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Contents

Acid-Base Regulation

Metabolic processes continually produce acid and, to a lesser degree, base. Hydrogen ion (H^+) is especially reactive; it can attach to negatively charged proteins and, in high concentrations, alter their overall charge, configuration, and function. To maintain cellular function, the body has elaborate mechanisms that maintain blood H^+ concentration within a narrow range—typically (pH 7.43 to 7.37, where pH = −log $[H^+]$) and ideally (pH = 7.40). Disturbances of these mechanisms can have serious clinical consequences.

Acid-base equilibrium is closely tied to fluid and electrolyte balance, and disturbances in one of these systems often affect another.

Lesser amounts of organic acid derive from the following:

- Incomplete metabolism of glucose and fatty acids into lactic acid, acetoacetic and β-hydroxybutyric acids.
- Metabolism of sulfur-containing amino acids (cysteine, methionine) into sulfuric acid
- The oxidation of proteins, nucleic acids and phospholipids produces phosphoric acid
- Hydrolysis of dietary phosphate
- Complete (aerobic) metabolism of fat and carbohydrates produces CO2. In solution, CO2 forms a weak carbonic acid which is **transported to the lungs in the blood** and is rapidly **excreted by the lungs**. Only if respiratory function is impaired do problems occur.

This “fixed” or “metabolic” acid load cannot be exhaled and therefore must be neutralized or excreted.

Background to Buffers

Before we can define a buffer, or describe what a buffer does and how it does it, there are certain concepts that must be understood.

Definition of Terms

ACID Substance that dissociates to produce H+ ions,

$HA \leftrightarrow H^+ + A^-$ e.g., $H3PO4 \leftrightarrow H^+ + H2PO4^-$.

BASE Substance that accepts H^+ ions, e.g., $H2PO4^- + H^+ \leftrightarrow H3PO4$.

Acid-Base Balance

Acid-base balance is maintained by chemical buffering and by pulmonary and renal elimination.

Chemical buffering: Chemical buffers are solutions that resist changes in pH. Intracellular and extracellular buffers provide an immediate response to acid-base disturbances. Bone also plays an important buffering role. A buffer is made up of a weak acid and its conjugate base. The conjugate base can accept H^+ and the weak acid can relinquish it thereby minimizing changes in free H^+ concentration.

The most important extracellular buffer is the HCO_3^-/CO_2 system, described by the equation:

$$H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow CO_2 + H_2O$$

An increase in H^+ drives the equation to the right and generates CO_2. This important buffer system is highly regulated; CO_2 concentrations can be finely controlled by alveolar ventilation, and H^+ and HCO_3^- concentrations can be finely regulated by renal excretion.

The relationship between HCO_3^- and CO_2 in the system can be described by the Kassirer-Bleich equation, derived from the Henderson-Hasselbalch equation:

$$H^+ = 24 \times Pco_2/HCO_3^-$$

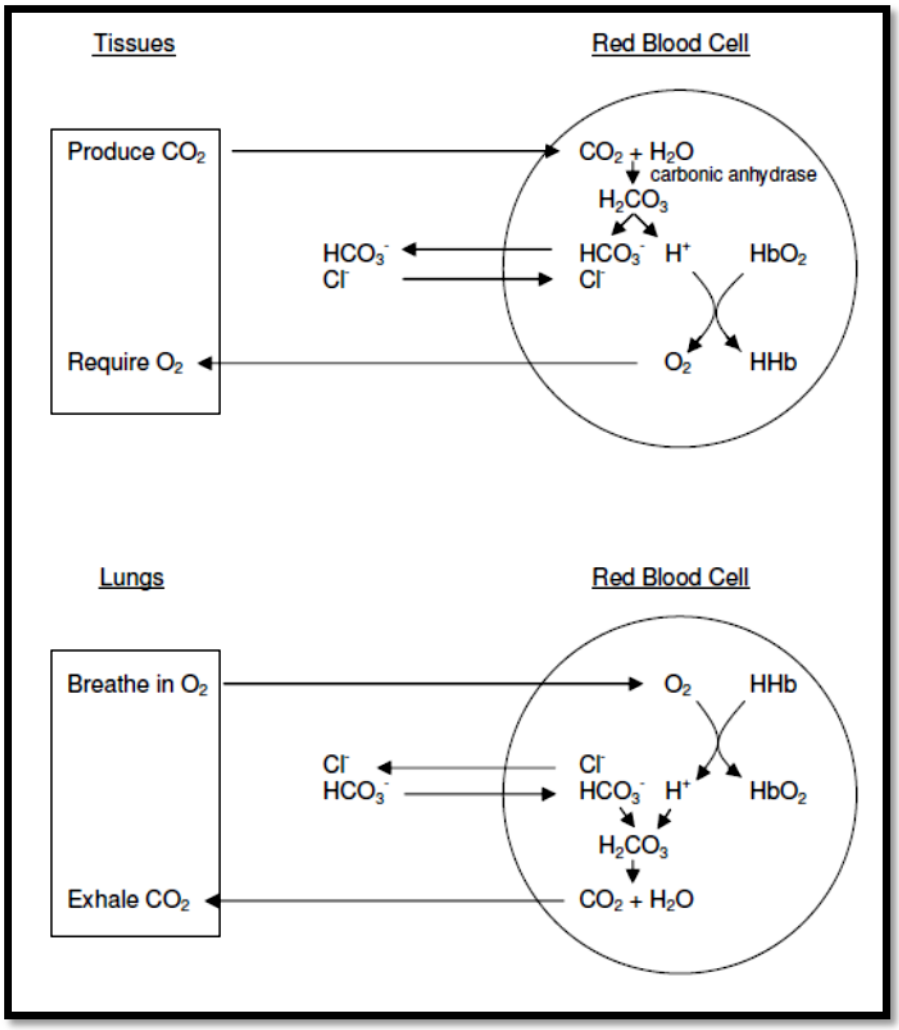
This equation illustrates that acid-base balance depends on the ratio of Pco_2 and HCO_3^-, not on the absolute value of either one alone. With this formula, any 2 values (usually H^+ and Pco_2) can be used to calculate the other (usually HCO_3^-).

- Other important physiologic buffers include intracellular organic and inorganic phosphates and proteins, including Hb in RBCs.
- Less important are extracellular phosphate and plasma proteins.
- Bone becomes an important buffer after consumption of extracellular HCO_3^-.
- Bone initially releases sodium carbonate ($NaHCO_3$) and potassium carbonate ($KHCO_3$) in exchange for H^+. With prolonged acid loads, bone releases calcium carbonate ($CaCO_3$) and calcium phosphate ($CaPO_4$). Long-standing acidemia therefore contributes to bone demineralization and osteoporosis.

Role of haemoglobin - transport of co2 and buffering

- CO2, produced by complete (aerobic) metabolism of fat and carbohydrates, diffuses out of cells into the ECF.
- In the ECF a small amount combines with water to form carbonic acid, thereby increasing the [H+] and decreasing the pH of the ECF.
- In RBCs metabolism is anaerobic and no CO2 is formed. CO2 therefore diffuses into RBCs down a concentration gradient. Then, the CO2 combines with water to form carbonic acid, due to the presence of carbonic anhydrase. The carbonic acid dissociates to form hydrogen ions and bicarbonate ions, and the hydrogen ions are bound by the haemoglobin.
- Deoxygenated haemoglobin binds hydrogen ions more strongly than oxygenated haemoglobin, and in fact the binding of hydrogen ions to haemoglobin facilitates the release of oxygen (the Bohr effect). The overall effect of this process is that CO2 is converted to bicarbonate in RBCs.
- The bicarbonate diffuses out of the RBCs along a concentration gradient, to be replaced by chloride ions (the chloride shift).
- In the lungs, the reverse occurs, because of the low partial pressure of CO2 in the alveoli: bicarbonate diffuses into the red cells; combines with hydrogen ions

released when haemoglobin binds oxygen, and is converted into CO2, which diffuses into the alveoli to be excreted. The role of haemoglobin is therefore to transport O2 and by converting CO2 to bicarbonate, to minimize changes in the HCO3 / CO2 ratio between venous and arterial blood, which helps to minimize pH changes.

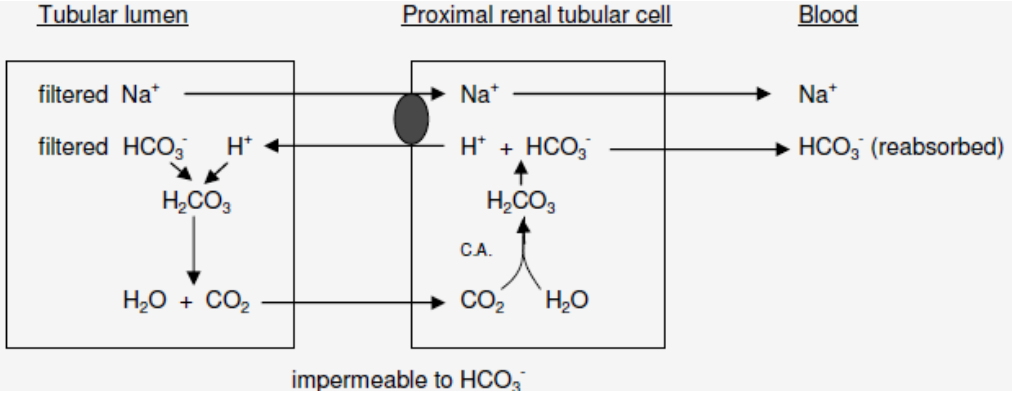


Role of the kidney in handling of bicarbonate and hydrogen

Reabsorption of bicarbonate

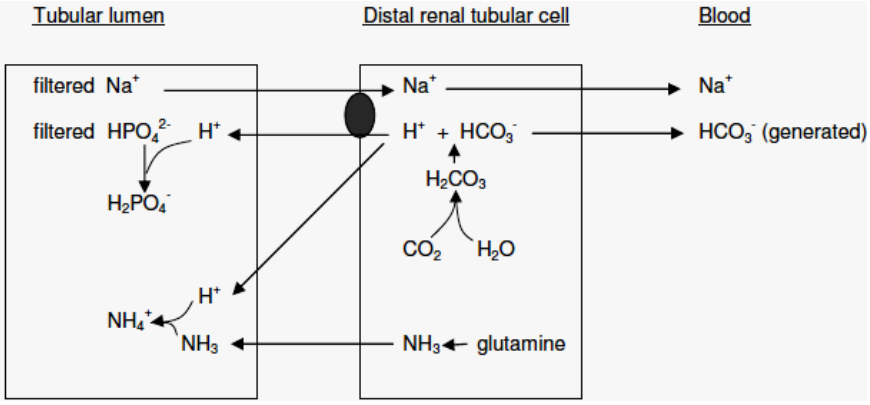
- The glomerular filtrate contains the same concentration of bicarbonate ions as the plasma.
- The luminal surface of the renal tubular cells is impermeable to bicarbonate, and direct reabsorption can thus not occur.

- Within the renal tubular cells, CO2 combines with water to form carbonic acid, due to the presence of carbonic anhydrase. The carbonic acid dissociates to form hydrogen ions and bicarbonate ions.
- The hydrogen ions are secreted into the tubular lumen in exchange for sodium ions, while the bicarbonate ions pass across the basal border of the cells into the interstitial fluid together with the sodium ions.
- In the tubular lumen the hydrogen ions combine with the filtered bicarbonate, form carbonic acid and then CO2 and water, some of which filters back into the renal tubular cell. The net effect of this process is that filtered bicarbonate is reabsorbed, but although there is hydrogen ion secretion, there is no net hydrogen ion excretion. This process takes place as long as filtered bicarbonate is present



Excretion of hydrogen and regeneration of bicarbonate

- This process uses the same mechanism as described above, but requires the presence of urinary buffers, otherwise the hydrogen ion gradient created would prevent further hydrogen ion secretion. **The main urinary buffer, phosphate ($HPO4^2$)** combines with secreted hydrogen ions to form H2PO4-.
- Another important urinary buffer is ammonia, produced by deamination of glutamine in renal tubular cells. The enzyme responsible, glutaminase, is induced by chronic acidosis, and there is thus an unlimited supply of NH3. The NH3 can diffuse across the renal tubular membrane, but NH4+ cannot. This process takes place as long as there is no more filtered bicarbonate present in the tubular lumen, which is essentially in the distal renal tubule.



- Decreases in effective circulating volume (such as occur with diuretic therapy) increase HCO_3^- reabsorption,
- while increases in parathyroid hormone in response to an acid load ↓ HCO_3^- reabsorption.
- ↑ Pco_2 leads to increased HCO_3^- reabsorption,
- while Cl^- depletion (typically from volume depletion) leads to increased Na^+ reabsorption and HCO_3^- generation by the proximal tubule

Concepts and vocabulary of acid-base imbalance

Acidosis: A condition characterised by a decrease in blood pH. The condition can be of metabolic or respiratory origin.

- a decrease in [HCO3-] (metabolic acidosis)
- an increase in pCO2 (respiratory acidosis)

Alkalosis: A condition characterised by an increase in blood pH. The condition can be of metabolic or respiratory origin.

- an increase in [HCO3-] (metabolic alkalosis)
- a decrease in pCO2 (respiratory alkalosis)

Compensation

- Metabolic compensation is slow to take effect, coming into effect over 2 - 4 days.
- In a respiratory acidosis, more H+ is excreted and more HCO3- is generated by the kidneys, increasing blood HCO3-

- In a respiratory alkalosis, less H+ is excreted and less HCO3- is generated by the kidneys, decreasing blood HCO3-
- When there are metabolic disorders, some compensation is possible by the lungs by altering the rate and depth of respiration, which is affected directly by the blood pH. This is known as *respiratory compensation* for the primary metabolic disorder.
- Respiratory compensation is quick to take effect, coming into effect within 15 - 30 minutes.
    - In a metabolic acidosis (decreased pH due to decreased HCO3-), the rate and depth of respiration are increased (hyperventilation), decreasing blood Pco2
    - In a metabolic alkalosis (increased pH due to increased HCO3-) the rate and depth of respiration are decreased (hypoventilation), increasing blood Pco2

Systematic list of metabolic acidosis

A. Gain of H+
    i. Increased production of fixed acid
        a. Ketoacidosis
        b. Lactoacidosis
    ii. Ingestion of acids or potential acids (rare)
    iii. Decreased excretion of H+ by kidney
        a. A chronic renal failure
        b. Distal renal tubular acidosis

B. Loss of HCO3-
    i. GIT loss
        a) Diarrhoea
        b) Transplantation of ureters into colon (extremely rare)
    ii. Renal loss
        a) Proximal Renal Tubular Acidosis (RTA type II)
        b) Some diuretics

Systematic list of metabolic alkalosis

1. Gain of bicarbonate
    i. Ingestion or infusion of alkali
        a) Antacids
        b) citrate, acetate or lactate
        c) bicarbonate
    ii. Too-rapid reversal of chronic respiratory acidosis
2. Loss of hydrogen
    i. GIT loss (could be considered a gain of bicarbonate instead of a loss of hydrogen)
        a) vomiting
        b) nasogastric suction
    ii. Renal loss
        a) diuretics
        b) excess mineralocorticoid action
        c) potassium depletion due to many causes, including purgatives

Systematic list of causes of respiratory acidosis

1. Depression Of Respiratory Centre

i) Drugs such as morphine, barbiturates, alcohol or general anesthetics

ii) Head injury

iii Intracerebral disease or tumours

2. Physical Inability To Ventilate

i) Crush injury to chest, flail chest

ii) Muscle paralysis, muscle relaxants

3. Airway Obstruction

i) Asthma

ii) Chronic obstructive airways disease (COAD) – emphysema

and chronic bronchitis

iii Foreign body inhalation

4. Pulmonary Disease Causing Decreased Co2 And O2 Exchange

i) Severe pneumonia

ii) Severe lung collapse

iii Severe pulmonary fibrosis

Systematic list of causes of respiratory alkalosis

1. Direct Stimulation Of Respiratory Centre

i) Drugs e.g., salicylates

ii) Anxiety, hysteria

iii) Brain stem disease

2. Mechanical Overventilation

3. Hypoxia

i) High altitude

ii) Anaemia

iii) Pulmonary disease causing decreased O2 diffusion

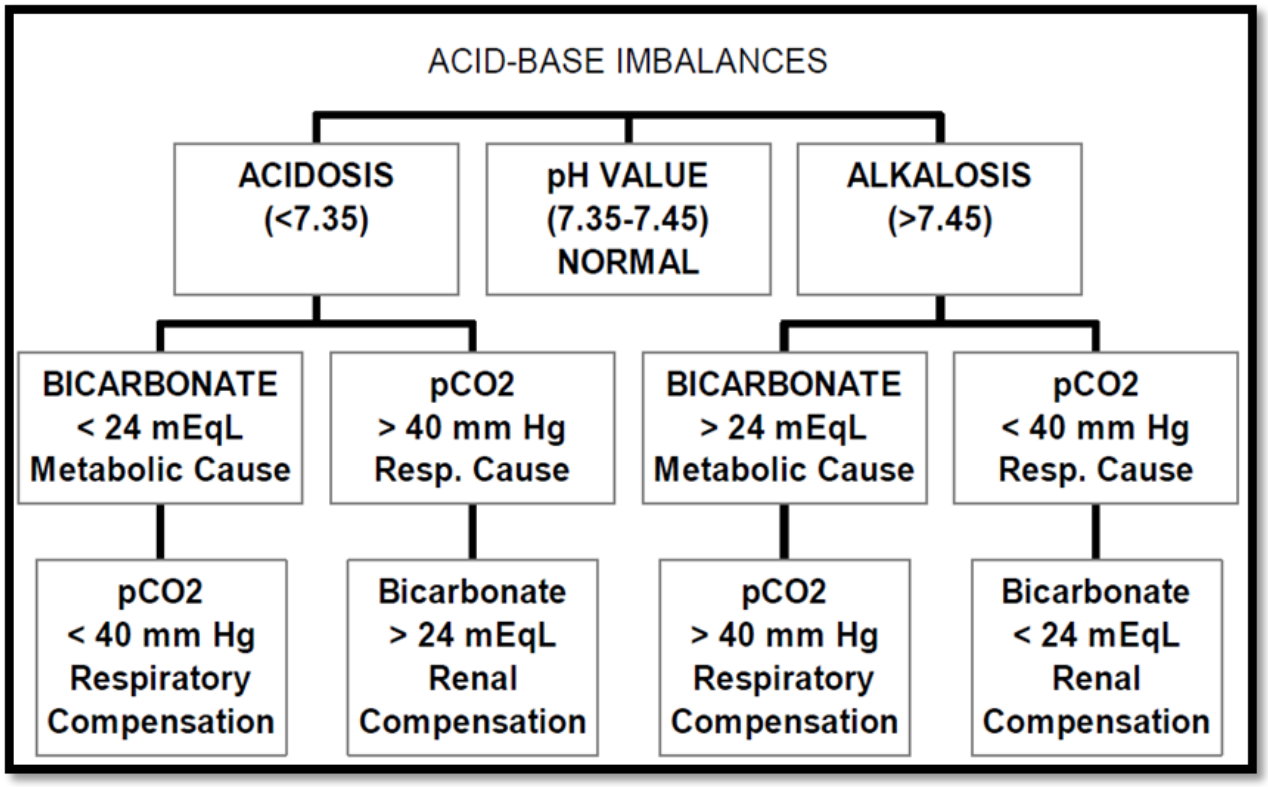
- Pulmonary oedema

iv) Pulmonary disease causing decreased O2 and CO2 exchange (ventilation/perfusion imbalance) *

- Mild pneumonia

- Mild lung collapse

- Mild pulmonary fibrosis



Interpretation of acid-base imbalance data

Mineral and electrolytes

Definition of Minerals

They are inorganic elemental atoms that are nutritionally essential.

