# CHAPTER TWO

# RENAL FUNCTION TEST

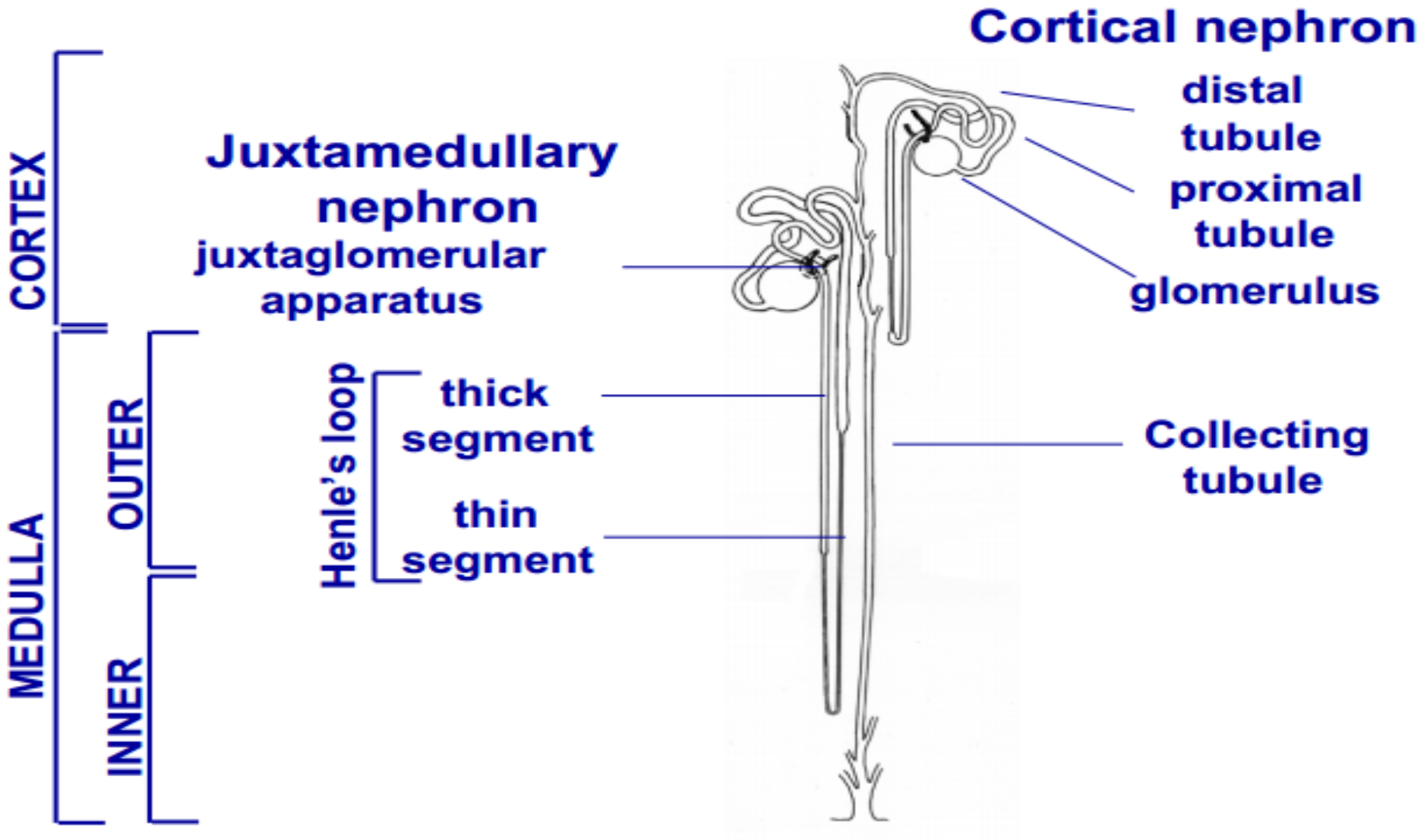
# Objectives of lesson

- After a successful completion of this lesson, you will be able to:
  - Explain the importance of kidney function test.
  - Describe the types of renal function tests.
  - Perform the various types of kidney function test.
  - Interpret the various components of the result to the underlined pathological condition

# INTRODUCTION

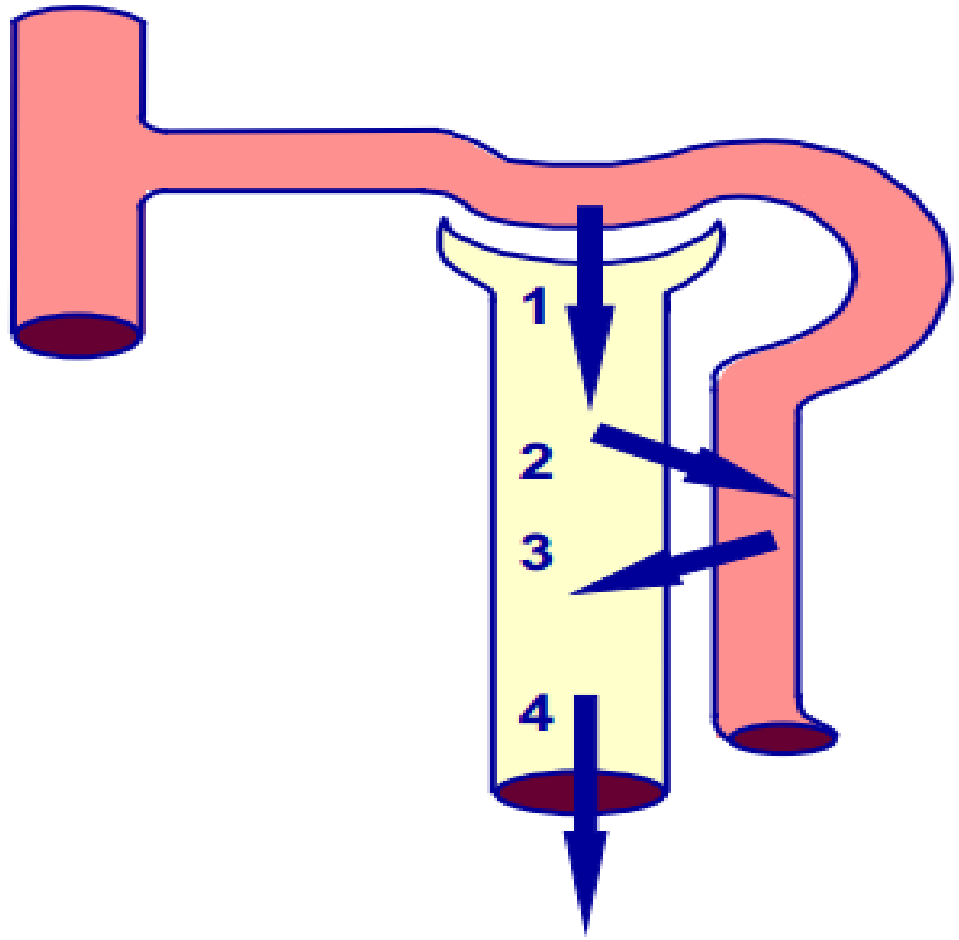
- Kidneys are retroperitoneal abdominal organs having an outer cortical and inner medullary region.
- They can be classified as either unilobular or multilobular based on the presence of pyramidal areas in the renal medulla.
- The basic functional unit of the kidney is the nephron.
- It consists of the renal capsules, the proximal and distal tubule, the loop of Henle, and the collecting ducts.
- There are usually about **400,000 nephrons** in the average kidney.