They are not changed by digestion or metabolism.

Functions of Minerals

- Some participate with enzymes in metabolic processes (cofactors)
- Some have structural functions (Ca, P in bone; S in keratin)
- Acid-base and water balance (Na, K, Cl)
- Nerve & muscle function (Ca, Na, K).
- Other functions:

Iron → help in building the vital oxygen carrier hemoglobin.

Iodine → builds thyroid hormone, regulates the rate of all body metabolism.

Cobalt → the central core of vitamin B12.

Chlorine→ in hydrochloric acid in the stomach.

Minerals in Foods

-Found in all food groups.

-More reliably found in animal products.

-Often other substances in foods decrease absorption (bioavailability) of minerals

**Oxalate*, found in spinach, prevents absorption of most calcium in spinach.

**Phytate,* form of phosphorous in most plants makes it poorly available.

Classification

1-Macro or Major Minerals

1. They are required in amounts greater than 100 mg/day.
2. They include 7 elements:

Sodium, potassium, magnesium, calcium, phosphorus, sulfur, chloride.

2-Micro or Trace minerals (body needs relatively less)

1. They are required in amounts less than 100 mg/day.

2. They include 10 elements: chromium, cobalt, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, zinc, silicon and nickel

Macrominerals

I. Calcium

Sources:

1. Milk and milk products (the richest sources).
2. Beans, leafy vegetables and egg yolk.

Absorption:

1. Site: upper small intestine.
2. Absorption of calcium is active process and requires calcium binding protein present in the intestinal mucosal cells.
3. Absorption is regulated by:

a) Vitamin D: [1,25 dihydroxy cholecalciferol]: through the formation of calcium binding protein (calbindin).

b) Parathyroid hormone: Increase calcium absorption through the conversion of vitamin D to 1,25 dihydroxy cholecalciferol in the kidney.

4. Factors affecting calcium absorption:

a) Factors promoting calcium absorption:

1) High protein diet: Amino acids form with calcium a soluble calcium salts which are easily absorbed.

2) pH: an acidic pH in the upper small intestine is essential for calcium absorption.

3) High dietary lactate or citrate: that form soluble salts with calcium.

***b)* Factors inhibiting calcium absorption:**

1) High dietary phosphate, oxalate and phytate: which form insoluble salts with calcium.

2) Alkalinity: excessive alkali intake (as during treatment of peptic ulcer) decreases calcium absorption.

3) Impaired fat absorption: fatty acids form insoluble calcium soaps with calcium.

Body calcium:

Calcium is the most abundant mineral in the body (about 1200 grams).

1) 99% present in bones and teeth: in the form of hydroxyapatite: 3 $Ca_3(PO_4)_2Ca(OH)_2$

a) Calcium salts in bones are not inert. They are in a constant state of turnover in skeleton being deposited in sites of bone formation and released at sites of bone resorption. In adult male about 700 mg calcium enter and leave bones each day.

b) Calcium in bones acts as a reservoir, which helps to stabilize calcium ions in plasma and extracellular fluid.

c) Parathyroid hormone and active vitamin D stimulate osteoblasts while estrogen hormone inhibits osteoclasts.

Thus after menopause= ↓estrogens -↓ bone mass (osteoporosis).

2) 1 %: of calcium is present in body fluids and other tissues.

Plasma calcium:

Level: 8.5 - 10.5 mg *I* dl.

1. Blood calcium lies entirely in the plasma (No calcium in RBCs).

2. **Forms**: Plasma. calcium is present in 2 forms; ionized and non ionized.

a) Ionized: (50 %)

- It is the active fraction. Its deficiency causes tetany.

b) Non ionized: (50 %)

• (Diffusible) Complexes with organic ions e.g. citrate: (5 %).

• (non diffusible) It is bound to protein mainly albumin (45%). Its deficiency occurs with conditions of hypoproteinemia and causes no tetany.

3**. Factors affecting plasma calcium (calcium homeostasis**):

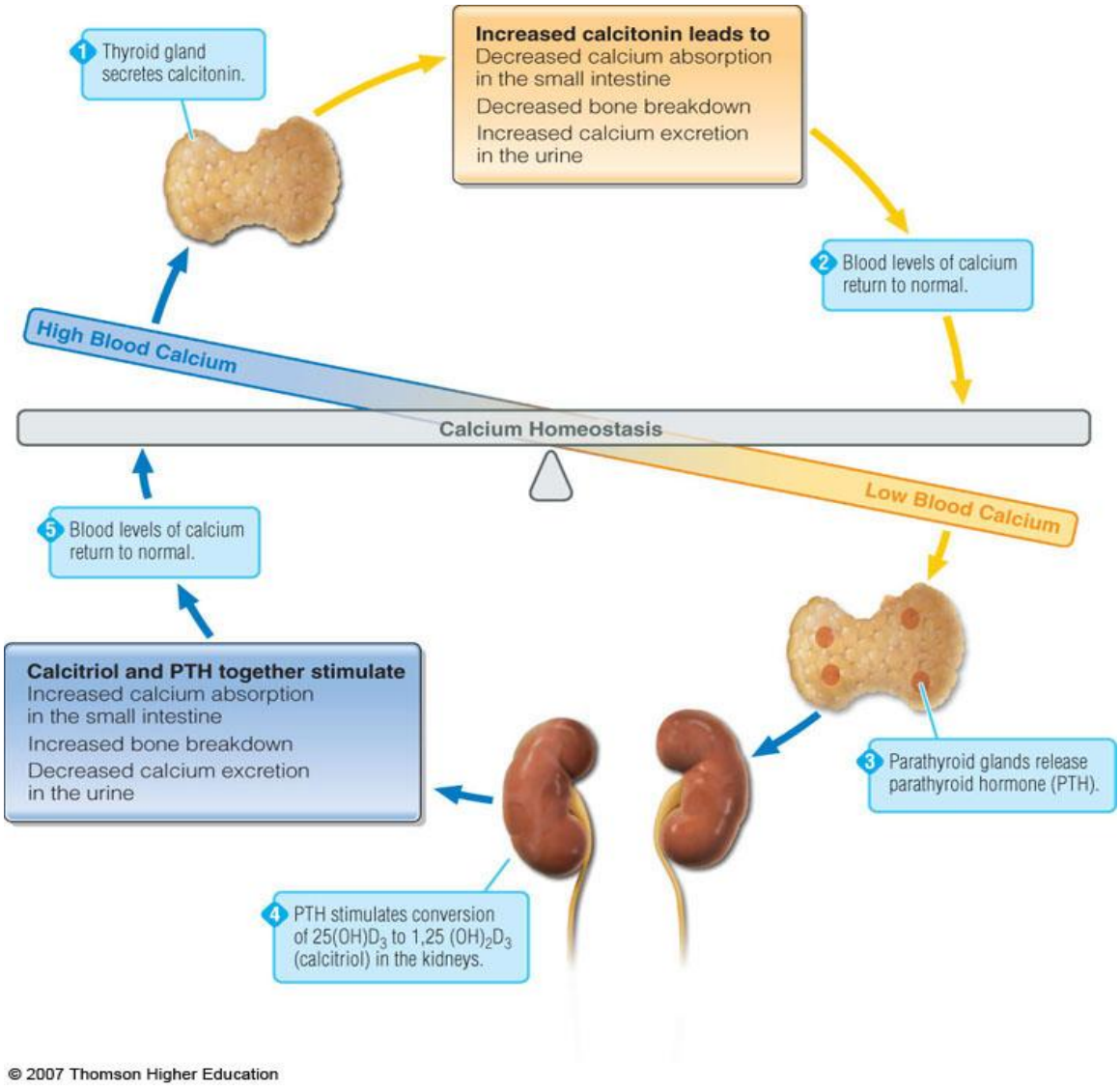
***a)* Hormonal regulation**: Three hormones are concerned with regulation of blood calcium. These are parathyroid hormone, active vitamin D (calcitriol) and calcitonin.

1) Parathyroid hormone (PTH): It increases plasma calcium level through:

i- Mobilization of calcium from bones (bone resorption).

ii- Absorption of calcium from intestine (through conversion of vitamin D into calcitriol "active vitamin D" in the kidney).

iii- Reabsorption of calcium by renal tubules i.e. ↓Ca2 • excretion in urine and ↑ phosphate excretion.



2) Calcitriol: 1,25 dihydroxycholecalciferol: It increases blood calcium level through:

i- Absorption of calcium from the intestine.

ii- Reabsorption of calcium by renal tubules.

iii- Mobilization of calcium from bones.

3) Calcitonin:

It is a hormone that is secreted by the parafollicular . or "C" cells of the thyroid gland.

It is released in response to hypercalcaemia and causes decrease of blood calcium level through inhibition of calcium mobilization from bones, or increasing calcium deposition in bones.

***b)* Other factors:**

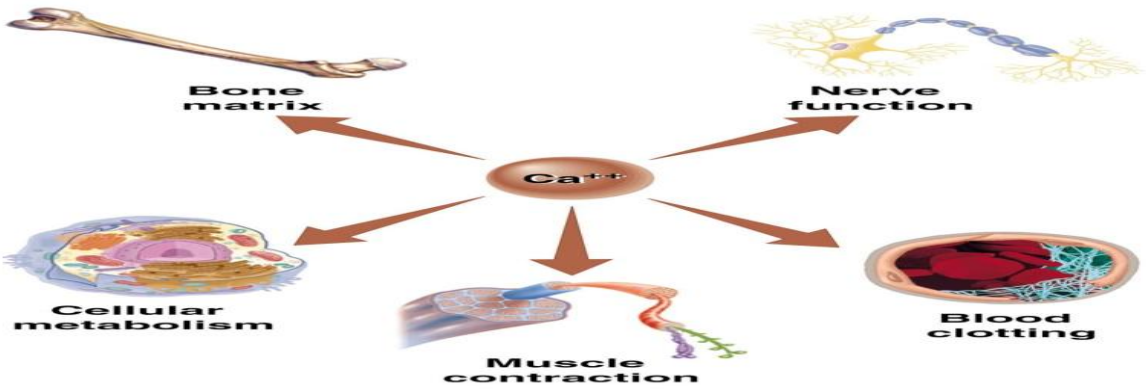
1) Solubility product: Normally Ca/P ratio must be constant. Ca x P in children is so and in adults are 40. If plasma phosphate increases {as in renal failure) the plasma, calcium decreases to keep the ratio constant.

2) Blood pH: Ionization of calcium occurs at normal blood pH, 7.4. The more the acidosis of the blood pH, the more formation of ionized calcium.

3) Plasma proteins: In cases of hypoproteinemia, the non-diffusible calcium decreases.

Functions of calcium:

1. Calcification of bones and teeth.
2. Regulation of transmission of nerve impulses.
3. Regulation of contraction of muscles.
4. Decrease of neuromuscular excitability. Deficiency of ionized calcium leads to tetany.
5. Cardiac conduction.
6. Calcium acts as a second messenger for hormonal action by acting together with calmodulin and cAMP.
7. Blood and milk clotting.
8. Maintenance of cell membrane permeability.
9. Activation of certain enzymes e.g. pyruvate kinase.



Excretion:

1. Most of calcium excretion is eliminated with feces.

2. Small amount of calcium is excreted in urine (about 200 mgfday).

Requirements:

- Adult man and women: 800 mg / day
- Pregnant, lactating and postmenopausal women: 1500 mg / day
- Infants (less than 1 year): 300 - 500 mg / day
- Children (1 – 18 years) : 800 - 1200 mg / day

Abnormal plasma calcium:

1. **Hypercalcemia:**

Causes:

1) Primary hyperparathyroidism: usually due to adenoma (benign tumor).

Serum calcium usually ranges 12-20 mg/dl.

2) Ectopic cells as in some malignancy -+ t PTH

3) Excess intake of vitamin D: or calcium or both. Usually it is due to over dosage or self-medication with vitamin D.

4) Milk-alkali syndrome: This is hypercalcemia present in patients who received, for long periods, excessive absorbable alkalies and milk (source of calcium), for the treatment of peptic ulcer:·

5) Bone diseases: (bone resorption) As in malignancy, leukemia, multiple myeloma.

6) Drugs: As thiazide diuretics.

7) Other causes: As thyrotoxicosis, Cushing's syndrome.

Effects:

- Stone formation: e.g renal stones.
- Calcification in different tissues.

Treatment

- diuretics,
- Corticosteroids.

2. Hypocalcemia:

Causes:

1) Hypoparathyroidism.

2) Alkalosis.

3) Kidney diseases where activation of vitamin D is inhibited.

Effects:

1) Acute deficiency: if ionized calcium is much decreased, tetany results.

2) Chronic deficiency: In children, Rickets and in adults, Osteomalcia(osteoporosis).

Rickets: Is a disorder of defective calcification of bone. This may be due to a *low levels of vitamin D* in the body or due to a dietary deficiency of Ca and P or both.

Osteoporosis: Is characterized by demineralization of bone resulting in the progressive loss of bone mass.

Occurrence:

The elderly people (over 60 yrs) of both sexes are at risk for osteoporosis. However, it more predominantly occurs in post-menopausal women which are the major cause of disability among the elderly.

Etiology: The ability to produce calcitriol from vitamin D is decreased with age, particularly in the postmenopausal women. Deficiency of sex hormones in women has been implicated in the development of osteoporosis.

Treatment: Estrogen administration along with Calcium supplementation in combination with vitamin D to postmenopausal women reduces the risk of fracture.

Milk fever (Parturient paresis):

- Milk fever, postparturient hypocalcemia, or parturient paresis is a disease, primarily in levels dairy cattle, beef cattle, ewes
- Characterized by reduced blood calcium Occurs within 72 hours following parturition during late stage of gestation.

Symptoms: loss of appetite, nervousness, turning head back to flanks

Grass tetany:

- A metabolic disease due to low Mg level in blood and in many cases low blood calcium.

- In ruminant (beef cattle, dairy cattle and sheep)
- Usually after grazing on pastures of rapidly growing grass, especially in early spring.

Symptoms: nervousness, staggering, convulsion, coma and death.

II. Phosphorus

Sources:

Milk and milk products, chicken, beans, salmon, fish, bread, egg yolk, whole wheat

Absorption:

1. Phosphorus (in the form of phosphate) is absorbed by an active transport mechanism in the mid jejunum and enters blood stream via portal circulation.
2. Absorption is regulated by active vitamin D (calcitriol).
3. Factors affecting absorption of calcium will affect-in the same manner-the absorption of phosphorus.

Body phosphorus: Metabolism of phosphorus follows calcium inversely.

1. Total body phosphorus is about 800 g.
2. Most of phosphorus (80%) is present in the skeleton (bones and teeth) in the form of hydroxyapatite: $3\ Ca_3\ (P0_4)2\ Ca(OH)_2$
3. The other 20 % is present in other tissues (mostly intracellular) and body fluids.

Blood phosphorus:

1. Normal plasma inorganic phosphorus: 3-5 mg/dl.
2. Other forms are present:
   - In plasma: phospholipids.
   - In RBCs: organic phosphate e.g. ATP, glucose-6-phosphate.

3. Factors affecting blood phosphorus:

***a)* Parathyroid hormone (PHT):**

It inhibits renal tubular reabsorption of phosphate → ↑ phosphate excretion in urine → ↓ plasma phosphate.

***b)* Active vitamin D "Calcitriol":**

Calcitriol increases blood phosphorus through stimulation of:

*Absorption of phosphorus from the intestine.

* Bone resorption i.e. mobilization of phosphorus from bones.

* Renal reabsorption by renal tubules.

c)Renal function: Renal failure → failure of excretion in urine → ↑ plasma inorganic phosphate.

Functions of phosphorus: (is the main intracellular anion). It enters in the structure of the following compounds:

1. Bones and teeth (in the form of hydroxyapatite).
2. Plasma buffers (phosphate buffers).
3. Cellular components:
    - Nucleic acids: DNA, RNAs.
    - Phospholipids: e.g. lecithin, cephalin.
    - Phosphoproteins.
    - Coenzymes: e.g. NAD, NADP
    - High energy phosphate compounds e.g. ATP, GTP, creatine and phosphate.
    - Cyclic AMP and cyclic GMP.
    - Carbohydrate intermediates e.g. glucose-6-phosphate, fructose-1-phosphate.

Excretion: Mostly (90%) is excreted in urine.

Alterations of serum phosphate:

1. Causes of Hyperphosphatemia

- Hypoparathyroidism.
- Acidosis.
- Hypervitaminosis D.
- RBCs Hemolysis.

Toxicity Signs:

- Diarrhea, nausea, and vomiting.

-Mineralization of soft tissues.

Treatment:

- Low-phosphorus diet
- IV saline.

2. Causes of Hypophosphatemia:

- Hyperparathyroidism.
- Vitamin D deficiency.
- Renal tubular disease.
- Chronic alcoholism.
- Excessive use of antacids.
- Malabsorption.

Treatment:

- Mild/ moderate: Dietary interventions, Oral supplements.
- Severe: IV replacement using potassium phosphate or sodium phosphate.

Requirements: Recommended Daily Allowance (RDA): 1000 mg / day

III. Magnesium

Sources: Leafy green vegetables (containing chlorophyll).

Absorption: Occurs in the upper small intestine.

Body magnesium:

1. Mostly (70%) in the skeleton (bones and teeth).

2. The remaining 30% is present in the other tissues and body fluids mostly intracellular.

Blood magnesium :

1. Plasma magnesium: 2-3 mg/dl.

2. RBCs content of magnesium is 3 times greater than plasma content.

Functions :

1. It enters in the structure of skeleton (bones and teeth).

2. It activates many enzymes e.g. kinase enzymes.

3. It is required for the active transport of other cations (Ca••, Na•, K•) across the cell membrane.

4. It is important for muscle contraction, nerve impulse transmission and it decreases neuromuscular excitability.

Hypomagnesemia:

- Mg deficiency causes neuromuscular irritation, weakness and convulsions.
- These symptoms are similar that observed in Ca deficiency, which are relieved only by Mg.
- Malnutrition, alcoholism and cirrhosis of liver may lead to Mg deficiency.
- Low level of Mg may be observed in uremia, rickets and abnormal pregnancy.

Hypermagnesemia is usual due to renal insufficiency. It causes muscle weakness, hypotension, sedation and confusion.

Excretion: Mostly (75 %} in feces.

Reguirements::For adults: 400 mg/day.

IV. Sodium

Sources: The main source is table salt.

Absorption: It occurs in small intestine (ileum}. It is nearly completely absorbed.

Body sodium: It is regulated by aldosterone.

1. 2/3 of sodium is present in tissues and body fluids (sodium is the main extracellular cation).

2. About 1/3 of sodium is present in skeleton.

Plasma sodium: 137-143 mmol/L.

Factors affecting plasma sodium:

1. Aldosterone and the rennin angiotensin system (t Plasm sodium).

2. Changes in glomerular filtrate and renal blood flow.

3. Atrial natriuretic peptide.

Functions:

1. Maintenance of osmotic pressure and volume of plasma and extracellular fluid.

2. Transmission of nerve impulses.

3. Contraction of muscles.

4. Regulation of acid base balance.

5. Sodium acts as substrate for Na+fK+ ATPase enzyme (sodium potassium pump).

Excretion: Mainly (95%) in urine and sweat.

Reguirements: For adults: 5 g/day.

Abnormal plasma sodium:

1. Hypernatremia (excess plasma sodium): It is caused by:

- Cushing syndrome: due to excessive glucocorticoids.
- Conn's disease: due to excessive aldosterone secretion.
- Diabetes insipidus (.I.ADH): due to rapid loss of water.
- Drugs: as ACTH or cortisone.

2. Hyponatremia (decrease plasma sodium) : It is caused by:

- Addison's disease: due to deficiency of aldosterone.
- Renal failure: where renal reabsorption of sodium is inhibited.
- Hypotonic dehydration: where loss of water and sodium (electrolytes) is treated by administration of water only.
- Edema which occurs in cirrhosis or congestive heart failure
- Diuretics: e.g. thiazides, which block tubular reabsorption of sodium.

Toxicity: Hypertension in susceptible individuals.

V. Potassium

Sources: Vegetables, fruits and nuts.

Absorption: Rapidly occurs in the small intestine.

Body potassium:

It is regulated by aldosterone.

1. 2/3 of potassium is present in tissues and body fluids (potassium is the main intracellular cation).

2. About t/3 is present in skeleton.

Plasma potassium: 3.5-5 mmol/L.

Functions:

1. Maintenance of osmotic pressure and volume of intracellular fluid.
2. Transmission of nerve impulses.
3. Contraction of muscles.
4. Regulation of acid base balance.
5. Substrate for Na+ /K+ ATPase.

Excretion: Mainly in urine.

Requirements: 4 g/day.

Alterations of plasma potassium:

1. Hyperkalemia (excess plasma potassium): it is caused by:

- Addison's disease: due to deficiency of aldosterone.
- Acidosis: (respiratory or metabolic): due to shift of K• from intra to extracellular in exchange with H +.
- Tissue necrosis: e.g. major trauma and burns due to leakage of tissue contents of potassium.
- Acute renal failure and advanced chronic renal failure, associated with oliguria.
- Uncontrolled diabetes mellitus: the lack of insulin and associated acidosis prevents K• from entering cells.

• Acute hyperkalemia: if plasma K• gets more than 6.5 mmol/L, cardiac arrhythmias and even cardiac arrest may result.

2. Hypokalemia: (decreased plasma potassium): it is caused by:

- Alkalosis: (respiratory or metabolic).
- Treatment of hyperglycemia: by insulin without giving potassium because insulin helps K+ to enter cells.
- Excessive vomiting and diarrhea.
- Cushing syndrome: due to excessive glucocorticoids.
- Primary and secondary hyper-aldosteronism.
- Diuretic therapy.

VI. Chloride

Sources: Table salt.

Absorption: Occurs in small intestine.

Plasma chloride: 96-106 mmol/L.

Functions:

1. Chloride is the main extracellular anion. Together with sodium, it maintains the osmotic pressure and volume of plasma and extracellular fluid.

2. Chloride ions are essential for formation of HCl in the stomach.

3. Activation of enzymes: Cl· activates salivary and pancreatic amylase enzymes.

Excretion: Mainly in urine.

Requirements: For adults: 5 g/day.

Alterations of plasma chloride:

1. Hyperchloremia:

- Hyperchloremic acidosis:
- Occurs when HC03• is lost in exchange with chloride as in renal tubular acidosis and hyperventilation.
- Glomerulonephritis.
- Eclampsia (toxicity of pregnancy).

2. Hypochloremia:

a) Hypochloremic alkalosis: decreased plasma chloride due to:

1) Intestinal obstruction ~ excessive vomiting.

2) This leads to decrease plasma chloride and increase plasma bicarbonate as compensatory mechanism, causing alkalosis.

b) Addison's disease.

c) Diabetes insipidus.

Microminerals (Trace elements)

I. Iron

Sources:

1. Liver, heart, kidney, spleen and fish.

2. Sugar cane syrup (molasses).

3. Dates and egg yolk.

4. Contrary to popular belief, spinach is a poor source of iron because it is bound to phytate, which is difficult to absorb.

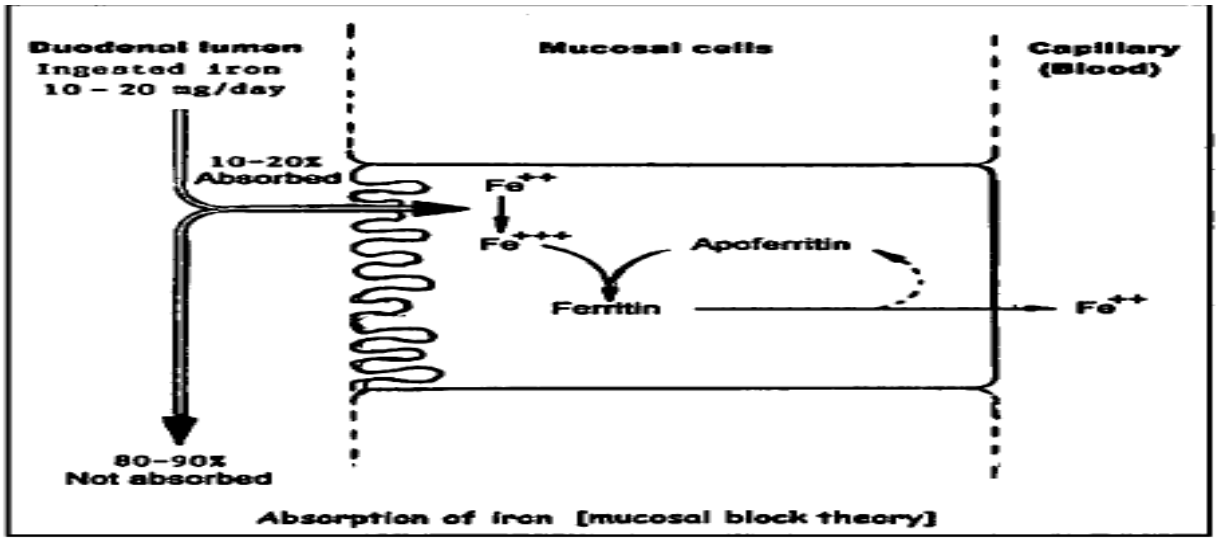
Absorption:

Absorption of iron occurs in the duodenum and the proximal part of the jejunum.

1. Diet contains about 10-20 mg iron/day. Usually only 10-20% of this amount is absorbed.

2. **Mechanism**: **Mucosal block theory:**

- According to this theory, iron is absorbed in the ferrous state (Fe++). Inside mucosal cells, it is oxidized to ferric state (Fe+++) and combines with apoferritin to form ferritin.
- Ferritin liberates ferrous ions into the capillaries (plasma) and apoferritin is regenerated again. The rate of this liberation depends on body needs.
- The intestinal content of apoferritin is limited and when all apoferritin molecules become saturated with iron, absorption is blocked.



Absorption of iron [mucosal block theory]

3. Factors affecting iron absorption: most of dietary iron is present in the ferric state (Fe+++) as ferric organic compounds.

a) **Factors increasing iron absorption:**

1) Cooking of food and gastric HCI facilitates the liberation of ferric ions (Fe+++) from organic compounds.

2) Reducing substances: vitamin C and cysteine (-SH) of dietary protein help the reduction of ferric ions (Fe+++) into the absorbable ferrous (Fe++) state.

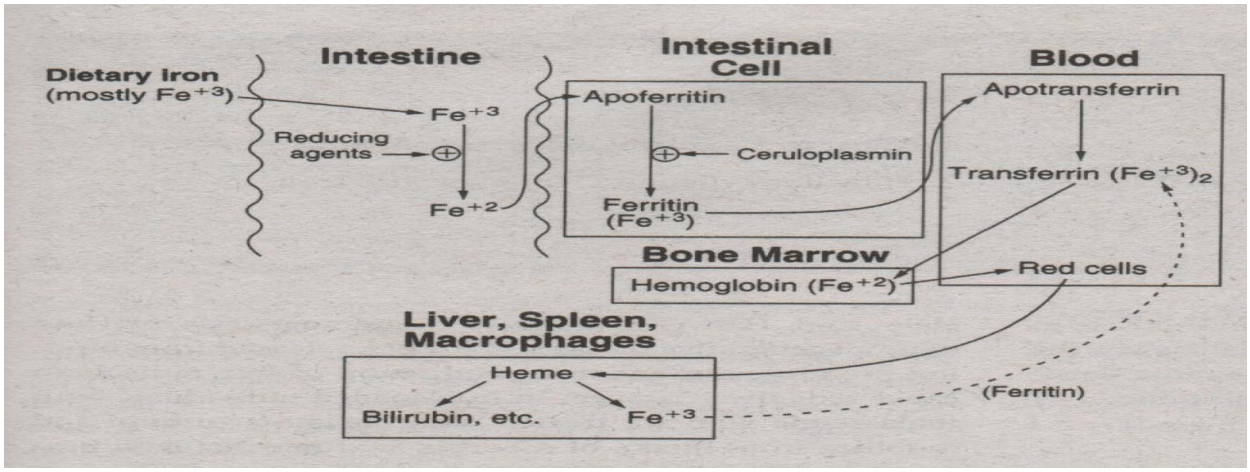
3) Body needs: absorption occurs only if the body is in need to iron. More iron is absorbed when there is iron deficiency or when erythropoiesis is increased.

***b)* Factors decreasing iron absorption:**

1) High dietary phosphate and phytate: They form insoluble, non-absorbable organic iron complexes.

2) Steatorrhea: Where fatty acids form non-absorbable iron soaps.

3) Alkalies and tea.



Body iron:

1. The total body iron of an adult male is 3-5 grams.

2. It is distributed as follows: RBCs iron (Hemoglobin): 6696, tissue iron (3396) and plasma iron 1%.

3. RBCs iron: (hemoglobin): see hemoglobin metabolism.

4. **Tissue iron**: it includes:

a) **Available iron forms (29 %):** can be used by tissues when there is body need.

1) Ferritin:

- It is the main storage form of iron.
- It is present in iron stores: liver, spleen, bone marrow and intestine.

2) Hemosedrin:

- When body contains very high content of iron more than the capacity of apoferritin, some of iron is found in granules called hemosedrin that deposited in tissues.
- These granules are composed of iron, protein, and polysaccharides.
- Hemosedrin may be a degraded ferritin.

***b)* Non-available iron forms (4%):** cannot be used even if there is body needs. All these forms arc hemoproteins i.e. contain heme ring.

1) Myoglobin:

- It is hemoprotein formed of a single heme ring attac!ted to one long polypeptide chain.
- It is present in muscles and heart.
- It acts as oxygen reservoir for quick utilization by contracting muscles.

2) Respiratory cytochromes (b, c., c, a, a 3):

- These are components of respiratory chain in mitochondria.
- They act as electron carriers.

3) Catalase and peroxidase:

-These are two enzymes that act on the toxic hydrogen peroxide (H_2O_2 converting it into H_2O.

4) Tryptophan oxygenase (pyrrolase):

-This enzyme is important for tryptophan metabolism.

5) Cytochrome P 45o:

i- These are a specific group of enzymes that present in liver, lung, kidney, gut, adrenal cortex, heart, and brain. They are used in xenohiotics metabolism.

Plasma iron:

a) **Plasma iron**: Ranges from 6o- 160 ug/dl.

b) **Plasma transferrin**:

1) This is a plasma glycoprotein that acts as carrier for iron. It is synthesized in the liver.

2) Each molecule can carry 2 atoms of iron in ferric state (Fe+++).

3) Transferrin may carry up to 180-450 ug iron/dl. This is known as total iron binding capacity of transferrin (TIBC). As the plasma iron is 6o-t6o ug/dl, thus only 30% of the TIBC of transferrin is saturated.

4) TIBC is therefore defined as maximum *amount of iron that* can be carried *by* transferrin per deciliter.

5) Abnormalities of plasma TIBC concentration:

- In iron deficiency anemia: Plasma iron is decreased. Liver synthesizes more transferrin with subsequent increase of TIBC.
- In liver diseases: Both plasma iron and transferrin synthesis tend to decrease (↓plasma iron and ↓TIBC).
- In iron overload: transferrin synthesis is inhibited. This leads to increased plasma iron and decreased Total iron binding capacity.

c) **Plasma ferritin:**

1) Ferritin is present mainly in iron stores: liver, spleen, bone marrow and intestine.
2) Very low concentration of ferritin is present in plasma.
3) Measurement of plasma ferritin gives a good idea about body iron stores.
    - A low plasma ferritin indicates the presence of depleted iron stores e.g. in iron deficiency anemia.
    - A raised plasma ferritin is found in iron overload and also in many patients with liver disease and cancer.

Functions of iron: Iron enters in the structure of the following compounds:

- Hemoglobin: which carries oxygen.
- Myoglobin: which stores oxygen.
- Respiratory enzymes: which use oxygen.
- Cytochrome P 45o: which detoxicates drugs and oxygen.
- Other enzymes: catalase, peroxidase and tryptophan oxygenase.

Transport and storage of plasma iron :

1. Absorbed iron enters in the portal blood in ferrous state (Fe++).
2. In the plasma, it is rapidly oxidized to ferric state (Fe+++). A protein containing copper called ceruloplasmin catalyzes this oxidation.
3. Then ferric ions are carried by a transferrin, which is taken mostly by bone marrow to synthesize hemoglobin.
4. Iron, from iron stores (ferritin) can be released into plasma and carried by transferrin to be utilized by bone marrow and other tissues.

Excretion:

1. Iron excreted in the feces is mainly exogenous i.e. dietary iron that has not been absorbed.

2. In males there is an average loss of endogenous iron of about 1 mg/day. It is derived from desquamated cells from skin and the intestinal mucosa.

3. In females, there are additional sources of loss, due to menstruation and pregnancy.

4. Urine contains negligible amount of iron.

Reguirements:

1. Adults: 10 mg/day.

2. Pregnant and lactating women: 30 mg/day.

Alterations of plasma iron:

1. Iron deficiency anemia:

Causes:

1) Deficient intake.

2) Impaired absorption: e.g. steatorrhea, abdominal surgery.

3) Excessive loss e.g. menstrual loss, gastrointestinal bleeding due to some parasites (anchylostoma).

Biochemical changes:

1) Plasma iron is decreased.

2) Plasma TIBC is increased.

2. Iron overload:

Causes:

1) Repeated blood transfusion.

2) Intravenous administration of iron.

3) Hemochromatosis (hemosiderosis, bronze diabetes):

a- This is a rare hereditary disease characterized by abnormal increase of iron absorption.

b- Iron is deposited in the form of hemosedrin in:

• Liver: causing liver cirrhosis.

• Pancreas: causing fibrosis and diabetes mellitus.

• Skin: causing bronze discoloration of skin.

Biochemical changes:

1) Plasma iron is increased.

2) Plasma TIBC is decreased.

3) Plasma ferritin is increased.

II. Copper

Sources: The richest sources are: liver, kidney, dried legumes and nuts.

Absorption: Mainly occurs in the upper small intestine.

Body copper:

1. The adult human body contains about 100-150 mg of copper.

2. 64 mg (50%) are found in muscles and the remaining present in other tissues including liver and bones.

Blood copper:

1. In the plasma: 90 ug/dl. It is present in association with 2 proteins:

***a)* Ceruloplasmin**: (90%) A copper binding protein. Each molecule can bind 6 atoms of copper. It acts as ferroxidase enzyme during iron metabolism ($Fe^{++} \rightarrow Fe^{+++}$),

***b)* Albumin**: (10%) It is loosely bound form of copper. It acts as a carrier for transport of copper in plasma.

2. In RBCs: 100 ug/dl. It is present in association with the enzyme superoxide dismutase (erythrocuprein}, which deals with the toxic free radical superoxide ion (O^{-}_{2}) generated during aerobic metabolism.

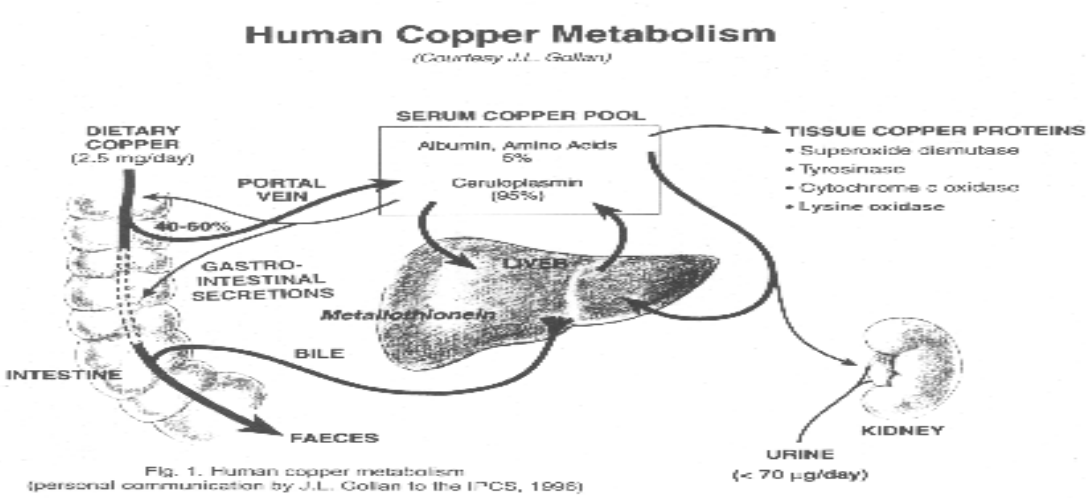


Fig. 1. Human copper metabolism (personal communication by J.L. Gollan to the IPCS, 1998)

Functions:

1. Copper is essential for:

- Hemoglobin synthesis.
- Bone formation.
- Maintenance of myelin of the nerves.