# ANATOMY OF THE NEPHRONS



# BASIC NEPHRON FUNCTIONS

- 1. Filtration
- 2. Reabsorption
- 3. Secretion
- 4. Excretion



# Basic Function.....

- The primary function of glomeruli is filtration through which an ultrafiltrate of plasma, containing only trace amounts of proteins passes into the Bowman's space.
- The proximal tubules are responsible for reabsorption of nutrients and other substances which the body needs to retain.
- The loops of Henle serve as a countercurrent multiplier system which concentrates or dilutes the filtrate and in doing so creates a hyperosmotic state in the medulla.

# Basic Function…..

- The distal convoluted tubule is influenced by antidiuretic hormone.
- It allows either the filtrate to pass in its diluted state or concentrates the filtrate by allowing water to be reabsorbed.
- The collecting tubules are also under the influence of ADH continue either to concentrate the urine or allow its passage in a dilute form.

# Major Function of Kidney

- **Urine Formation**
  - By 4 process: Glomerular filtration, Tubular reabsorption, Tubular secretion and Excretion
- **Excretion of harmful/toxic substances:**
  - Non-nitrogenous substances
    - Bilirubin, metabolites and drugs/toxins
  - Non-protein nitrogenous (NPN) substances
    - Urea, Creatinine and Uric Acid

# Major Function.....

- **Production of Hormone:**
  - Erythropoietin (EPO)
    - Secreted in response to low blood oxygen content.
    - It acts on bone marrow, stimulating the production of RBCs (Erythropoesis)
  - Renin
    - Regulates blood pressure, fluid and electrolyte balance via the Renin-Angiotensin-Aldosterone System (RAAS).

# Major Function.....

- **Homeostasis:**
  - Acid/Base Balance
  - Electrolytes Balance
    - It is governed by the 4 process which permit rapid removal of unwanted substances and retention of important molecules
    - Each of these processes is regulated according to the body needs.
    - E.g. when there is excess sodium→↑ Na filtration →↓Na reabsorption resulting in increased urinary excretion of sodium.

# Renal excretion system

- In renal system, a substance may be cleared by:
  - Glomerular filtration alone
  - Glomerular filtration plus tubular secretion
  - Glomerular filtration plus tubular reabsorption
- As the degree of tubular reabsorption diminishes, the substance may appear in the urine and its clearance could be increasing

# Renal excretion system…

- The manner by which a substance is excreted and reabsorbed by the kidney directly affects the renal clearance rate.

  - If a substance is completely filtered at the glomerulus and completely reabsorbed by the tubules, its clearance value is zero (**e.g., glucose**)

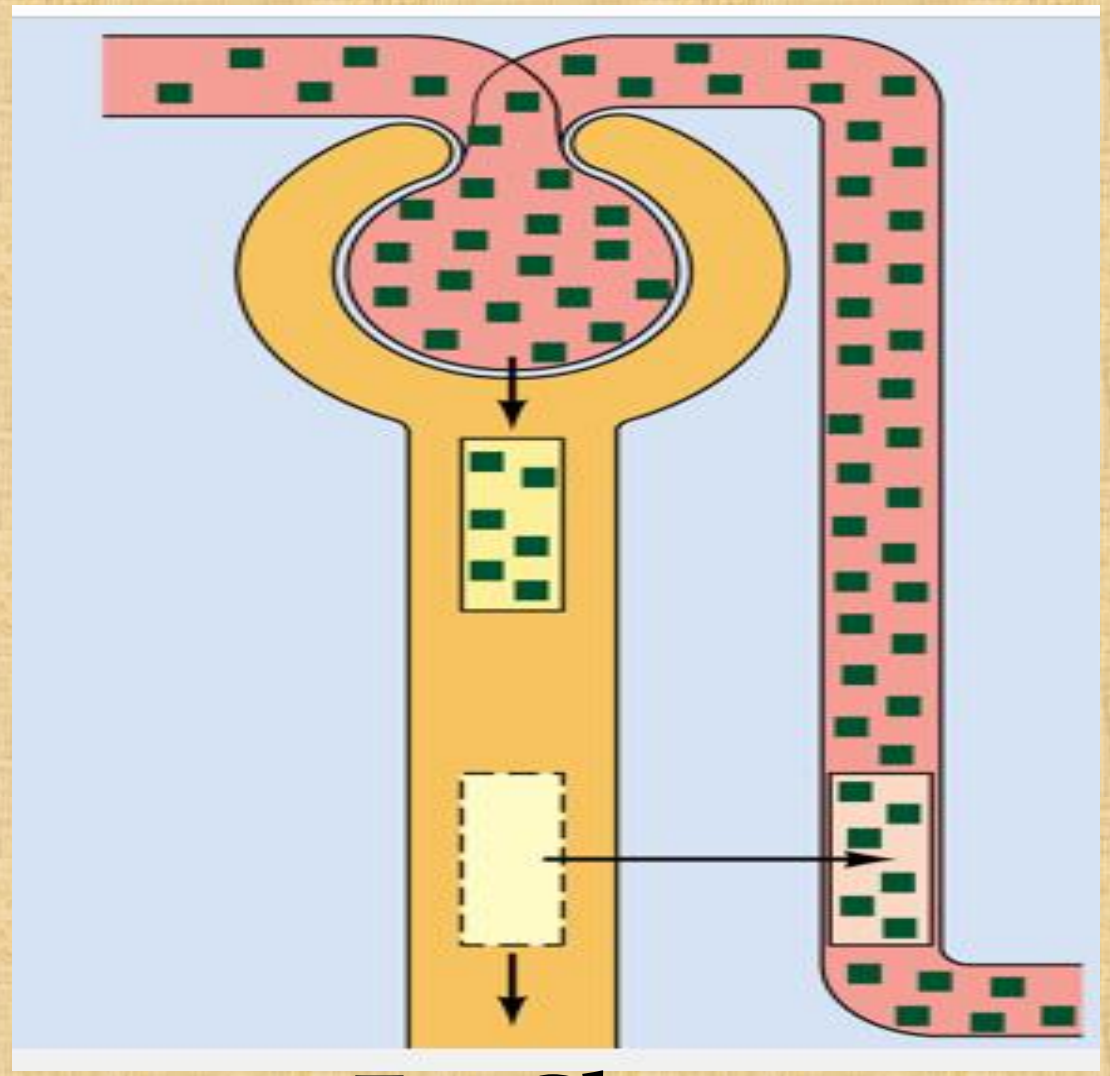
  - If a substance is completely filtered at the glomerulus but partially reabsorbed by the tubules, its clearance value is its difference (**e.g., urea**)