2. Copper is essential constituent of several metaloenzymes:

- Ceruloplasmin: which oxidizes Fe++into Fe+++ in the plasma.
- Superoxide dismutase: which eliminates the toxic effect of superoxide ions (O^-_2).

* Superoxide dismutase is present in RBCs (erythrocuprein), liver (hepatocuprein) and brain (cerebrocuprein).

- *c)* Cytochrome oxidase.

3. Copper activates many enzymes: e.g. tyrosinase, uricase and dopamine hydroxylase.

Excretion:

1. Mainly with bile.

2. Urinary excretion is minimal due to large molecular weight of ceruloplasmin.

Requirements: Adults: 2- 3 mg/day.

Alterations of plasma copper:

1. Hypercupermia: (excess plasma copper and ceruloplasmin):

Ceruloplasmin is considered as acute phase protein i.e. its plasma level is increased in infections and malignancy.

2. Hypocupermia: (decreased plasma copper and ceruloplasmin):

a) Anemia: Hypochromic and microcytic anemia.

b) Impaired bone mineralization.

c) **Wilson's disease** (hepatolenticular degeneration): characterized by accumulation of large amounts of copper in:

- Liver causing hepatic cirrhosis.
- Lenticular nucleus of the brain causing lenticular degeneration with abnormal movement.
- Cornea: Causing greenish-brown discoloration of the corneal margin, which is called: Kayser - Fleisher rings.

- Kidney causing renal tubular damage which leads to:
  - Increased excretion of copper and ceruloplasmin.
  - This results in low serum copper (hypocupremia) and ceruloplasmin.
  - Increased excretion of amino acids. This results in aminoaciduria.

The cause of Wilson's disease is most probably due to either:

- Excessive copper absorption from intestine.
- Inadequate excretion of copper in bile.

III. Zinc

Sources: Meat, liver, eggs, seafood, milk, and whole grain cereals.

Absorption: Zinc absorption occurs mainly in small intestine, especially from the duodenum.

Body zine:

1. The adult male body contain about 2 g of zinc.
2. About 20 % of total body zinc is present in the skin.
3. The remaining is present in skeleton (bones and teeth), spermatozoa, prostate, epididymis and pancreas.

Plasma zine: Adults: 70-150 ug/dl.

Functions of zinc:

1. Zinc is essential for growth and reproduction.
2. It plays a role in tissue repair and wound healing.
3. Zinc forms a complex with insulin in p islet cells of the pancreas. This helps crystallization, storage and release of insulin.
4. Zinc is required for mobilization of vitamin A from the liver and subsequently maintains the normal concentration of vitamin A in plasma.
5. Zinc is essential component of a number of enzymes e.g.:
   - Alkaline phosphatase.
   - Carbonic anhydrase.
   - Superoxide dismutase
   - Carboxyp-eptidase.
   - RNA polymerase.

Reguirements: An adult male: 10-20 mg/day.

Excretion: Mainly in feces (mostly unabsorbed dietary zinc).

Zinc deficiency: It causes:

1. Hypogonadism.
2. Poor healing of wounds.
3. Poor appetite and retard growth in children.
4. Liver cirrhosis.
5. Diarrhea and dermatitis.
6. Confusion, apathy and depression.

IV.Iodine

Sources: 1. Iodinized table salt will provide daily body needs.

2. Fish, seafoods, weeds, and vegetables grown near seaboard.

Absorption: Occurs mainly from small intestine.

Body iodine:

1. The adult male body contains about 2s-so mg iodine.
2. It is present in:
    - Thyroid gland: (50%): as thyroglobulin.
    - Other tissues and body fluids (50%): as T3 and T4

Plasma iodine:

1. Organic iodine: 4-8 ug/dl.
2. Inorganic iodine: 1-2 ug/dl.

Functions:

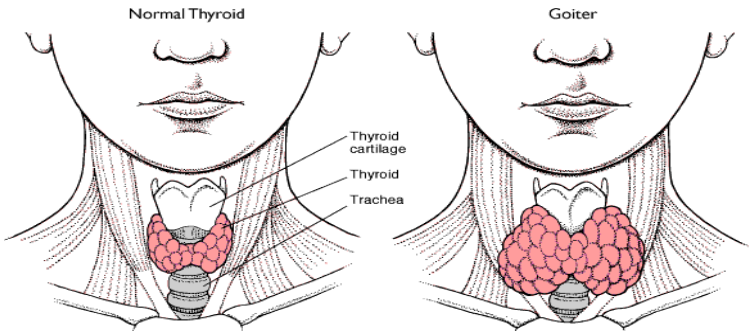
The only known function of iodine is the formation of thyroid hormones (T3 - T4).

Excretion: Mainly (70%) in urine.

Reguirements: For adult: 100- 1'so ug/day.

Deficiency: Hypothyroidism (myxodema in adults and cretinism in children).

*__Goitrogens__: occurring naturally in foods can cause goiter by blocking absorption or utilization of iodine (cabbage, turnips, peanuts, soybeans)



V. Selenium

- Selenium is an antioxidant. It is an essential component of the enzyme glutathione peroxidase (GSH-Px) which. Catalyzes the reaction:

$$2\ GSH + H2O2 \xrightarrow{GSH\text{-}Px} GSSG + 2\ H2O$$

- This reaction acts as protective mechanism against the oxidative damage of hydrogen peroxide (H202) and fatty acid hydroperoxide by destroying them:

1. In RBCs, it protects hemoglobin and red cell membranes.
2. In liver, it is important for detoxifying lipid hydroperoxides.
3. In lens of the eye, it prevents its oxidative damage.

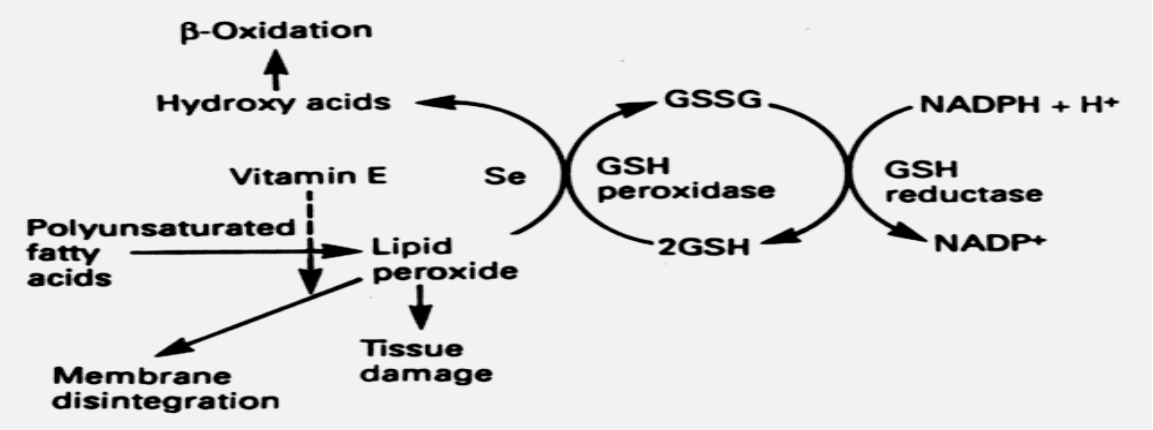
Deficiency of selenium (GSH-Px): It causes:

1. Hemolytic anemia.
2. Liver cirrhosis.
3. Cataract.

Metabolism

- Selenium is stored in the body as selenocysteine in selenoproteins
- Excreted in urine and in breath as dimethyl selenide with a garlic-like odor

Relationship of glutathione peroxidase, selenium, and vitamin E.



VI. Manganese

- Manganese is essential for:

1. Normal bone structure.

2. Reproduction (spermatogenesis and ovulation).

3. Normal function of the central nervous system.

- Manganese is a component of:

1. Pyruvate carboxylase enzyme.

2. Superoxide dismutase enzyme.

-Manganese activates: the arginase enzyme.

VII. Cobalt

Functions:

1. Cobalt is a component of vitamin Ba:a, which is necessary for normal blood cell formation. Cobalt gives vitamin Bu its red color.

2. Enzymes requiring vitamin Ba:a for their activities are:

- Methylmalonyl CoA mutase.
- Methyltetrahydrofolate oxidoreductase.
- Homocysteine methyltransferase.
- Ribonucleotide reductase.

Deficiency of cobalt ↓vitamin B12 causes pernicious anemia.

VIII. Chromium

• It acts only together with insulin to promote glucose utilization.

• Its deficiency leads to impairment of glucose utilization by tissues.

IX. Molybedenum

• It is a component of oxidase enzymes e.g. xanthine oxidase.

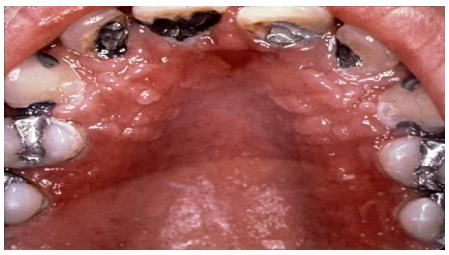
X. Flouride

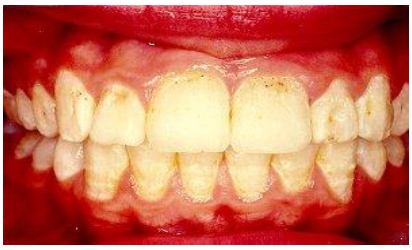
• It increases the hardness of bones and teeth.

• Its deficiency causes dental carries and osteoporosis.

• It is supplied in drinking water to support bones and teeth.

• Excess flouride leads to flourosis: mottling and discoloration of the enamel of teeth and changes in bones.





Nutrient and Adult RDA	**Sources**	**Functions**	**Signs of Deficiency**
Calcium (Ca) 1000- 1200mg/day	1.Milk and milk products (the richest sources). 2. Beans, leafy vegetables and egg yolk	-bone and teeth formation, -blood clotting, - nerve transmission, - muscle contraction -heart action	-Tetany, - Rickets, -Osteoporosis
Phosphorus (p) 700 mg/day Tetany, - Rickets, -Osteoporosis	High- protein foods Milk ,milk products, grains	-bone and tooth formation, -acid-base balance, - energy metabolism	- bone loss - poor growth
Magnesium (Mg) 310-420 mg /day	Grains, nuts, beans, Green vegetables,	-aids thyroid hormone secretion -activator and coenzyme in protein and carbohydrate metabolism	-tremor, spasm, - muscle weakness and cramp, -blood vessel construction in the heart and brain -hypertension -Muscle cramps -Cold skin
Sodium (Na)	table salt	- water balance	-Weakness of

(500 mg/day)	natural food (milk, meat , egg, celery) processed foods	- acid-base balance -muscle action - glucose absorption	respiratory muscle with difficult breathing -poor intestinal muscle - cardiac arrest
Potassium (K) (2000-3500 mg /day)	Whole grains, meat legumes, fruits, vegetables,	-water balance - metabolic reaction -muscle action -Insulin release	Hypochloremia:
Chlorine (Cl) 750 mg/day	Table salt	-Component of HCl in stomach, fluid balance and acid-base balance.	General protein , malnutrition
Sulfur(S) Diet adequate in protein contains adequate sulfur	Meat , egg, cheese Milk, nuts, legumes	-essential constituent of cell protein - hair, skin, nails - vitamin structure - collagen structure -high- energy sulfur bonds in energy metabolism	

Nutrient and Adult RDA	Sources	Functions	Signs of Deficiency
Iron (Fe) (10-15 mg/day)	Liver, lean meats, enriched and whole grain breads and cereals	Oxygen transport by way of hemoglobin, constituent of enzyme systems	Microcytic anemia, pallor, decreased work capacity, fatigue, weakness
Iodine (I) (150 µg/day)	Iodized salt, seafood, food additives	Component of thyroid hormones	Goiter
Copper (Cu) (1.5-3 mg/day)	Liver, seafood,, grains, nuts	-Aids in iron metabolism and activity of some enzymes -hemoglobin synthesis -absorption and transport of iron	-Anemia - Altered bone formation,

liver and heme metabolism

Biochemical function of Liver:

•Metabolism of Proteins, Carbohydrates and Lipids.

•Detoxification of endogenous and exogenous compounds (as drugs and toxins).

•Formation of Bile.

•Storage of Glycoge, vitamins A, D, and B12 , iron..

•Formation of Urea and Ketone Bodies.

•Conjugation of steroid hormones.

•Biosynthesis of Plasma Proteins and clotting factors.

•Inactivation of Polypeptide hormones.

Liver Function Tests (LFT)

LFT provide useful information about the presence and severity of hepatobiliary injury or impairment of liver function. Some of these tests based on substances that are produced or synthesized by Liver (as albumin and coagulation factors). Others based on substances released by damaged hepatocytes (as ALT and AST) and some test based on substances cleared from plasma by liver (as bilirubin, bile acids, ammonia)

Biochemical parameters in LFT are:

•Alanine Aminotransferase (ALT)

•Aspartate Aminotransferase (AST)

•Alkaline Phosphatase (ALP)

•Gamma Glutamyl Transpeptidase (GGTP or **GT**)

•Serum or Plasma Albumin

•Total Protein

•Prothrombin Time

•Bilirubin (Conjugated and Unconjugated),

AST (Aspartate Aminotransferase)

Old name is: Serum Glutamate Oxaloacetate Transaminase (SGOT)

AST is high in Heart muscle, Liver and Skeletal muscle.

Damage tissues releases AST in blood and its level in blood are directly related to extent of cellular damage or injury.

AST level in plasma is elevated 8 hrs. after cellular injury, peak at 24 to 36 hrs., and return to normal in 3 to 7 days

•**AST** level in blood is always high in case of chronic Hepatocellular disease and Acute Extra-hepatic Obstruction (e.g. Gallstone).

ALT (Alanine Aminotransferase)

Old name Serum Glutamate Pyruvate Transaminase (SGPT)

ALT found mainly in Liver, lesser quantities are in Kidneys, Heart and Skeletal muscle.

Liver injury causes elevation of ALT level in blood. **ALT** is sensitive and specific indicator of liver disease,

ALT level in plasma is elevated 8 hrs. after cellular injury, peak at 24 to 36 hrs., and return to normal in 3 to 7 days.

ALT/AST ratio is usually less than 1in most Hepatocellular disease other than Viral Hepatitis. In viral hepatitis the ratio is usually greater than 1.

ALP (Alkaline Phosphatase)

•ALP is highest in Liver, Biliary Tract Epithelium, Bone, Placenta

•Plasma ALP level is use to detect disorders in Liver and Bone.

Liver: ALP level in plasma is greatly increased in both Extra-hepatic and Intra-hepatic Obstructive Biliary Disease and Cirrhosis.

Bone: New bone growth causes elevated blood levels of ALP

•Healing fractures.

•Rheumatoid Arthritis.

•Hyperparathyroidism.

Placenta: Placental ALP appears in maternal blood usually in the third trimester of pregnancy.

GGTP (Gamma Glutamyl Transpeptidase):

Catalyzes transfer of Amino Acids and Peptides across membrane and involve in Glutathione metabolism.

•GGTP level is very high in Liver and Biliary Tract, but low in Kidney, Spleen, Heart, Intestine, Brain and Prostate gland,

Test for GGTP is used to detect Liver cell dysfunction as GGTP test is highly accurate in indication of Cholestasis.

•Elevation of GGTP parallels that of ALP in Liver disease.

•GGTP is not elevated in Bone disease.

GGTP is useful in screening and evaluation of alcoholics as GGTP is elevated in about 75% of patients who chronically drink alcohol.

Total Protein (Albumin and Globulins):

Albumin and Globulins constitute most of the proteins in blood and are measured as Total Protein.

•Albumin is synthesize in the Liver and transports important blood constituents, such as drugs and hormones.

•Globulins: key components of Antibodies, ?

•Some Globulins are synthesize in Liver, but most are made in Reticuloendothelial System,

•Albumin and Globulins can be measured separately,
Albumin is the major protein synthesized in liver, thus can be used to assess hepatic function.

•Half-life of albumin is 12 to 18 days, thus, severe impairment of hepatic albumin synthesis may not be recognized for several weeks or even months,
•Hypo-albuminaemia is a feature of malnutrition, advanced chronic liver disease and severe acute liver damage,
In some Chronic liver diseases, Albumin level is low, but Globulin level is high given normal Total Protein level. The reason might be that liver cannot produce Albumin (low albumin level) but Globulins are mostly made in Reticuloendothelial system, thus their levels may increase during infection.
•These changes can be detected by measuring the Albumin/Globulin (A/G) ratio or performs Protein Electrophoresis.
Normal A/G ratio: 1.2/1 – 1.5/1

Prothrombin Time

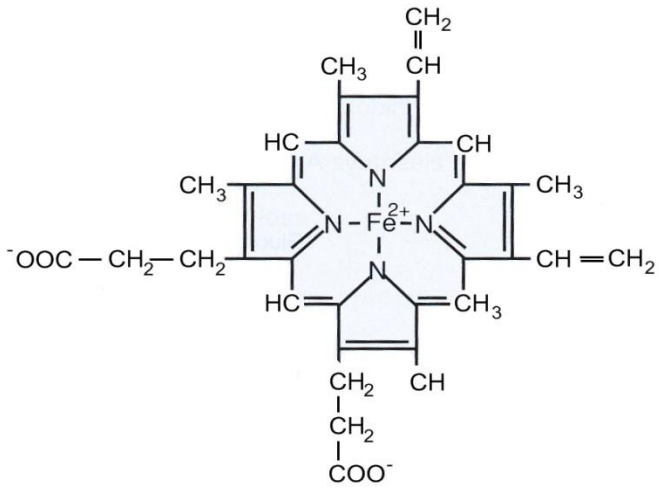
It is a measure of the activities of certain Coagulation Factors made by the Liver

•It is used as indicator of Hepatic Synthetic Function

•Prothrombin has a very short half-life (6hrs.), and an increased Prothrombin time may be the earliest indicator of hepatocellular damage. Vitamin K deficiency also causes prolonged PT.

Bilirubin

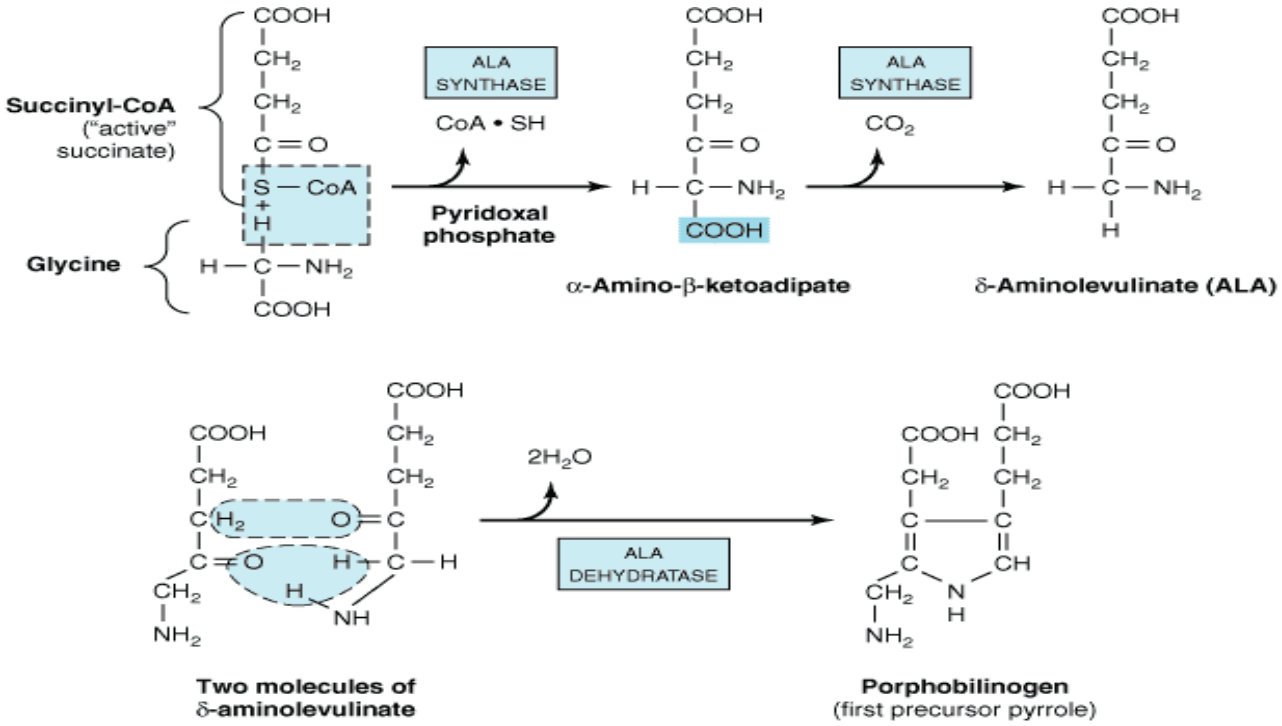
Heme is a derivative of the porphyrin. Porphyrins are cyclic compounds formed by fusion of 4 pyrrole rings linked by methenyl bridges. The side chains of porphyrin are methyl, vinyl and proponyl. The porphyrin ring coordinated with an atom of iron.

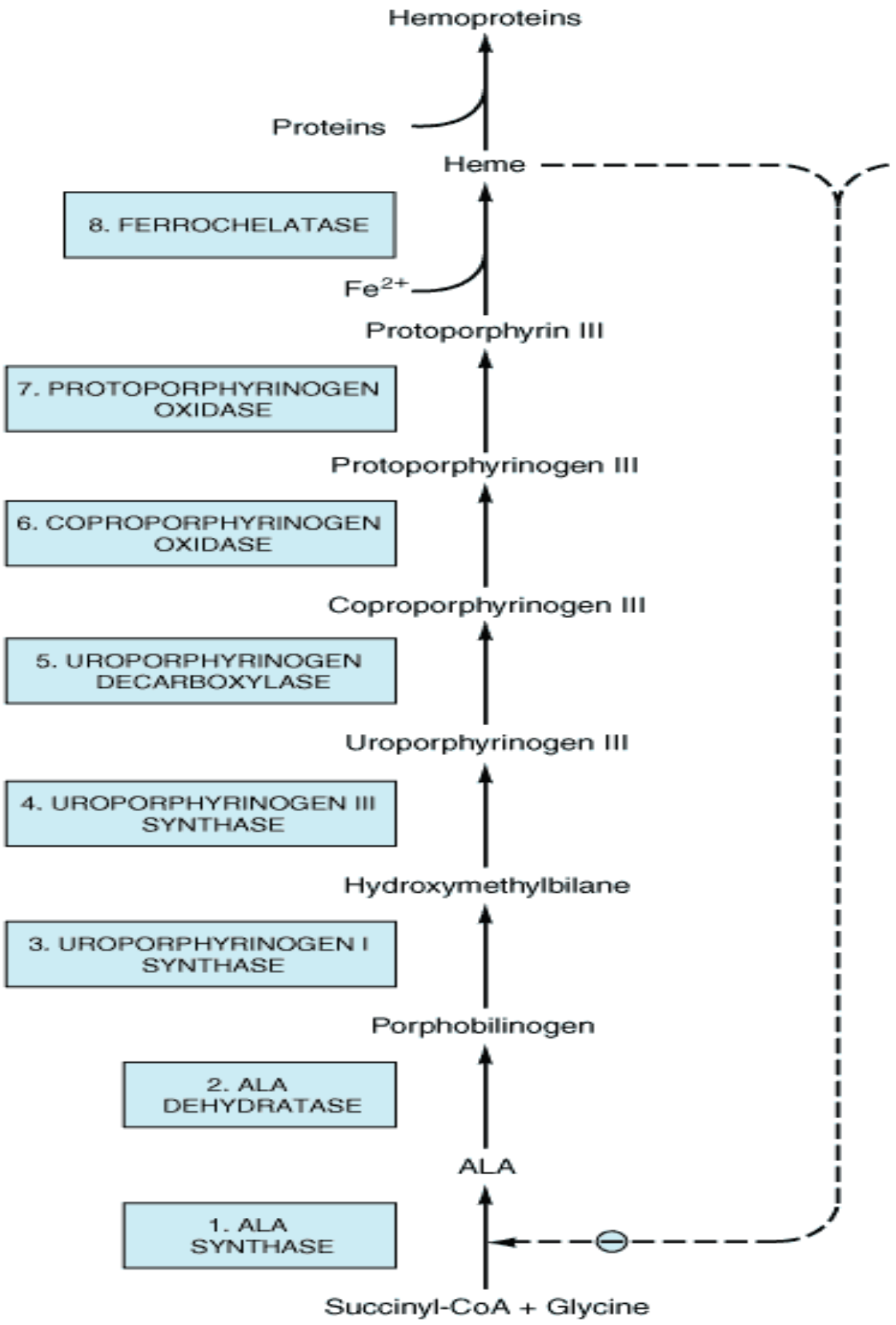


Heme is conjugated with proteins to form Hemoglobin, myoglobin and cytochromes

Heme synthesis

Heme synthesis begins with condensation of glycine & succinyl-CoA, with decarboxylation, to form gamma aminolevulinic acid (ALA).




Hemoproteins
Proteins
Heme
8. FERROCHELATASE
Fe^{2+}
Protoporphyrin III
7. PROTOPORPHYRINOGEN OXIDASE
Protoporphyrinogen III
6. COPROPORPHYRINOGEN OXIDASE
Coproporphyrinogen III
5. UROPORPHYRINOGEN DECARBOXYLASE
Uroporphyrinogen III
4. UROPORPHYRINOGEN III SYNTHASE
Hydroxymethylbilane
3. UROPORPHYRINOGEN I SYNTHASE
Porphobilinogen
2. ALA DEHYDRATASE
ALA
1. ALA SYNTHASE
Succinyl-CoA + Glycine

Bilirubin metabolism

RBCs are phagocytized in the **spleen**. Hemoglobin is catabolized into amino acids, iron and **heme**.

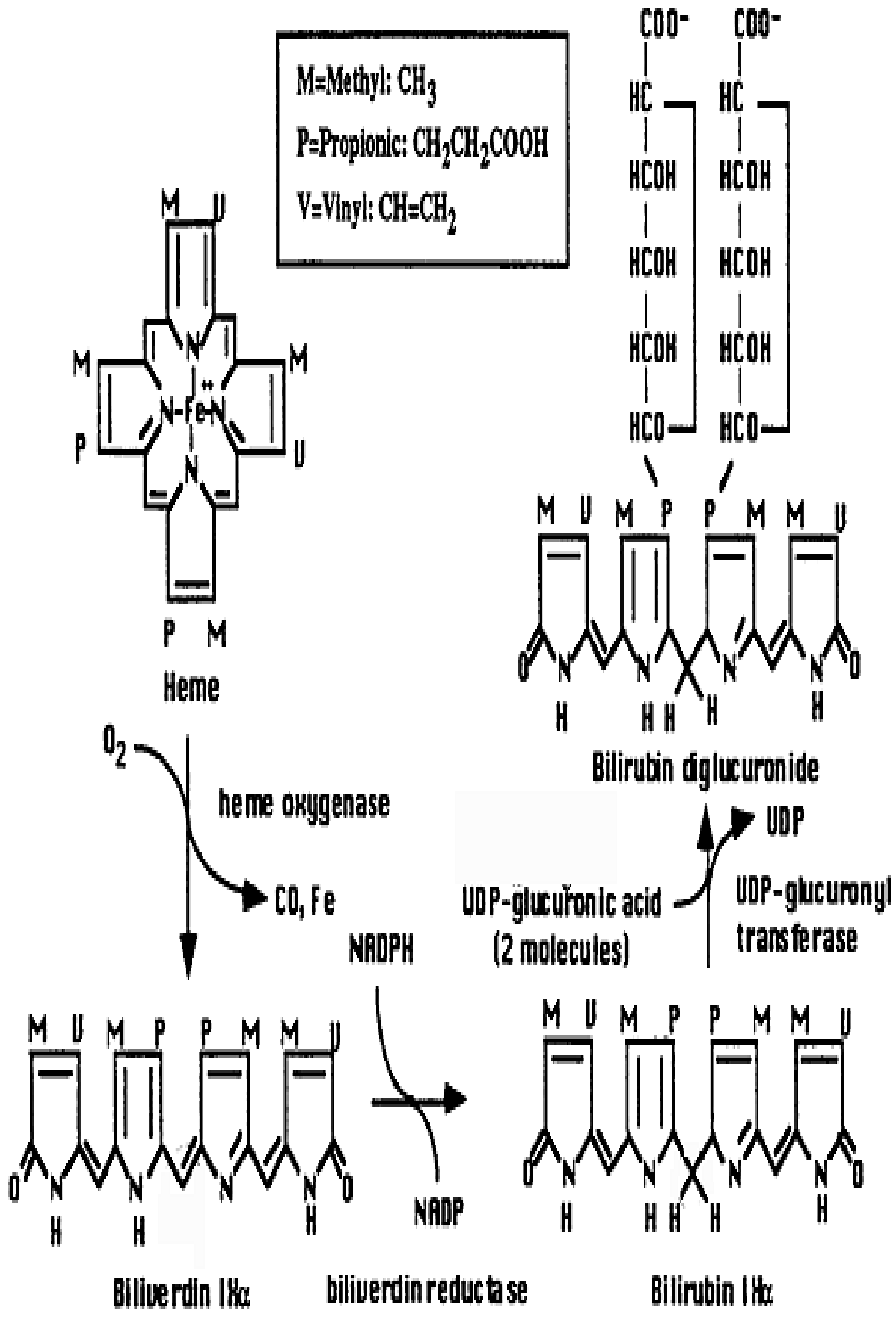
Heme ring is broken and converted to **unconjugated (indirect) bilirubin.**

This unconjugated bilirubin is not soluble in water. It is bound to albumin and sent to the liver.

In liver: Bilirubin is conjugated with glucouronic acid to produce bilirubin diglucuronides, which is water soluble and readily transported to bile. Then conjugated bilirubin is excreted in bile through bile duct to help in food digestion (mainly fat).

About 95% of the secreted bile is reabsorbed by the small intestine. This bile is secreted again by the liver into the small intestine. This process is known as enterohepatic circulation

About half of the conjugated bilirubin remaining in the large intestine (about 5% of what was originally secreted) is metabolized by colonic bacteria to form urobilinogen, which may be further oxidized to urobilin and stercobilin. Urobilin, stercobilin and their degradation products give feces its brown color.


M=Methyl: CH3
P=Propionic: CH2CH2COOH
V=Vinyl: CH=CH2
Heme
Bilirubin diglucuronide
O2
heme oxygenase
CO, Fe
NADPH
NADP
UDP
UDP-glucuronic acid
(2 molecules)
UDP-glucuronyl
transferase
Biliverdin IXα
biliverdin reductase
Bilirubin IXα

Types of bilirubin in serum

a)-Direct bilirubin: is conjugated (water soluble bilirubin) in aqueous solution it reacts rapidly with reagent (direct reacting).

b)-Indirect bilirubin: is unconjugated (water insoluble bilirubin) because it is less soluble in it reacts more slowly with reagent (reaction carried out in methanol).

Both conjugated and unconjugated bilirubin are measured to give **total bilirubin**. Unconjugated will calculated by subtracting direct from total and so called indirect.

Jaundice

It is a medical term describes the elevation of bilirubin in blood result in yellow color of skin, visible mucus membranes and sclera. According to the cause of jaundice, it is classified to three main types:

- Pre-hepatic jaundice
- Hepatic jaundice
- Post-hepatic

1)-Pre-hepatic jaundice

Massive lysis of red blood cells (for example, in patients with sickel cell anemia or malaria) may produce bilirubin faster than the liver can conjugate it.

More bilirubin is excreted into the bile, the amount of the urobilinogen entering the enterohepatic circulation is increased, and urinary urobilinogen is increased.

Unconjugated bilirubin is elevated in blood.

2)-Hepatic jaundice

Damage to liver cells (cirrhosis or hepatitis) causes a decrease in both bilirubin uptake and production of conjugated bilirubin. Unconjugated bilirubin occurs in the blood and increased urobilinogen in the urine.

The urine is dark in color and stool is pale, clay color. Plasma level of AST and ALT are elevated.

3)-Post-hepatic jaundice

In this instance jaundice is results from obstruction of the bile duct. For example, the presence of a hepatic tumor or bile stone may block the bile ducts, preventing passage of bilirubin into the intestine. Stool is pale.

Sample	**Indices**	**Normal**	**Obstructive Jaundice**	**Hemolytic Jaundice**	**Hepatic Jaundice**
Serum	Total Bil	＜1mg/dl	＞1mg/dl	＞1mg/dl	＞1mg/dl
	Direct Bil	0～0.8mg/dl	↑↑		↑
	Indirect Bil	＜1mg/dl		↑↑	
Urine	Color	normal	deep	deeper	deep
	Bilirubin	—	＋＋	—	＋＋
	Urobilinogen	a little	↓	↑	uncertain
	Urobilin	a little	↓	↑	uncertain
Stool	Color	normal	Argilous (complete obstruction)	deeper	lighter or normal

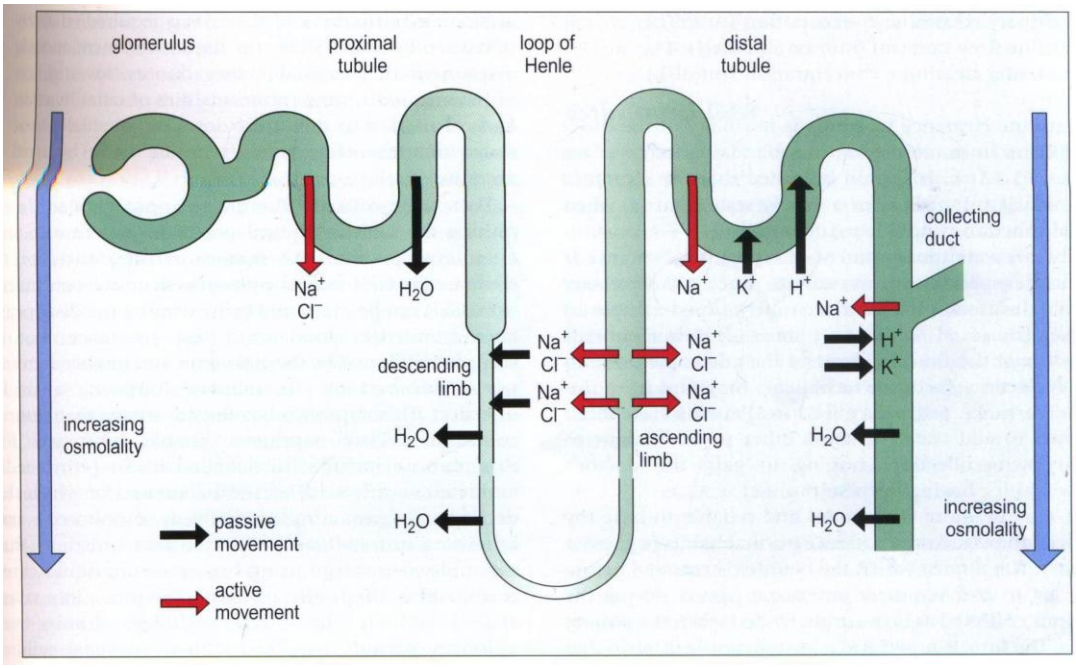
Neonatal (Physiological jaundice)

High bilirubin level is common in newborns age (1-3 days). After birth the newborns breaking down the excess RBCs they are born with and, because the newborn's liver is not fully mature, (unable to process the extra bilirubin) lead to elevate its level in blood and other body tissues. This situation usually resolves itself within a few days.

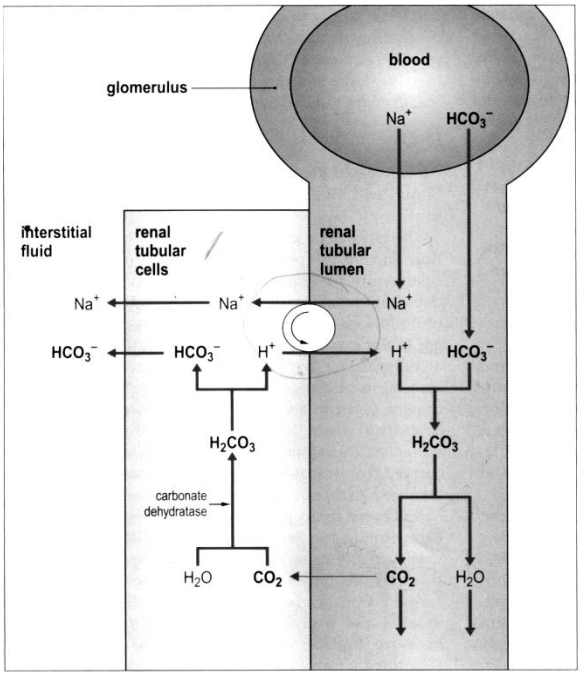
Usually newborn is treated by phototherapy which breakdown bilirubin (ID→D) and convert it to the photo isomer forms which is more soluble.