# Renal excretion system…

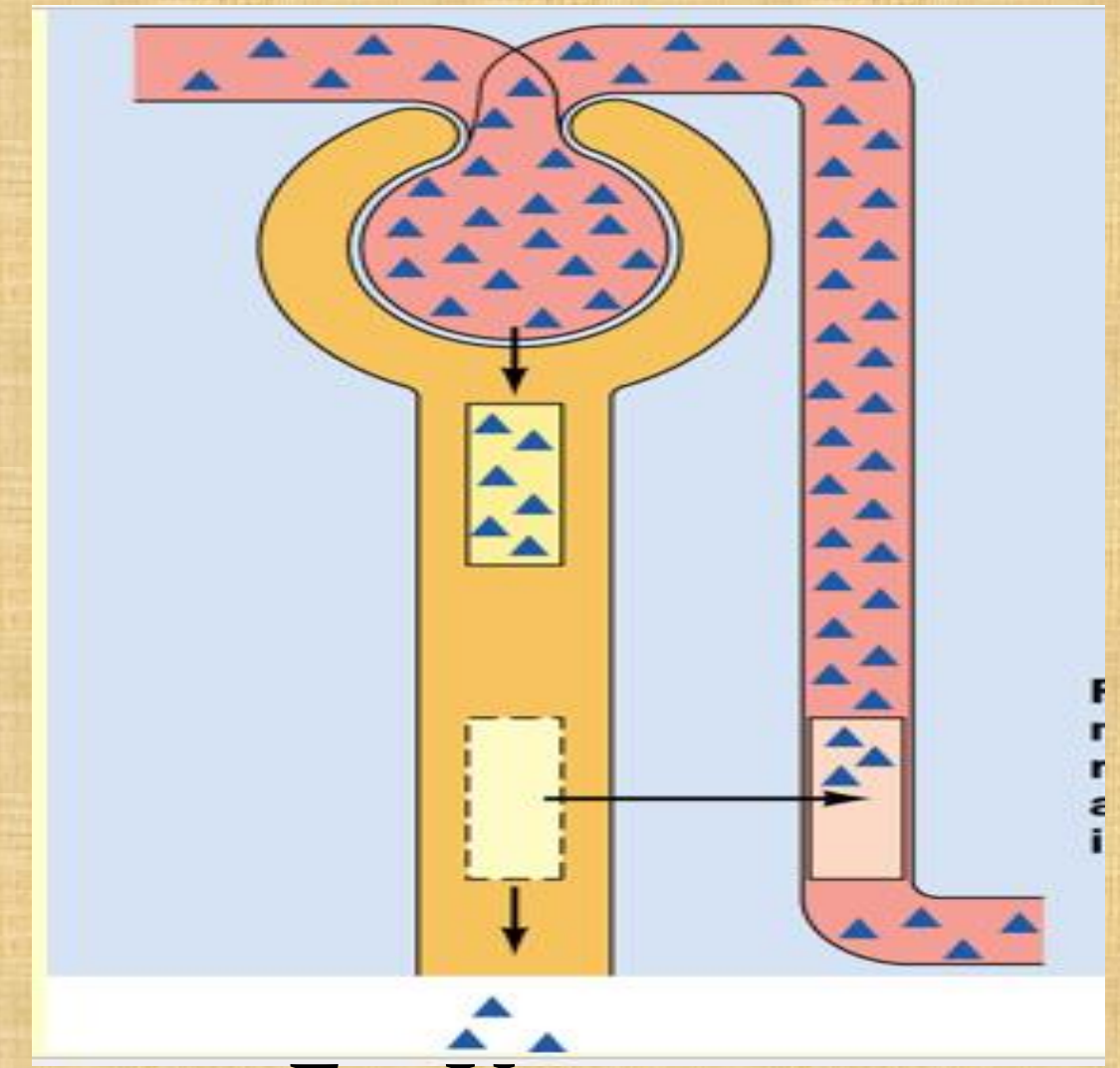
- If there is no reabsorption of a substance then its clearance will be equivalent to the rate of glomerular filtration (**e.g., inulin and creatinine**)

- In addition to being filtered through the glomerulus, the substance is also secreted by the tubular epithelium, its clearance will exceed the rate of glomerular filtration by an amount equal to the extent of tubular secretion (**e.g., phenol red**)

# Renal excretion system…

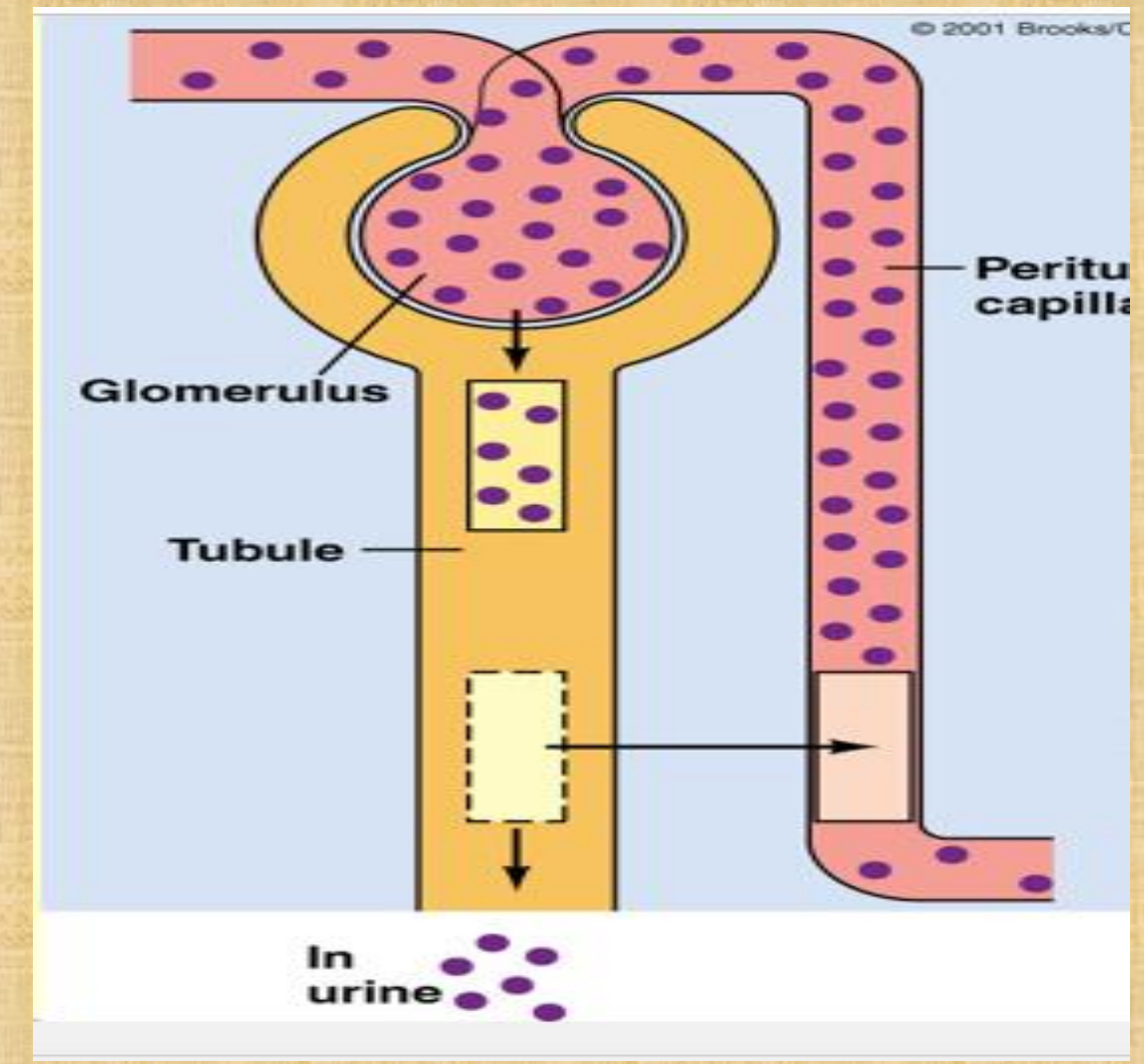


Eg. Glucose

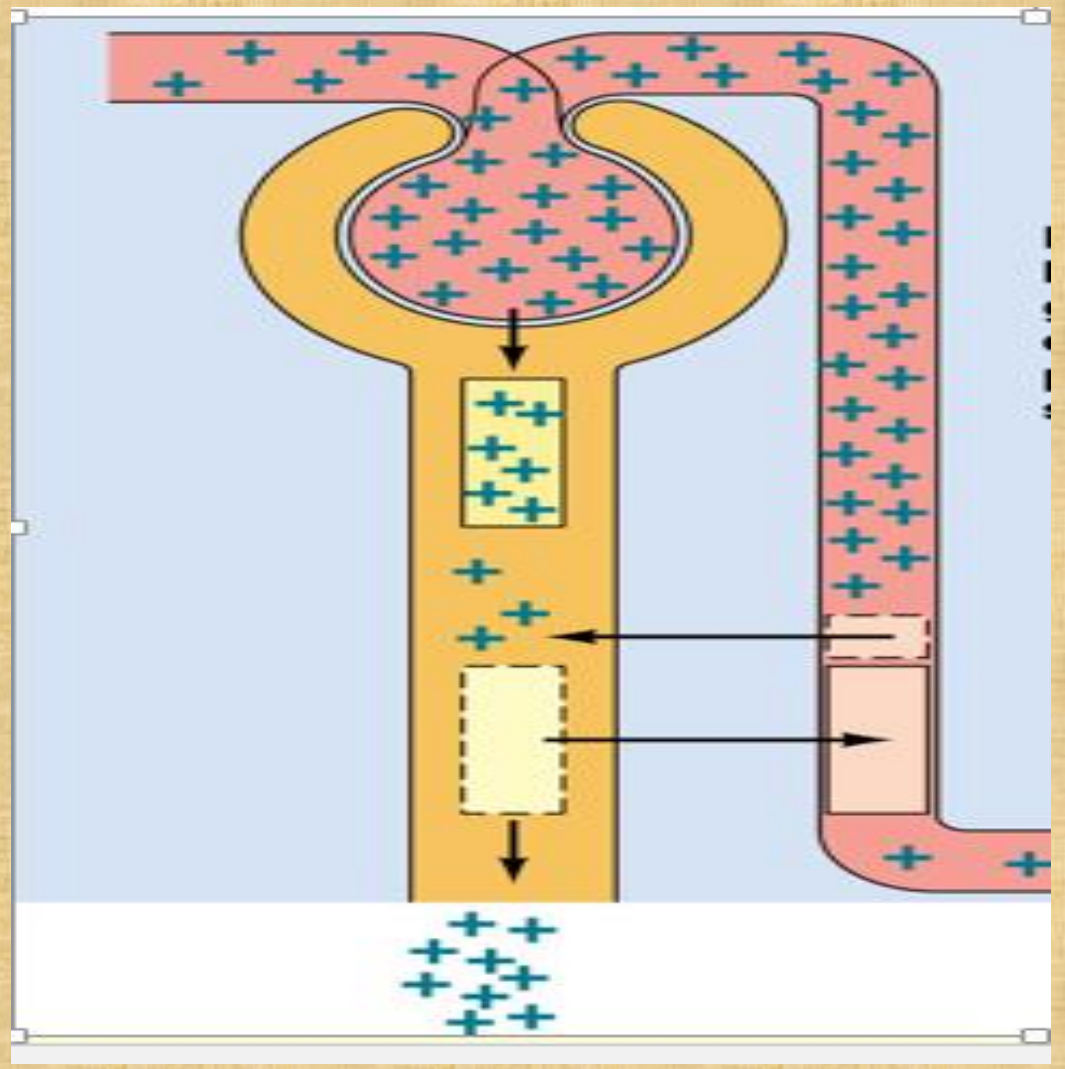


Eg. Urea

# Renal excretion system…




Eg. Inulin and creatinine



Eg. Phenol red

# QUANTITIES OF SOLUTE FILTERED AND EXCRETED

| | Plasma Conc. mM | Filtered/day mmoles | Excreted/day mmoles | Percent Reabsorbed |
|---|---|---|---|---|
| Na | 140 | 25,200 | 103 | 99+ |
| Cl | 105 | 18,900 | 103 | 99+ |
| $HCO_3$ | 25 | 4,500 | 2 | 99+ |
| K | 4 | 720 | 100 | 86+ |
| Glucose | 5 | 900 | trace | 100 |
| Urea | 5 | 900 | 360 | 60 |

# Why Test Renal Function?

- To identify renal dysfunction
- To diagnose renal diseases
- To monitor disease progress
- To monitor response to treatment
- To overcome complication induced by drug administration and other intervention

# Desirable characteristics of tests for Glomerular function

- GFR (the rate of glomerular filtration) mostly serves as a test to assess glomerular function.
- To measure the rate of filtration, it is best to use a substance which:
  - ☞ Can be measured in urine and plasma
  - ☞ Can pass the glomerular membrane
  - ☞ Must not be reabsorbed or excreted by the tubules to any appreciable degree.