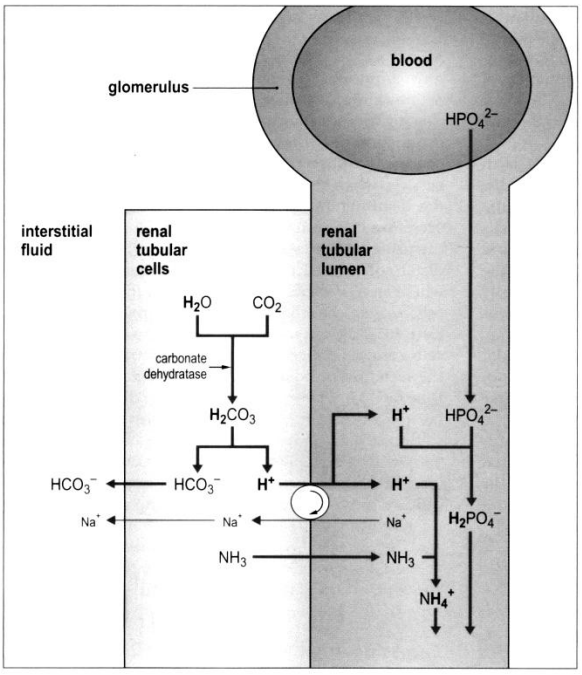
High levels of unconjugated bilirubin are toxic to the newborn – due to its hydrophobicity it can cross the blood-brain barrier and cause a type of mental retardation known as **kernicterus.**

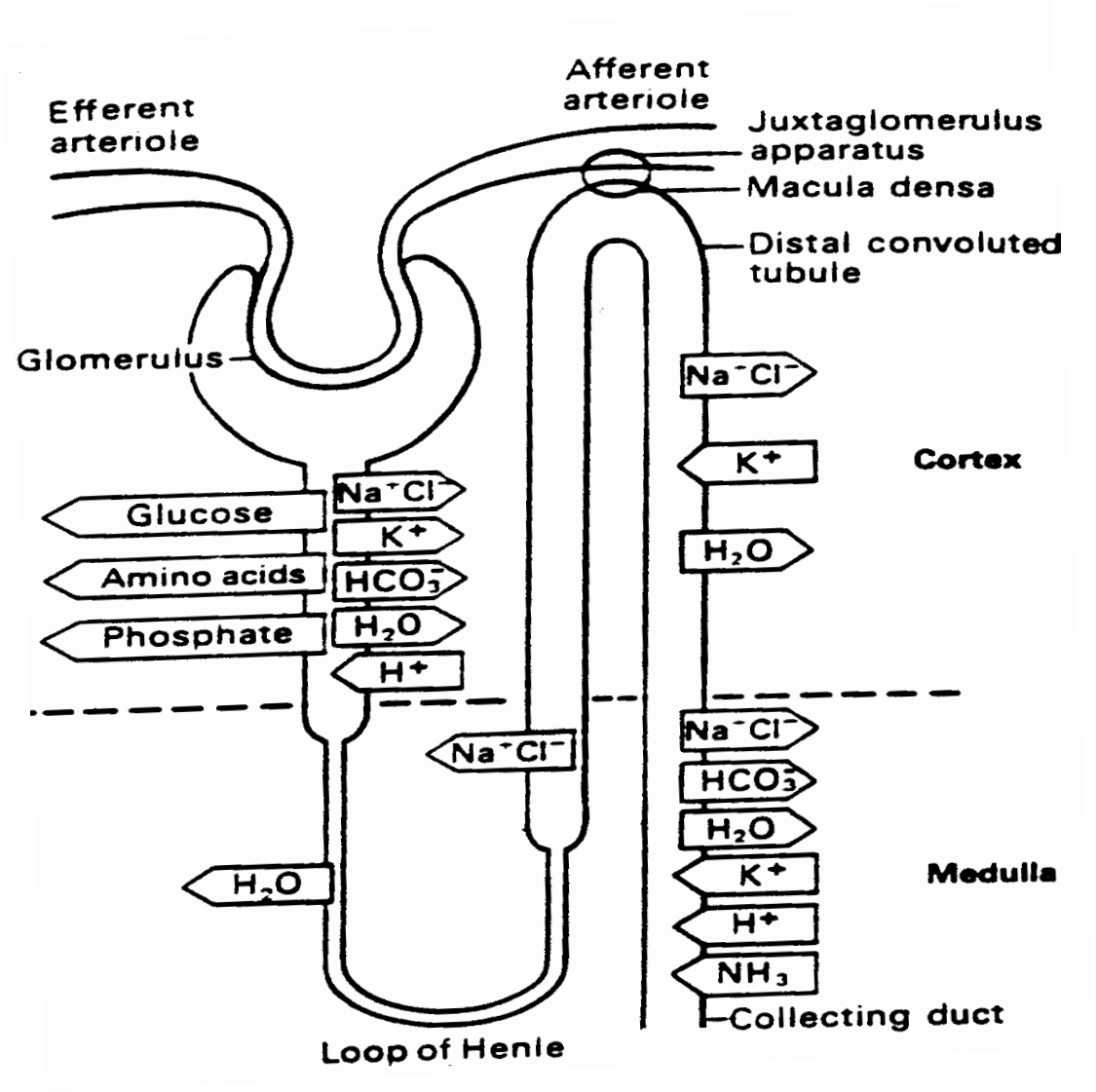
The kidney



Movement of major ions







Reabsorption of filtered bicarbonate by renal tubular cells & renal H^+ excretion

Functions of the kidney:

- Regulation of water, electrolyte and acid-base balance
- Excretion of the products of protein and nucleic acid metabolism: urea, creatinine, uric acid, sulphate and phosphate.
- The kidneys are also endocrine organs, producing a number of hormones, and are subject to control by others.
  - ❖ Arginine vasopressin (AVP) acts to influence water balance,
  - ❖ Aldosterone affect sodium reabsorption in the nephron.
  - ❖ Parathyroid hormone (PTH) promotes tubular reabsorption of calcium, phosphate excretion and the synthesis of 1,25 dihydroxycholecalciferol, which regulates calcium absorption by the gut.

- ❖ Renin catalyzes the formation of angiotensin I from angiotensinogen in the process which leads to aldosterone synthesis.
- ❖ Erythropoietin, a peptide hormone, promotes haemoglobin synthesis.

Where renal disease is advanced, the endocrine functions of the kidneys are deranged.

It is convenient to discuss renal function in terms of the assessment of glomerular and tubular function.

Tests of glomerular function

- The glomerular filtrate is an ultrafiltrate of plasma, and has the same composition as plasma without most of the proteins.
- Plasma is filtered by the glomeruli at a rate of approximately 120 ml/ minute.
- GFR is directly related to body size, and consequently is higher in men than women. It is also affected by age.
- Glomerular filtration rate (GFR) depend on the normal renal blood flow and pressure.
- If the GFR falls due to restriction of the renal blood supply, or as a result of destruction of nephrons by renal disease, there is retention of the waste products of metabolism in the blood. In chronic disease, a new 'steady state' is reached with a constant elevation in the serum concentration of substances such as urea and creatinine. As the renal disease progresses, urea and creatinine concentrations may increase slowly over many months.

Creatinine clearance

An estimate of the GFR can be calculated from the creatinine content of a 24-hour urine collection, and the plasma concentration within this period. The 'clearance' of creatinine from plasma directly related to the GFR provided that

- The urine volume is collected accurate!
- There are no ketones or heavy proteinuria Present to interfere with the creatinine determination.

The GFR is calculated as follows:

GFR (creatinine clearance) = $\frac{U\,xV}{P}$

U = urine concentration of creatinine (µmol/1)

P = serum or plasma concentration of creatinine (µmol/1)

V = urine flow in (ml/min or (L/24h)/1440)

Plasma creatinine

- Plasma creatinine concentration is the most reliable simple biochemical test of glomerular function
- It is related to the muscle bulk
- It is inversely related to the GFR (GFR = $\frac{U\,xV}{P}$)
- Changes in plasma creatinine concentration can occur independently of renal function, owing to changes in muscle mass

Plasma urea

- Like Plasma creatinine, urea in plasma samples are used as convenient, but insensitive, measures of glomerular function.
- Dietary protein intake affects serum urea concentration. Gastrointestinal bleeding will cause serum urea to be elevated, and this does not indicate that glomerular filtration is compromised.
- Urea is reabsorbed in the tubules. This reabsorption increases at low urine flow rates.

Most laboratories will measure both serum creatinine and urea. The ratio of the two is of value in the investigation of renal disorders.

Factors affecting the ratio of plasma urea to creatinine are summarized in table 1

Table 1:

Causes of an abnormal plasma urea to creatinine ratio	
Increased	**Decreased**
• High protein intake • Gastrointestinal bleeding • Hypercatabolic state • Dehydration • Muscle wasting* • Amputation*	• Low protein intake • Dialysis • Severe liver disease

*Indicates causes of decreased creatinine synthesis; other conditions primarly affect urea concentration

Proteinuria

- The glomerular basement membrane does not usually allow passage of albumin and large proteins.
- A small amount of albumin, usually less than 30 mg/24h, is found in urine. When larger amounts, in excess of 300 mg/24h, are detected, significant damage to the glomerular membrane has occurred. Quantitative urine protein measurements should always be made on complete 24-hour urine collections.
- Albumin excretion in the range 30-300 mg/24h is termed micro albuminuria.

Renal tubular function

To ensure that important constituents such as water, sodium, glucose and amino acids are not lost from the body, tubular reabsorption must be equally efficient.

Investigation of tubular function

Osmolality measurements in plasma and urine

- The most frequently affected by disease is the ability to concentrate the urine.

- If the tubules and **collecting ducts** are working efficiently, and if AVP is present, they will be able to reabsorb water.
- Determine the osmolality, and then compare this to the plasma.
- In normal individuals on an average fluid intake the urine: plasma osmolality ratio is usually between 1.0 and 3.0. In other words, the urine is more concentrated than the plasma. When the urine: plasma osmolality ratio is 1.0 or less, the renal tubules are not reabsorbing water.

Osmolarity= 2 x [Na+] + [urea] + [glucose]

The water deprivation test

- The causes of polyuria and inability to concentrate urine include:
  - ❖ Increased osmotic loade.g. due to glucose
  - ❖ Increased water ingestion
  - ❖ Cranial diabetes insidipus
  - ❖ Nephrogenic diabetes insidipus
  - ❖ Hypercalcemia
  - ❖ Hypokalemia
  - ❖ Certain drugs, notably lithium
- Occasionally it may be necessary to deprive a patient of water in an effort to find the cause of excessive polyuria.
- The water deprivation test involves complete fluid deprivation during a 24-hour period, with measurement of the osmolality of all the urine specimens passed during the second 12 hours of the test. An osmolality of greater than 700 mmol/kg should be attained and the urine: plasma osmolality ratio should be 2.0 or above.
- Administration of AVP as the synthetic analogue DDAVP will result in increased urinary concentration (osmolality greater than 700 µmol/kg)
- No response will be obtained if the AVP receptors cannot respond to the hormone. 'nephrogenic' diabetes insipidus.

The acid load test

- The acid load test is occasionally used for the diagnosis of renal tubular acidosis, which arises from diminished tubular secretion of hydrogen ions.
- Ammonium chloride is administered orally in gelatin capsules. Urine samples are collected for the following 8 hours. With normal renal function, the pH of at least one sample should be less than 5.3. If necessary in a difficult diagnosis, the excretion rates of titratable acid and ammonium ion, and serum bicarbonate concentration, are all measured. This test should not be performed on patients who are already acidotic or who have liver disease.

Renal tubular acidosis (RTA) may be characterized as follows:

- **Type I**. There is a defective hydrogen ion secretion in the distal tubule which may be inherited or acquired.
- **Type II**. The capacity to reabsorb bicarbonate in the proximal tubule is reduced.
- **Type IV**. Bicarbonate reabsorption by the renal tubule is impaired as a consequence of aldosterone deficiency, aldosterone receptor defects, or drugs which block aldosterone action.

Specific proteinuria

- Mention has already been made of protein in urine as an indicator of leaky glomeruli. (**β_2- microglobulin** and **α_1 - macroglobulin)** are small proteins which are filtered at the glomeruli and are usually reabsorbed by the tubular cells.
- The presence of these proteins in urine is a sensitive indicator of renal tubular cell damage.

Glycosuria

The presence of glucose in urine when blood glucose is normal usually reflects the inability of the tubules to reabsorb glucose because of a specific tubular lesion.

Aminoaciduria

- Normally, amino acids in the glomerular filtrate are reabsorbed in the proximal tubules.
- **Causes:**
  - ❖ The plasma concentration exceeds the renal threshold
  - ❖ There is specific failure of normal tubular reabsorptive mechanisms, such as cystinuria (cystine is relatively insoluble and leads to formation of renal calculus)
  - ❖ Acquired renal tubular damage.

Specific tubular defects

The Fanconi syndrome

The Fanconi syndrome is used to describe the occurrence of generalized tubular defects such as renal tubular acidosis, aminoaciduria and tubular proteinuria.

Causes

- Inherited metabolic diseases
  - ❖ Cystinosis
  - ❖ Galactosemia
  - ❖ Fructose intolerance
  - ❖ Glycogen storage diseases
  - ❖ Tyrosinemia
  - ❖ Wilson's disease
- Nephrotoxins
  - ❖ Heavy metals
  - ❖ drugs

Affected infants with Cystinosis fail to thrive, develop rickets and polyuria with dehydration and eventually progress to renal failure

Hypophosphatemic rickets

- also known as vitamin D-resistant rickets
- a defect in tubular phosphate reabsorption leads to severe rickets
- it is treated with phosphate supplements and vit. D

Renal stones

Renal stones (calculi) produce severe pain and discomfort, and are common causes of obstruction in the urinary tract. Types of stone include:

- **Calcium phosphate**: may be a consequence of primary hyperparathyroidism (increased intestinal calcium absorption) or renal tubular acidosis.
- **Magnesium, ammonium and phosphate**: these are often associated with urinary tract infections.
- **Oxalate**: may be a consequence of hyperoxaluria.
- **Uric acid**: may be a consequenceof hyperuricaemia.
- **Cystine**: these are rare and a feature of the inherited metabolic disorder cystinuria.

Primary hyperoxaluria: it is rare inherited metabolic disorder, and is characterized by:

- Increased hepatic oxalate biosynthesis
- Increased urinary excretion of oxalic, glyoxalic acids
- Renal failure and calcium oxalate crystals develop in many body tissues

Secondary hyperoxaluria:

It is an increased intestinal absorption of dietary oxalate with or without increased oxalate ingestion.

Cause: Gastrointestinal disorders and malabsorption conditions

Mec.: 1. non absorbed free fatty acids bind to calcium

2. This will limit the combination of calcium with oxalate to be excreted normally in feces as insoluble calcium oxalate

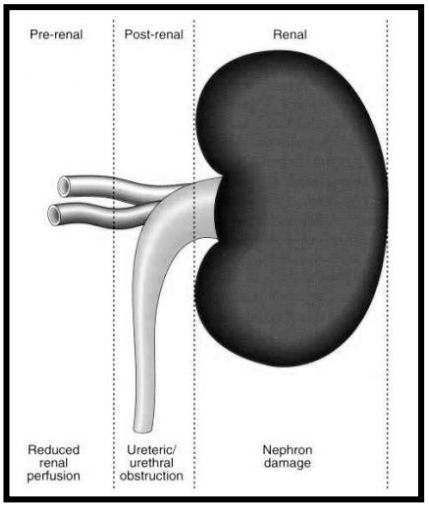
3. Oxalate will increased and reabsorbed

Biochemical investigation of renal stones

Plasma: calcium, phosphate, bicarbonate, urate

Urine: pH, qualitative test for cystine, 24 h volume and excretion of calcium, oxalate, urate, citrate

Management of renal stones



- Treatment of urinary infection
- A high Fluid intake
- Hyperuricaemia is treated with allopurinpol
- Alkalinization of urine increases the solubility of both cystine and uric acid
- Thiazide diuretics decrease urinary calcium excretion
- Citrate forms soluble complexes with calcium and is an endogenous inhibitor of calcium stone formation

Acute renal failure

Renal failure is the cessation of kidney function. In acute renal failure (ARF), the kidneys fail over a period of hours or days. Chronic renal failure (CRF) develops over months or years and leads eventually to end stage renal failure (ESRF). ARF can be reversed and normal renal function regained, whereas CRF is irreversible.

Aetiology

- It usually presents as a sudden deterioration of renal function indicated by rapidly rising serum urea and creatinine concentrations.
- Usually, urine output falls to less than 400 ml/24 h, and the patient is said to be oliguric. The patient may pass no urine at all, and be anuric. Occasionally urine flow remains high when tubular dysfunction predominates.
- The term ‘uraemia’ (meaning urine in the blood) is often used as synonym for renal failure (CRF &ARF)

Kidney failure or uraemia can be classified as (Fig. 1):

- **Pre-renal**: is due to impairment of renal blood flow.
- **Post-renal**: is due to urinary obstruction.
- **Renal (intrinsic)**: intrinsic damage to the kidney tissue. This may be due to a variety of diseases, or the renal damage may be a consequence of prolonged pre-renal or post-renal problems.

Diagnosis

Factors which precipitate **pre-renal uraemia** are usually associated with a reduced effective extracellular fluid (ECF) volume and include:

- Decreased plasma volume because of blood loss, burns, prolonged vomiting, or diarrhoea
- Diminished cardiac output
- Local factors such as an occlusion of the renal artery.

Pre-renal factors lead to decreased renal perfusion and reduction in GFR. As a result, both AVP and aldosterone are secreted maximally and a small volume of concentrated urine with a low sodium concentration is produced (a fact that may be helpful in distinguishing between pre-renal and intrinsic ARF).

	Pre-renal failure	**Intrinsic failure**
Urine sodium concentration	↓(<20 mmol/l)	↑(>40 mmol/l)
Urine: plasma urea concentration	↑(>20:1)	↓(<10:1)
Urine: plasma osmolality	↑(>1.5:1)	↓(<1.1:1)

Post-renal factors cause decreased renal function, because the effective filtration pressure at the glomeruli is reduced due to the back pressure caused by the blockage. Causes include:

- Renal stones
- Carcinoma of cervix, prostate, or occasionally bladder.