# Desirable characteristics of tests for tubular reabsorption

- To measure the reabsorptive ability of the tubule, it is necessary to use a substance which:
  - Can be measured in urine and plasma
  - Can pass through the glomerular membrane and is largely reabsorbed by the tubules (**Glomerular function must simultaneously be measured**)
  - Has little or no tubular secretion

# Desirable characteristics of tests for tubular secretion

- To measure the rate of tubular excretion, it is best to use a substance which:
  - Can be measured in urine and plasma
  - Has little or no glomerular filtration or tubular reabsorption
  - Is predominantly removed from blood plasma by tubular secretion

# Types of Renal function tests

- Renal function tests in common use for clinical purposes fall into the following categories:
  - Urinalysis
  - Urine concentration test
  - Estimations of NPN levels in the blood
  - Clearance test
  - BUN/ creatinine ratio
  - Dye excretion tests

# Urine Concentration Tests

- **Could be done in to two ways**
  1. Water Deprivation (Fishburg Concentrate)
  2. Vasopressin Test (Pituitrin Concentration Test, Pitressin Conc Test)

# Water Deprivation (Fishburg Conc)

- Withholding water for a period of time causes hyperosmolality of plasma.
- This stimulates pituitary gland to release of antidiuretic hormone (**ADH**).
- ADH acts on the renal tubular epithelial cells causing reabsorption of water thereby increasing urine specific gravity.
- If the tubules are nonfunctional, water will not be reabsorbed and the specific gravity will remain low.

# Water Deprivation…..

- **Procedures**
  - Withhold water for 12-24 hours.
  - Empty the bladder at 12 hours and determine the urine specific gravity.
  - If it is above 1.020 discontinue the test because the animal can concentrate urine.
  - If the specific gravity remains below 1.020, continue water deprivation for a total of 24 hours.
  - If the specific gravity remains below 1.020 at this point, an impairment of concentrating ability is indicated.

# Water Deprivation.....

Failure of urine conc occurs with:

1) Renal disease due to
   - Approximately **2/3** of the nephrons are nonfunctional
2) Diabetes insipidus
   - Pituitary disease causes a lack of **ADH** secretion.
   - In this condition, the renal tubules are normal but are not stimulated to reabsorb water.

# Water Deprivation…..

- Contraindications include:
  - Uremia - a diagnosis of renal disease is already established.
  - Debilitated animals
  - Dehydration: because maximal stimulation for **ADH** release is already in effect.

# Vasopressin Test (Pituitrin Concentration Test, Pitressin Concentration Test)

- The test is similar to the urine concentration test except exogenous **ADH (pitressin)** adminstration
- Indications
  - To differentiate between diabetes insipidus and renal disease.
  - To evaluate tubular reabsorption when prolonged water deprivation is contraindicated.
- Contraindications
  - Uremia
  - Pregnancy

# Vasopressin Test.....

- **Procedures**
  - Inject IM/SC of pitressin tannate (up to 5 units).
  - Empty bladder at 30 minutes
  - Check urine specific gravity at 3, 6, and 9 hours
  - A specific gravity remaining less than 1.020 is abnormal.
- **Interpretation**
  - Concentration to a specific gravity of 1.020 or greater indicates diabetes insipidus.
  - Failure to concentrate to a specific gravity of 1.020 indicates renal disease.

# Tests for Non-protein Nitrogen Blood Levels

- Determination of the non-protein nitrogenous substances is important especially (urea and creatinine) to assess renal diseases.

- Because significant elevation are usually the result of accumulation of these substances in the blood due to defective elimination by kidney.

# Non Protein Nitrogen

- The majority of these compounds arise from the catabolism of proteins and nucleic acids.
- Most important NPNs includes:
  - ✓ Urea /BUN ( Blood Urea Nitrogen )
  - ✓ Creatinine
  - ✓ Uric acid
  - ✓ Ammonia
- Measurements of plasma or serum concentrations of these metabolites are commonly used as indicators of kidney function and conditions.

# UREA

- It is one of the NPN compound present in highest concentration in the blood.
- Urea is the major excretory product of protein metabolism.
- It is formed in the liver from amino groups (-NH2) and free ammonia generated during protein catabolism.
- It is excreted from the body by the kidney following glomerular filtration
- The blood urea nitrogen (BUN) has been commonly used to calculate urea conc.
- Urea nitrogen level can be converted to urea concentration by multiplying BUN with 2.14 factors.

# Urea Level

- Plasma concentration depends on:
  - Renal function and perfusion
  - Protein content of the diet
  - The amount of protein catabolism
  - Liver function
  - State of hydration

# Clinical Application

- Measurement of urea is used to
  - Evaluate renal function
  - Assess hydration status
  - Determine nitrogen balance and
  - Verify adequacy of dialysis
- An elevated concentration of urea in the blood is called **azotemia.**
- Very high plasma urea concentration accompanied by renal failure is called **uremia, or uremic syndrome.**

# Clinical applicn….

- According to its causes, azotemia are classified into three main categories: Pre renal,  Renal, Post renal
- **Prerenal azotemia** is caused mostly by reduced renal blood flow or rarely due to high rates of protein catabolism.
- Less blood is delivered to the kidney; consequently, less urea is filtered.
- High protein catabolism causing high level of blood urea due to its rate beyond the threshold level of filtration
- Causative factors include: CHF, shock, hemorrhage, dehydration that decrease in blood volume and high-protein diet

# Clinical applicn….

## Renal azotemia

- It is related to renal diseases result in compromised urea excretion.
- Causes include acute and chronic renal failure, glomerular nephritis, tubular necrosis, and other intrinsic renal disease.

## Post-renal azotemia

- This is mostly due to obstruction of urine flow anywhere in the urinary tract
- Causes: renal calculi, tumors in the bladder or prostate, or severe infection.

# Methods of Urea Measurement

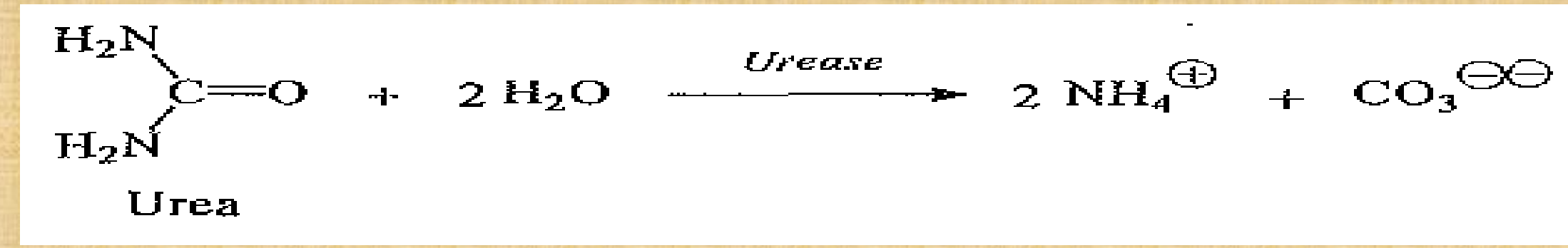
- Urea can be determined by Enzymatic (indirect) method and Chemical (direct) method

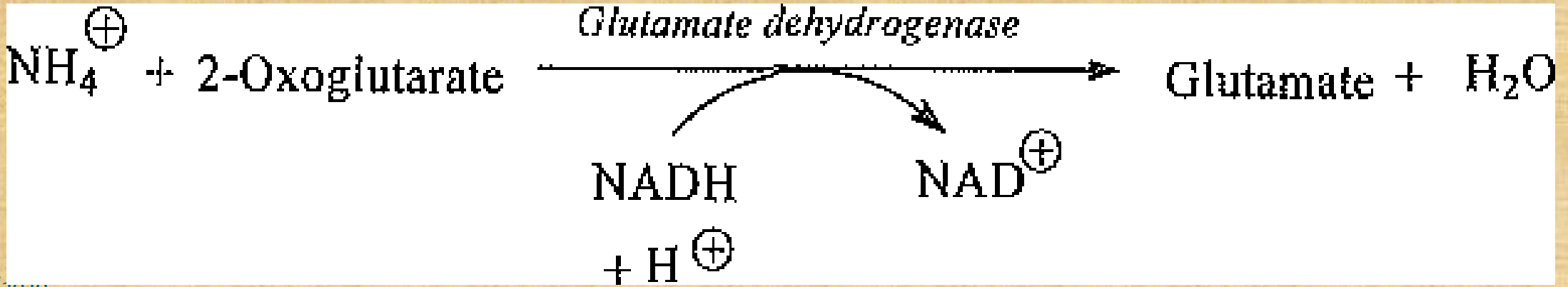
## 1. Enzymatic Methods (indirect method)

- The enzyme urease hydrolyzes urea in the sample to produce ammonium ion (NH4) and bicarbonate.
- In the second reaction ammonium ion react with oxoglutarate in the presence of glutamate dehydrogenase (GLDH) give rise glutamate and $H_2O$.

# Methods….

- The rate of disappearance of nicotinamide adenine dinucleotide (reduced, NADH) to facilitate the reaction at 340 nm is measured.



# Methods…..

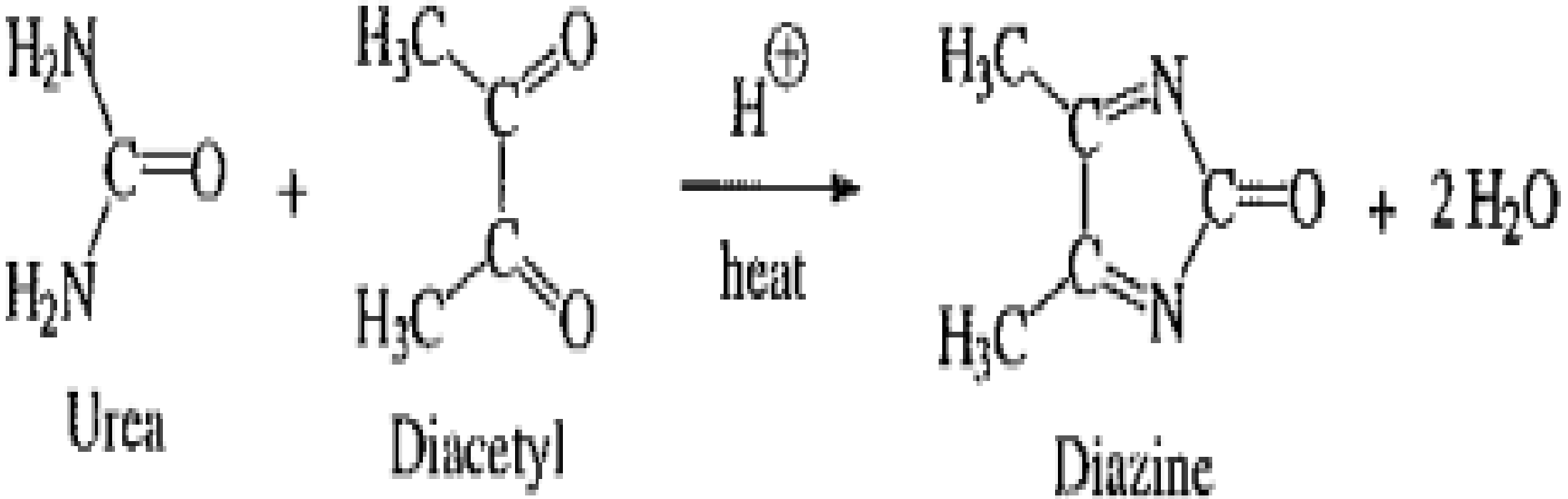
- The amount of $NAD^+$ produced is directly proportional to the amount of ammonium ion.
- This is directly proportional to the amount of urea present
- Disadvantage: endogenous ammonia will interfere

# Methods….

## 2. Chemical Methods

- It is called ‘direct’ methods because urea in the sample directly reacts with a color causing reagent called diacetylmonoxime.
- In the reaction diacetyl react with urea to form the chromogen diazine.
- It can be measured spectrophotometrically at 540 nm.
- Advantage: endogenous ammonia does not interfere

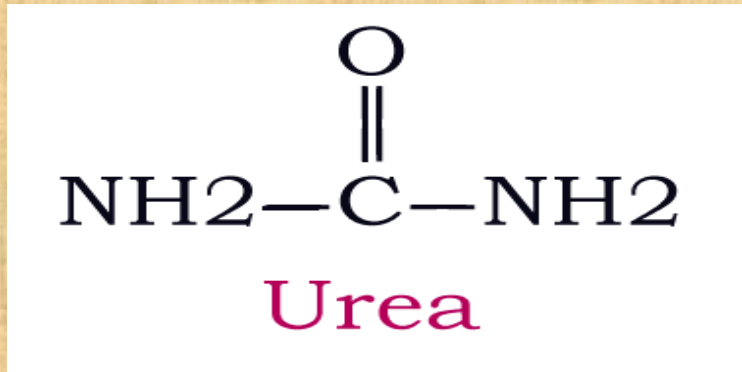
# Methods….