If these pre- or post-renal factors are not corrected, patients will develop intrinsic renal damage (acute tubular necrosis).

Acute tubular necrosis

Acute tubular necrosis may develop in the absence of pre-existing pre-renal or post-renal failure. The causes include:

- acute blood loss in severe trauma
- septic shock
- specific renal disease such as glomerulonephritis
- nephrotoxins such as the aminoglycosides, NSAIDs, cephalosporins, cisplatinum and heavy metals
- Deposition of protein within the nephrons.

Patients in the early stages of acute tubular necrosis may have only a modestly increased serum urea and creatinine which then rise rapidly over a period of days, in contrast to the slow increase over months and years seen in chronic renal failure.

Biochemical changes in plasma in acute renal failure	
Increased	**Decreased**
• Potassium (hyperkalemia) • Urea • Creatinine • Phosphate (hyperphosphataemia) • Magnesium (hypermagnesaemia) • Hydrogen ion (acidosis) • Urates	• Sodium (hyponatraemia) Bec. of excess of water relative to sodium • Bicarbonate • Calcium (hypocalcaemia)

Management

Important issues in the management of the patient with ARF include:

- *Correction of pre-renal factors*, if present, by replacement of any ECF volume deficit. In cardiac failure, inotropic agents may be indicated.

- Biochemical monitoring. Daily fluid balance charts provide an assessment of body **fluid volume**. Serum **creatinine** indicates the degree of impairment of the GFR and the rate of deterioration or improvement. Serum **potassium** should be monitored closely.
- *Dialysis.* Indications for dialysis include a rapidly rising serum potassium concentration, severe acidosis, and fluid overload.
- *Treatment of the underlying disease* (e.g. to control infection).

Consequences of crf

All of the activities of the kidneys are affected in end stage renal failure, with important metabolic consequences. The areas involved and the clinical biochemical features are described below.

Biochemical changes in plasma in and end stage renal disease (CRF)		
Metabolic features	**Increased**	**Decreased**
• Impairment of urinary concentration and dilution • Impairment of electrolyte and hydrogen ion homoeostasis • Retension of waste product of metabolism • Decreased calcitrol synthesis • Decreased Erythropoietin synthesis • Dyslipidaemia • Reduced degradation of insulin and insulin resistance • Other endocrine abnormality	• Potassium • Urea • Creatinine • Phosphate • Magnesium • Hydrogen ion	• Sodium • Bicarbonate • Calcium

Management

In some cases it may be possible to treat the cause of the CRF and at least delay the progression of the disease. Conservative measures may be used to alleviate symptoms

before dialysis becomes necessary, **and these involve much use of the biochemical laboratory. Important considerations are**:

- Water and sodium intake should be carefully matched to the losses. Dietary sodium restriction and diuretics may be required to prevent sodium overload.
- Hyperkalaemia may be controlled by oral ion-exchange resins (Resonium A).
- Hyperphosphataemia may be controlled by oral aluminium or magnesium salts which act by sequestering ingested phosphate in the gut.
- The administration of hydroxylated vitamin D metabolites may prevent the development of secondary hyperparathyroidism. There is a risk of hypercalcaemia with this treatment.
- Dietary restriction of protein, to reduce the formation of nitrogenous waste products, may give symptomatic improvement.

Most patients with CRF will eventually require dialysis, in which case these conservative measures must be continued. In contrast, after a successful kidney transplant, normal renal function is re-established.

The nephritic syndrome

Hypoproteinaemia with oedema may develop if large amounts of protein are excreted in the urine. For this to occur, proteinuria must usually exceed 5g/24h.

Mechanisms of proteinuria

Overflow: Due to presence in plasma of a low molecular weight protein, which is filtered in a quantity exceeding tubular reabsorptive capacity?

Glomerular: Due to increased glomerular permeability, e.g. albumin

Tubular: Due to impaired or saturated reabsorption of protein filtered by normal glomeruli, e.g. β2-microglobulin

Secretory: Due to secretion by kidneys or epithelium of urinary tract.

The nephritic syndrome		
Causes	**Clinical and biochemical feature**	
	Feature	**Mechanism**
Minimal change glomerulonephritis	Proteinuria	Glomerular damage
Idiopathic membraneous glomerulonephritis	Edema	Low plasma albumin secondary hyperaldosteronism
Associated with carcinoma, drugs or infection, e.g. malaria, hepatitis	↑susceptibility for infection	↓ plasma immunoglobulins and complemen
Systemic lupus erythematosus	Thrombotic tendency	hyperfibrinogenaemia and low antithrombin III
Other forms of glomerulonephritis	Hyperlipidemia	↑ apolipoprotein synthesis
Diabetic nephropathy		

Cardiac function tests

- Cardiac function tests are biochemical markers for diagnosis of myocardial infarction and risk stratification of patients with chest pain and suspected acute coronary syndrome (ACS).
- These are the troponins as indicators of myocardial infarction and natriuretic peptides for patients with left ventricular dysfunction and heart failure.
- Other types of markers that have been described as being valuable in patients with cardiovascular disease include markers of endothelial dysfunction, and markers of inflammation.

Myocardial ischemia

*Most often caused by atherosclerosis *Inflammation of the coronary arteries

*Thrombosis *Coronary vasospasm

Myocardial Infarction

Myocardial ischemia results from the reduction of coronary blood flow to an extent that leads to insufficiency of oxygen supply to myocardial tissue.

When this ischemia is prolonged & irreversible, myocardial cell death & necrosis occurs, this is defined as:

Myocardial infarction (MI): is the death & necrosis of myocardial cells as a result of coronary prolonged & irreversible ischemia.

Diagnosis of Myocardial Infarction

1- Clinical Manifestations

2- Electrocardiogram (ECG)

3- Biochemical Markers

Cardiac profile

Types of Biochemical Markers*:*

A-Cardiac Enzymes (isoenzymes): are blood-based biomarkers of myocardial cell injury (leakage markers)

1-Total CK (sum of CK-MM, CK-MB & CK-BB)

2-CK-MB activity

3-AST 4-LDH 5- Myeloperoxidase (MPO)

B- Cardiac proteins: are specific cardiac function proteins (functional markers)

1-Troponins

2 -Myoglobin

3-B-type natriuretic peptide (BNP)

4-CRP

A) Cardiac Enzymes:

1-Creatine kinase (Total CK) (EC 2.7.3.2)

A cytoplasmic and mitochondrial enzyme. CK is most abundant in cells of cardiac and skeletal muscle and in brain, but also occurs in other tissues such as smooth muscle.

Function: Catalyzes reversible phosphorylation of creatine by ATP for skeletal muscle cell contraction.

Clinical significance

Normal range for total CK: Male : 46-171 U/L

Female: 34-145 U/L

- Enzyme activity in serum is highest in infancy and childhood (7-10 years of age) and may increase long before the disease is clinically apparent.
- Serum CK activity characteristically falls as patients get older and as the mass functioning muscle diminishes with the progression of the disease.
- Measurement of isoenzyme is more useful for diagnosis of MI. in myocardial infarction; it gets its maximum level after 24 hours, and returns to normal level within 2-3 days.

Isoenzymes of CK:

- CK consists of two protein subunits, M (for muscle) and B (for brain), which combine to form three isoenzymes. BB (CK-1), MB (CK-2) and MM (CK-3).
- CK-MM is the predominant isoenzyme in skeletal and cardiac muscle and is detectable in the plasma of normal subjects.
- CK-MB accounts for about 35 % of the total CK activity in cardiac muscle and less than 5% in skeletal muscle: its plasma activity is always high after myocardial infarction. It is the current gold standard biochemical marker for MI.
- CK-BB is present in high concentrations in the brain and in the smooth muscle of the gastrointestinal and genital tracts. Although they have also been reported after brain damage and in association with malignant tumours of the bronchus, prostate and breast, measurement is not of proven value for diagnosing these conditions. In malignant disease plasma total CK activity is usually normal.

2-Lactate Dehydrogenase (EC 1.1.1.27) (LD) (LDH):

Reaction: It catalyzes the reversible interconversion of lactate to pyruvate with the oxidation of NADH to NAD+ .

- The enzyme is widely distributed in the body, with high concentrations in cells of cardiac and skeletal muscle, liver, kidney, brain and erythrocytes.
- Measurement of plasma total LD activity is therefore a non-specific marker of cell damage.
- LD has a molecular weight of 134 kDa and is composed of four peptide chains of two types: M (or A), H (or B). Each under separate genetic control.
- The subunit compositions of the five isoenzymes are listed below in order of their decreasing mobility in an alkaline medium.
- LD-1 (HHHH; H4) = migrates fastest towards the anode
- LD-2 (HHHM; H3M)
- LD-3 (HHMM; H2M2)
- LD-4 (HMMM; HM3)
- LD-5 (MMMM; M4)

Clinical significance

- Normal range of total LDH: 180-360 U/L.
- It is increased in plasma in Myocardial injury, acute leukemias, generalized carcinomatosis and in acute hepatitis. Estimation of its isoenzymes in more useful in diagnosis of hepatic disease and Myocardial injury.

Causes of Raised Plasma Total LD Activity

1-Artefactual: Due to in vitro haemolysis or delayed separation of plasma from whole blood.

2-Marked increase (more than 5 times the upper reference limit in adults):

- Circulatory failure with 'shock' and hypoxia:
- Myocardial infarction
- Some haematological disorders. In blood diseases such as megaloblastic anaemia, acute leukaemias and lymphomas. Very high levels (up to 20 times the upper reference limit in adults) may be found.

3-Moderate increase: viral hepatitis: malignancy of any tissue: skeletal muscle disease: pulmonary embolism: infectious mononucleosis.

Isoenzymes of LD

LD1 fraction predominates in cells of cardiac muscle, erythrocytes and kidneys.

LD5 is the most abundant form in the liver and in skeletal muscle. \Whereas in many conditions there is an increase in all fractions, the finding of certain patterns is of diagnostic value.

1-Predominant elevation of LD1 and LD5. (LD1 greater than LD5 occurs after myocardial infarction, in megaloblastic anaemia and after renal infarction.

2-Predominant elevation of LD2 and LD3 occurs in acute leukaemia: LD3 is the main isoenzyme elevated due to malignancy of many tissues.

3-Elevation of LD5 occurs after damage to the liver or skeletal muscle.

3-Aspartate Transaminase (EC 2.6.1.1) (AST):

These enzymes are present in most tissues, but especially in cardiac muscle and liver.

It is also called Serum Glutamate Oxaloacetate Transaminase (SGOT)

Clinical Significance

- Normal values of AST: Male: <35 U/L

  Female: <31 U/L

- AST is used for diagnosis of hepatocellular damage. It is increased in myocardial infarction. It appears in plasma within 12 hours of infarction, gets its maximum level after 2 days of attack, then return after 5 days.

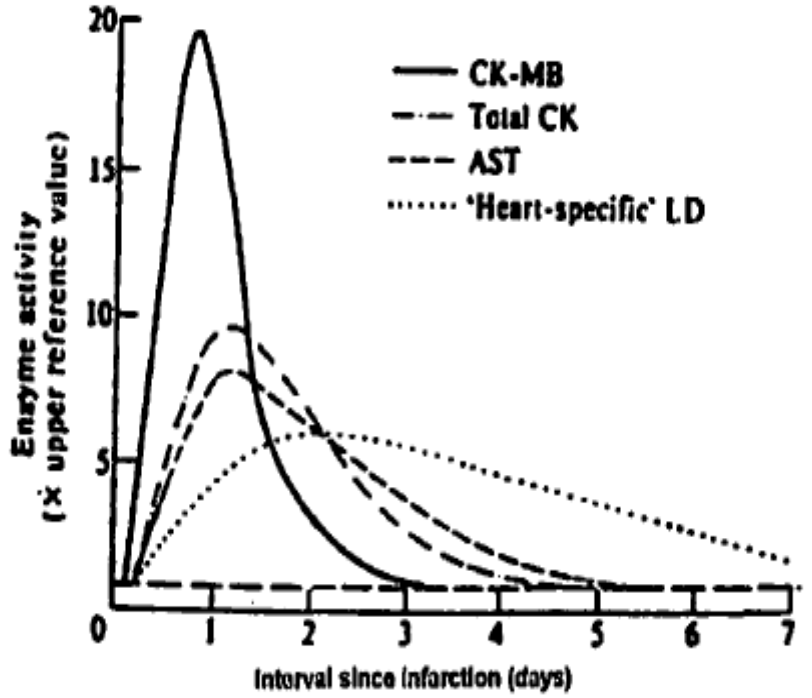
  It elevated to 4-10x the upper limit of normal in MI .

  Secondary rise may reflect extension or recurrence of MI

  It is not routinely used for the diagnosis of MI due to its distribution in many tissues and lack of myocardial-specific isoenzyme.

Note:
Enzymes for diagnosis of myocardial infarction:

1- First 24 hours: CPK
2- 2-3 Days: AST
3- 5-7 Days: LDL



Typical pattern of enzyme elevation following attack of myocardial Infarction.

4-Myeloperoxidase (MPO):

- Myeloperoxidase is a leukocyte enzyme that generates reactant oxidant species and has been linked to pro-thrombotic oxidized lipid production, plaque instability and vasoconstriction from nitrous oxide depletion.
- Early studies showed significantly increased MPO levels in patients with coronary artery disease; So MPO is a novel cardiac marker.

B) Cardiac Proteins:

1- Cardiac Troponins (cTn):

The troponins are regulatory proteins found in skeletal and cardiac muscle.

The troponin complex is located on the thin filament of the contractile apparatus and regulates the calcium-mediated interaction of myosin and actin.

The complex is made up of three structurally and functionally different proteins: Cardiac Troponin I (cTnI), Cardiac Troponin T (cTnT), and Cardiac Troponin C (cTnC).

cTnC has calcium-binding properties and has limited diagnostic value as the cardiac and skeletal muscle forms are identical. However, the skeletal and cardiac subforms for TnI and troponin TnT are distinct, and immunoassays have been designed to differentiate between them.

Clinical Significance

Damage to myocardial cellular integrity, brought about by ischemia, infarction, inflammation or degenerative disease, results in the leakage of troponin into the extracellular space and hence into the plasma where it can be detected. cTnI is an inhibitory protein that prevents contraction when calcium is not present.

The cardiac troponins have become the cardiac markers of choice for patients with ACS.

However, although elevations in the serum levels of TnI, TnT, and CK-MB indicate the presence of injury-associated necrosis of myocardial cells.

Up to 80% of patients with acute MI will have an elevated troponin level within 2-3 hours after onset of infarction versus 6-9 hours or more with CK-MB and other cardiac markers. The level reachs a **peak** around 24 hours.

cTnI disappears from blood after about one week, but cTnT disappears 14 days (stays longer), So, useful for diagnosis of delayed admission cases

The advantage of this biomarker lies in its ability to provide an early, highly cardiac specific diagnosis of considerable cardiac injury with prognostic risk assessment and ability to guide therapy quickly.

cTnI is now considered the gold standard test for acute myocardial infarction in humans.

Other promising applications of troponin analysis include detecting myocardial injury in clinically suspected myocarditis in the dog, monitoring for cardiotoxicity in animals undergoing chemotherapy, and utilizing the predictive ability of troponin analysis to identify patients at high risk for fatal outcomes.

2- Myoglobin

Myoglobin is cytosolic oxygen binding heme protein found in skeletal and cardiac muscle that has attracted considerable interest as an early marker of MI.

Its low molecular weight accounts for its early release profile: myoglobin typically rises 2-4 hours after onset of infarction, peaks at 6-12 hours, and returns to normal within 24-36 hours.

Rapid myoglobin assays are available, but overall, they have a lack of cardio-specificity.

This significantly diminishes its utility, and a number of studies have indicated that contemporary cardiac troponin assays render the use of myoglobin measurements unnecessary.

3-Natriuretic peptides:

The natriuretic peptides are a family of structurally similar hormones that act as key regulators of salt/water homeostasis and blood pressure control by

antagonizing the renin angiotensin-aldosterone system and the sympathetic nervous system.

The natriuretic peptides include Atrial natriuretic peptide (ANP), Brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), Dendroaspis natriuretic peptide (DNP), and urodilatin.

BNP is secreted predominantly by the ventricular myocardium in response to increased ventricular stretch including volume expansion and pressure overload.

C-type natriuretic peptide is produced by the endothelium.

D-type natriuretic peptide has been found in the venom of the green mamba (genus Dendroapsis).

B-type natriuretic peptide (BNP):

Multiple studies have demonstrated that BNP may also be a useful prognostic indicator in ACS. The mortality rate nearly doubled when both TnI and BNP levels were elevated.

The severity of ischemia was directly proportional to the elevation in BNP.

BNP is primarily produced by the ventricles with chronic pressure or volume overloads and is stored to a much lesser extent.

BNP appears to be more sensitive than that of ANP at detecting chronic left ventricular systolic and diastolic dysfunction with left ventricular hypertrophy of any cause.

The majority of clearance of natriuretic peptides occurs through enzymatic degradation and renal elimination.

In general, the plasma concentrations of natriuretic peptides are increased in disease states characterized by ventricular hypertrophy, tachycardia, hypoxia, expanded fluid volume, or reduced renal clearance of the peptides.

Measurements of BNP can be used in a number of different clinical situations for the diagnosis of suspected cardiac failure.

4-C-Reactive Protein:

- C-reactive protein (CRP), a non-specific marker of inflammation, is considered to be directly involved in coronary plaque atherogenesis.
- Data indicate that CRP is a useful prognostic indicator in patients with ACS, as elevated CRP levels are independent predictors of cardiac death and acute MI.
- CRP reflects the extent of ischemia, necrosis, and atherosclerosis.

Body fluids

1-Blood

Blood is the mirror for metabolic and pathological events in the cells and tissues of the body and this is of great importance today in modern medical diagnosis and therapy.

Physical properties of blood

1-Color: arterial blood is a bright crimson

Venous blood is darker red not purple or blue

2-Blood more viscous than due to many RBCs and high protein

3-pH ranges from 7.3 to 7.5 (7.4)

4- Specific gravity 1.035 to 1.075

5-Sedimentation rate (the rate of settling out of RBCs when defibrinated blood removed from circulation). It increases in menstruation, normal pregnancy septicemia and pulmonary tuberculosis. (Increase due to increase globulin & fibrinogen lead to clumping and agglutination of RBCs).

General function of the blood:

1) Transportation of foods or the products resulting from their digestion, from intestine to the tissues & from one tissue to another.

2) Transportation of waste products arising from metabolism such as; urea , uric acid to the kidney ,skin , intestine, liver for excretion.

3) Exchange of the respiratory gases between the lungs and the tissues.

4) Distribution of hormones, enzymes, vitamins and other substances.