Measured spectrophotometrically at 540 nm

# BUN (Blood Urea Nitrogen)

- The chemical methods also measured the nitrogen content of plasma after precipitating out the proteins.
- The result was reported as the amount of ‘blood urea nitrogen’ or BUN
- A conversion factor is needed to correlate the nitrogen content to urea (2.14)



# Specimen Collection/Handling

- Serum or heparinized plasma can be used
- Stability: up to 24 hours at room temperature and 1 week at 2-4$^{o}$C
- It should not be hemolyzed
- Fasting is not required
- Ammonium heparin cannot be used for methods using urease due to its interference
- Fluoride and citrate containing anticoagulants cannot be used because they inhibit enzyme activity

# Creatinine

## Introduction

- Creatinine is formed as a final product of creatine and creatine phosphate as a function of muscle contraction
- Creatine is synthesized primarily in the liver and to some extent in kidney and pancreas from arginine, glycine, and methionine.
- It is then transported to muscle and converted to creatine phosphate, which serves as a high-energy source.
- When creatine phosphate dephosphorylated and creatine loses water, they form the cyclic compound known as creatinine

# Creatinine.....

- This creatinine diffuses into the blood and goes to the kidney for filtration.
- It is released into the circulation at a relatively constant rate proportional to muscle mass.
- It is filtered from the circulation only by glomerular filtration and excreted through urine.
- Daily creatinine excretion is fairly stable.
- It show little or no response to dietary changes

# Clinical Application

- Measurement of creatinine concentration is used to determine
  - Sufficiency of kidney function
  - Severity of kidney damage and
  - Progress of kidney disease
- Blood/ urine creatinine concentration is a function of
  - Relative muscle mass
  - The rate of creatine turnover and
  - Renal function especially GFR.

# Methods of Creatinine Measurement

## 1. Chemical method: Jaffe Reaction

Creatinine + alkaline pictrate → Janovski complex

(yellow) (red-orange color)

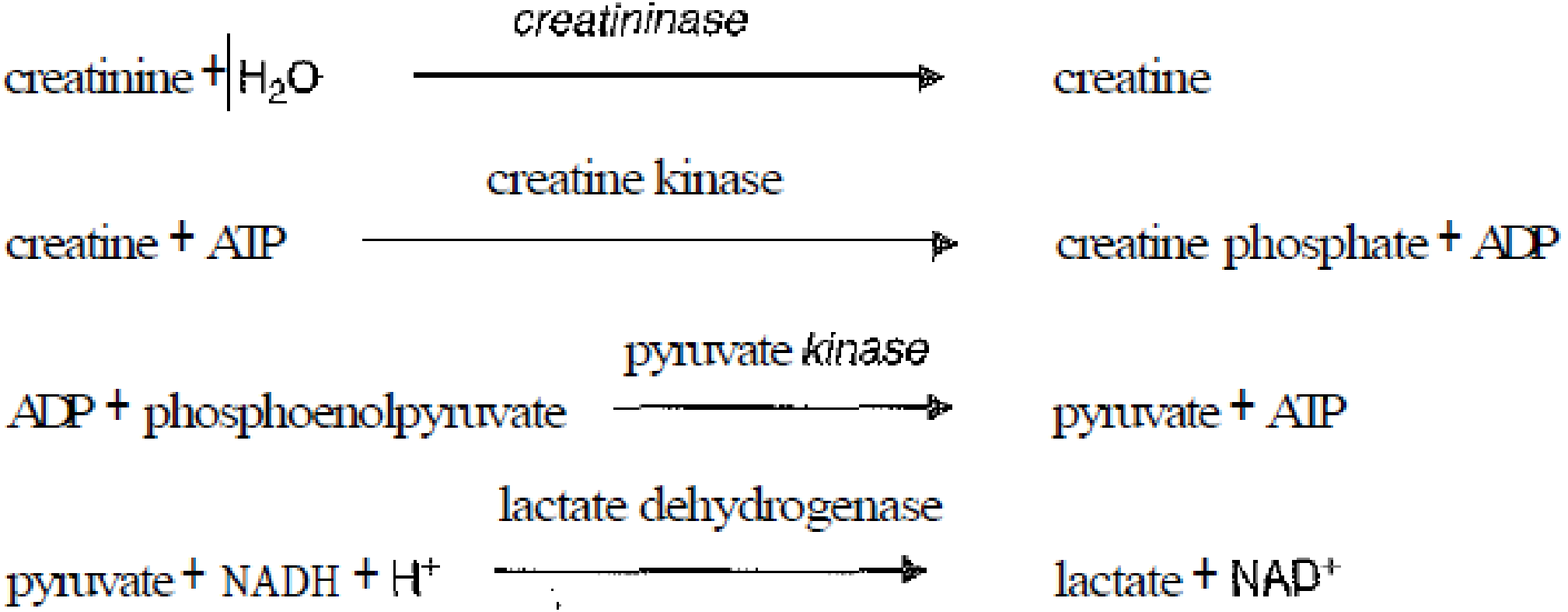
- This reaction lacks specificity:
  - Falsely increased : with high levels of protein, ascorbic acid, ketones, glucose, pyruvate and uric acid
  - Falsely decreased : with bilirubin

# Methods of …..

## 2. Coupled enzymatic method

- Using creatininase that catalyzes the conversion of creatinine→ creatine
- The creatine is then detected with a series of enzyme-mediated reactions involving creatine kinase, pyruvate kinase, and lactate dehydrogenase
- It has a potential to replace Jaffe reaction and most widely used method
- Less interference as compared to Jaffe reaction

# Mthods of…..




The decrease in absorbance at 340 nm proportional to the amount of creatinine in the sample

# Creatinine Specimen Collection and Handling

- Urine
  - Timed collection preferred and random is acceptable
  - Stability: up to 4 days in refrigeration
  - Longer when it is deep frozen
- Serum or heparinized plasma
  - Avoid  hemolysis
  - Avoid lipemia
  - Stability: one week at refrigeration

# Interpretation of creatinine result

- Normal values range from 1 to 2 mg/dl
- Low values have no significance
- Increased values occur in:
  - Primary renal disease with reduced GFR
  - Impaired renal blood flow
  - Obstruction of urinary system
  - Muscular damage

# Interpretation of creatinine result …

- Creatinine is helpful in making a prognosis.
- Studies states that if creatinine is between:

  ①1-2 mg/dl and BUN is normal - prognosis is excellent

  ②2-5 mg/dl and BUN is elevated - prognosis is good

  ③5-7 mg/dl and BUN is elevated - prognosis is guarded

  ④Greater than 7 mg/dl - animal will probably die

# Clearance Tests

- Clearance means the blood volume (ml) from which a biomarker substances is totally cleared in unit time (minute) "ml/min"
- Eg. GFR = Clearance of a Substance

  **= (USubs/SSubs) x Urine flow rate (Urine Vol. ml/min)**

Where

- USubs= Conc. of the bio marker substance in Urine mg/dl
- SSubs= Conc. of the marker substance in Plasma mg/dl

# Clearance Tests…..

- Marker substance used to measure GFR may be Exogenous (e.g. inulin) or Endogenous (e.g. Creatinine, Urea…)
- Ideal biomarker for measurement GFR:
  - Cst rate of production (endogenous) or of delivery (IV) (exogenous).
  - Freely filtered in the glomerulus i.e. no protein binding.
  - Metabolically inactive
  - No tubular reabsorption
  - No tubular secretion
  - Can be estimated in the Lab

# Inulin Clearance

- Inulin is a fructose polysaccharide that gives very accurate estimations of GFR since it meets all the criteria mentioned above.
- A loading dose of inulin can be administered IV, followed by a sustaining infusion
- This is done first by infusing 30 ml of 10% inulin in 250 ml of normal saline infused at a rate of 20 ml/ min to achieve desired concentration.

# Inulin Clearance……

- Then 70 ml of 10% inulin in 500 ml saline in infused at a rate of 4 ml/ min to maintain the desired concentration.
- Inulin concentration in urine and plasma is determined and hence GFR can be calculated.
- Normal inulin clearance is estimate from 120 to 130 ml/minute
- Below the normal shows an impaired glomerular function.

# Creatinine Clearance

- Creatinine is filtered at the glomerulus and its reabsorption at the tubular level is insignificant.
- Because of this, creatinine clearance can be used to measure Glomerular Filtration Rate (GFR).
- It is measured over a period of 24 hrs.
- The concentration of creatinine is measured both in the urine and serum sample.

# Creatinine Clearance.....

- Creatinine clearance is measured by the following method:

$$\frac{(\text{Conc. of creatinine in urine} \times \text{volume of urine})}{\text{Conc. of creatinine in serum}} / 1440$$

- It is a more sensitive indicator of renal function than serum creatinine and BUN alone.
- The normal range of creatinine clearance is estimated between 90-120ml/min

# Urea clearance

- Urea clearance is the hypothetical amount of blood from which kidney clears urea in one minute.
- This is measured by determining the concentration of urea in blood, concentration of urea in urine and amount of urine excreted over one hour interval.
- Urea clearance is less than its glomerular filtration since some of the urea is reabsorbed at the tubules.

# Urea clearance.....

- To measure urea clearance first the animal has to be urinate and then to drink water.
- Then the urine and blood specimen is collected after an hour at the same time.
- The urine volume is calculated as

$$\frac{(\text{Urine urea conc.} \times \text{Urine volume per minute})}{\text{Urea conc. in serum}}$$

- A urea clearance below 60% considered as impairment for tubular reabsorption.

# BUN/Creatinine Ratio

- Used by clinicians to differentiate causes of azotemia:
  - Pre-renal
  - Renal
  - Post-renal
- **Azotemia** is the condition of increased NPN in blood most often due to increased BUN and creatinine.
- Evaluating their ratio is crucial to differentiate cause of azotemia.

# BUN/Creatinine Ratio …..

- Calculated: $\frac{\text{serum BUN (mg/dl)}}{\text{serum creatinine (mg/dl)}}$

- Normal ratio: 10-20 with majority around 12-16

# BUN/Creatinine Ratio…..

Increased ratio with increased BUN and normal creatinine

- Tend to be caused by pre-renal conditions:
  - Congestive Heart Failure
  - Shock, hemorrhage
  - Dehydration
  - Increased protein metabolism
  - Increased protein catabolism

# BUN/Creatinine Ratio Cont.….

- Increased ratio with dysproportionate increased BUN, slightly increased creatinine
  - Tend to be caused by post-renal conditions that obstruct urine flow:
    - Stone
    - Tumor
    - Sever infection

# BUN/Creatinine Ratio…..

- Increased ratio with increased BUN, increased creatinine

- Tend to be caused by renal conditions that decrease kidney function:
  - Acute renal failure
  - Chronic renal failure
  - Glomerulonephritis
  - Tubular necrosis

# BUN/Creatinine Ratio …..

- Decreased ratio with decreased BUN

- Tend to be caused by conditions of decreased urea production:
  - ☛ Low protein diet
  - ☛ Liver disease

# Dye Excretion tests

- Dye Excretion tests - The most commonly used dye is Phenosulfonphthalein

**Phenolsulfonphthalein (PSP)**

- When PSP is injected IV, most of the dye (**approximately 95%**) is bound to albumin and excreted by the tubules.
- The small amount not bound to albumin is filtered by the glomerulus.
- Therefore, it is a rough index to measure tubular function.
- Two methods can be used :
  - Excretion Test
  - Clearance Test

# Dye Excretion tests…..

## 1) Excretion Test

- Inject 6 mg of dye IV after the bladder has been emptied **(catheterization).**
- After 20 minutes the contents of the bladder are gently aspirated with a syringe and all urine is collected in a flask.
- Dilute and mix with sodium hydroxide in equal amount
- Then the quantity of dye excreted is measured spectrophotometrically **(at 560 nm)** or by visual comparison with color standards.
- Normal animal can excrete 21 to 66 percent of the dye in 20 minutes.

# Dye Excretion tests…..

## 2) Clearance Test

- Inject 1 mg/kg body weight IV.
- Measure blood levels at 30 or 60 minutes following injection.
- This is very useful during acute nephritis since only a small amount of urine is being produced.

- **Interpretation: Less than 20% clearance rate is related to**
  - Renal disease (Approximately 2/3 of the nephrons are nonfunctional).
  - Reduced renal perfusion due to various factors


Thank
you !