5) Protection against microorganisms (WBCs and antitoxin).

6) Aid in acid-base balance & water electrolyte balance.

7) Heating regulation of the body.

8) Prevent excessive haemorrhage by coagulation.

Composition of blood:

Blood is composed of a liquid portion, the plasma, in which the formed elements are suspended. The formed element is the erythrocytes, leukocytes and blood platelets.

RBCs average number is 5.4 million /cu mm for men &4.9 /cu mm for women

This number, 1- increase at high altitudes than at sea level 2-High RBCs count follow muscular exercise, emotional excitement, increased atmospheric temperature, this temporary changes from flow of concentrated blood from the spleen.

* If blood is removed from an artery or vein, it coagulates leaving straw color fluid called serum

Serum = Plasma - clotting factors

Plasma = Whole blood - formed elements.

Composition of plasma:

Plasma is a light straw- colored fluid with specific gravity 1.015-1.035, which related to protein content but in whole blood 1.090, which is due to RBCs.

* Plasma contains **90-92 % water** which carry water-soluble & water dispersible substances, it also is needed for maintaining blood pressure, osmotic conditions & heat regulation.

Also plasma contains **8-10 % Solid matter**.

The solid matter includes:

1) Plasma proteins

2) Non-protein nitrogenous constituents (NPN) e.g. urea, amino acid, uric acid , creatine, creatinine ammonia & indican.

3) Carbohydrates e.g. glucose, lactic acid & fructose

4) Lipid e.g. Neutral fat, phospholipids, cholesterol, free fatty acids & lipoprotein complexes.

5) Ketone bodies 6) Minerals e.g. Na, K, Ca, Mg, I2 etc.

7) Hormones e.g. insulin 8) Bitamines 9) Enzymes.

Hemolysis

It is the liberation of hemoglobin from RBCs. The red cells are in osmotic equilibrium with a solution of 0.9 % NaCl. In hypotonic solution, they swell and rupture to liberate hemoglobin.

Causes of hemolysis:

1-Shaking

2- Repeated freezing and thawing.

3-Lysolecithin produced by phospholipase A2 in snake venom.

4-Bile salts, soap, saponins.

5-Strong acids, alkalies, urea.

6-Oxidizing agents: H2O2, nitrites.

7-Bacterial and malarial toxins.

I) Plasma proteins

The total amount of plasma proteins is from 6-8 gm% & They includes:

1- Pre-albumin: 25 mg/dl, It is responsible for transport of T3 T4 and retinal.

2-Albumin : it is about 5 gm%

3-Globulins :- it is about 1-2 gm% They divided into subtypes :-α-globulin include α1 & α2 globulin β-globulin & γ-globulin

4- Fibrinogen : it is about 0.3 gm% 5- Prothrombin : it is about 0.15 gm%

Site of formation :-

All the plasma proteins are produced by liver except the γ-globulin, which is produced by lymphocytes.

Separation of plasma proteins:

1. Direct chemical measurement e.g. Biuret method for detecting the presence of peptide bonds. This method measures the total concentration of proteins.
2. Measurement of biological activity e.g. enzymatic activity, coagulation properties.
3. Immunological methods using antigen-antibody reactions.

4. Measurement after separation by techniques such as electrophoresis, isoelectric focusing, chromatography, ultracentrifugation, precipitation (by salts or alcohol) and dialysis.

Function of plasma proteins:-

1) **Nutritive** function which is due to albumin.

2) Maintain **osmotic pressure** & blood volume & water balance by drawing water from the surrounding tissue fluid into the capillaries which excreted inward & this balances the H.P. (due to heart beat, elasticity of arteries) exerted outward & this id due to albumin.

3) Necessary for normal blood **clotting** because of fibrinogen.

4) Supply the body with **antibodies** (immunoglobulin) due to γ-globulin.

5) Have an active role in the **buffering** activity of blood keeping its pH constant.

6) Act **as carrier** for many important substances in blood e.g.

a- Free fatty acids carried in blood by albumin

b- Non ionisable fraction of calcium carried in blood by albumin.

c- Drugs (Sulfonamide) & dyes transported in combination with albumin.

d- Bilirubin is carried in blood by albumin and α1-globulin.

e-Thyroxin carried by α-globulin called thyroxin-binding globulin.

f- Copper & iron is carried by β-globulin to form ceruloplasmin & siderophilin.

g- Fat-soluble vitamins & steroids hormones are carried by α- and β lipoproteins.

Deficiency of plasma protein:-

Lead to drop in osmotic pressure, more water forced out & blood water would pass into the tissues resulting in Oedema.

Plasma proteins decrease in :-

1) Loss of protein via kidney (nephrosis). 2) Malnutrition

3) Inhibition of its synthesis (liver cirrhosis liver fascioliasis-viral hepatitis).
Albumin increase in case of dehydration, homoconcntration and Shock.

Serum globulins, increase in case of infectious hepatitis, liver cirrhosis, acute and chronic infectious disease while decrease in case of malnutrition.

Albumin I globulin ratio (A/G ratio): It is about 1.6/1. This ratio is inverted in:
1. Liver diseases (due to decreased albumin synthesis)
2. Kidney diseases (due to loss of more albumin than globulins as albumin has smaller molecular weight).

II) Non-protein nitrogenous constituents of plasma (NPN):

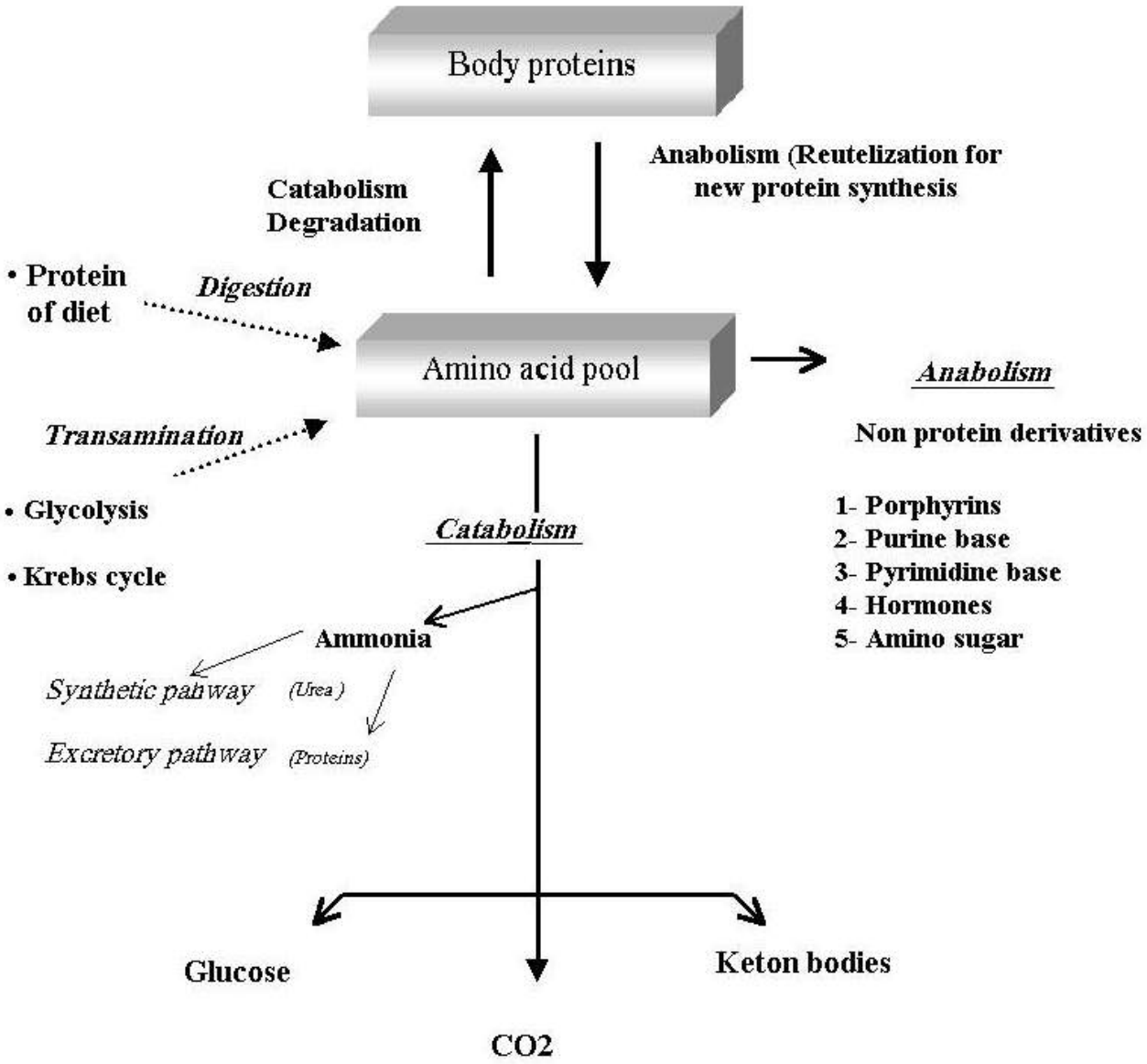
They are nitrogenous substances usually derived from the catabolism of proteins.
50% represented by urea 25% represented by free amino acids
25% remaining include large number of substance e .g creatin, creatinine, uric acid, bilirubin, cholin, epinephrine, thyroxin, nucleotides, ATP & glutathione.

1) Blood urea :-

* It is the chef nitrogenous end product of protein metabolism.
* It is formed in the liver from amino acid, ammonia liberated through deaminaion of amino acid especially arginine. It passes to bloodstream & lastly excreted in the urine.
*In blood it is about 20-40 mg%. If increase above this normal level, a condition called uraemia will result which is a fetal condition.
This increase occur in, high protein diet and renal failure (decrease excretion), while that level decrease in low protein diet and liver failure.
*In urine it is the main solid & is about 20-30 gm/day.

2) Amino acid of plasma: -

In the plasma amino acids exists in the form of **pool** which is the result of the different catabolic reactions of tissue proteins & the various anabolic reactions by which theses amino acids are continuously withdrawn.



Overall source and utilization of A.As

-The amino acid content of plasma :-

1-Rises sharply after a protein meal, return o the resting level in a few hours

2-It falls during stimulation of protein anabolism e.g. by growth hormones or androgen and also after insulin administration

3-A marked increase of plasma A.As occur in case of sever liver damage which is accompanied by failure of urea formation and by a decrease in its concentration in blood.

3) Uric acid in of plasma

It is the end produce of purine metabolism in man

Normal blood level about 3-6 mg%, increase by excess intake of diet rich in purine e.g liver, brain and kidney, and in case of renal insufficiency.

If increase above 6 mg% lead o precipitation in the small joint especially of big toe with the result of severe pain in such join , this condition called **gout**

The normal level decrease in case of acute hepatitis and treatment with allopurinol drug.

4) Creatin and Creatinin of plasma:

It is about 0.2-0.7 mg% *Creatinin level 0.8-1 mg%

III) Carbohydrates:-

-Postabsorpativ state blood glucose level 80-100 mg/dl

-After ingestion of carbohydrate meal, the level reaches 120-130 mg/dl

- Fasting blood glucose level 60-80 mg/dl

In ruminant, Sheep 40 mg% while in cattle 60 mg/dl

IV) Lipids:-

Normal concentration of total lipids 500-600 mg/dl, Triglycerides 100-250 mg/dl

Phospholipides 200 mg/dl Free fatty acids 8-20 mg/dl

Total cholesterol 150-250 mg/dl

Anticoagulants

The clotting of blood may be prevented by the action of substances called anticoagulants, which interfere with the conversion of prothrombin into thrombin.

The most important anticoagulants are:-

1} Heparin:-

1- I is acidic mucopolysaccaride

2- It isolated from liver but occur in many tissues specially the lung.

3-It secreted by the mast cells, which are distributed widely along the wall of the blood vessels.

Action of heparin: a- Antiprothrombin. B- Antithrombine (It delays the conversion of fibrinogen into fibrin under the influence of thrombin.

2} Antithromboplastin:- It inhibit the first phase of the process by which prothrombin is activated.

3} Antithrombin:- It cause destruction of thrombin by irreversible conversion to metathrombin, it present in the plasma.

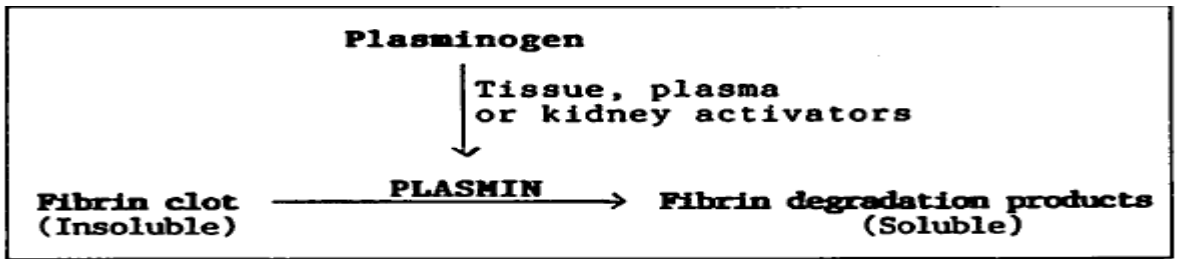
4} Oxalate citrate fluorides:- These substances remove the Ca^{++} with he formation of unionized calcium salts . Ca ions are necessary for conversion of prothrombin into thrombin.

5} Dicumarol:-It is competitive inhibitor to vitamin K, as they have nearly the same chemical structure, so it prevents prothrombin synthesis in liver

6} EDTA: Ethylen diamin tetra acetic acid.

Fibrinolysis:

This is the dissolution of clotted blood after their formation by a blood enzyme called plasmin. Plasmin is present in plasma in an inactive form, which is called plasminogen. Plasminogen is activated by a number of activators, which are derived from tissue, plasma or kidney as urokinase and streptokinase enzymes. These factors are used in treatment of recent blood clots as in myocardial infarction.



Hemophilia:

These are a group of inherited diseases in which one of clotting factors is deficient. Patient suffering from hemophilia shows frequent bleeding even from minor traumas. Tests that measure whole clotting time are all prolonged.

Types: *a)* **Hemophilia A**: is the most common type due to deficiency of factor VIII. The disease is an X-chromosome linked disease. It affects only ma les.

b) **Hemophilia B:** is also present, due to deficiency of factor IX.

Treatment of hemophilia is by repeated blood or plasma transfusion. Factor VIII prepared from pooled donors plasma may also be given. Factor VIII is produced also by recombinant DNA technology.

Types of enzymes of clinical importance:

1. Transaminases (ALT and AST):

These enzymes are present in most tissues, but especially in cardiac muscle and liver.

a) ALT activity: is widely used as a test for diagnosis of hepatocellular damage e.g. acute viral hepatitis.

b) AST activity:

1) It is also used for diagnosis of hepatocellular damage.

2) It is increased in myocardial infarction. It gets its maximum level after 2 days of attack.

2. Alkaline phosphatase:

It shows its maximum activity in the range of pH 9.0-10.5.

Liver, bone, placenta and intestine are important sources of plasma alkaline phosphatase:

a) Physiological increase of alkaline phosphatase occurs in growing children (bone) and in pregnancy (placenta).

b) Pathological increase: occurs in rickets and hyperparathyroidism (bone) and in obstructive jaundice (liver).

3. Acid phosphatase:

It shows its maximum activity in the range of pH 4-5. The prostate contains high concentrations of acid phosphatase, and its measurement is used mainly for the diagnosis of prostatic carcinoma.

4. Lactate dehydrogenase (LD):

It is present in most tissues especially liver, heart and muscles.

Its activity is increased in hepatitis, myocardial infarction and muscle diseases.

• In myocardial infarction, LD gets its maximum level after 5 days and returns to normal after 5-7 days of attack.

5. Amylase:

It is produced by pancreas and parotid glands. Its activity increases in acute pancreatitis and parotitis.

6. Lipase:

It is produced by pancreas. I~s activity increases in acute pancreatitis and pancreatic carcinoma.

7. Creatine kinase (CK):

Also known as creatine phosphokinase (CPK). It is increased in myocardial infarction and in myopathies, in myocardial infarction; it gets its maximum level after 24 hours, and returns to normal level within 2-3 days.

8.Acetvlcholinesterase:

An enzyme of postsynaptic membrane that degrades the neurotransmitter acetylcholine.

There are 2 types of the enzyme:

1) **Plasma acetylcholinesterase**: known as pseudocholinosterase.

2) **Tissue acetylcholinoesterase**: known as true cholinoesterase.

Succinyl choline apnea: Some patients during anesthesia and after administration of succinyl dicholine as muscle relaxant develop prolonged apnea, often lasting for several hours. The plasma of these patients is usually deficient in pseudocholinesterase enzyme essential for hydrolysis of succinyl dicholine.

9. Gamma-glutamyl transferase CGGTl:

Also known as gamma glutamyl transpeptidase. It is found in a number of tissues especially kidney and liver. Its activity increases in cholestasis (i.e. impairment of bile flow) and in 70- 8o% of chronic alcoholics.

2- MILK

I- General introduction to milk
II- physical properties of milk
III- Composition of milk
IV- Nutritive value of milk
V- Dairy product

I- General introduction to milk

Milk is the fluid secreted from the mammary gland after labor
Milk is the natural diet for young mammals.

Hormonal control for milk secretion; -

1- *Prolactin:* -From he anterior pituitary initiate and maintain milk secretions on condition that the mammary gland was previously developed under the effect of estrogens (promote development of duct system) and progesterone (promote development of alveolar system).
2- *Thyroxin*: increase milk flow
3- *Oxytocin*; from posterior pituitary cause contraction of myoepithelial cells and consequently let down of milk
4- *Adrenaline,* Fair cause sudden stop of milking.

II- physical properties of milk

A-Color: -

White due to: i-) presence of fat in the form of emulsion. ii-) presence of proteins in the form of colloidal solution

Reflection of light from such fat and protein give the white color of milk.
The yellow color of cow milk is due to the presence of milk carotene and xanthophyle, which is yellow pigment.

B-Reaction: -

Fresh milk has a pH of 6.6-6.9, when milk is lifted for a long time at atmosphere especially in presence of warmth, the pH of milk become lowered due to the production of lactic acid from lactose by the effect of bacteria always present in milk called lactobacillus lactis and streptococcus lactis/

Bacteria

Lactose --------→ → Lactic acid. This condition is called souring.

C- Specific gravity: -

It is measure the total solid of milk.
Normal specific gravity is from 1.027-1.037 at 15 C^{o} and if the total solid increased specific gravity increase.

D-Taste: Has characteristic taste, souring causes changes of the normal taste.

III- Composition of milk

a- Normal milk composition b-Variations in milk composition

A- Normal milk composition: -

milk is formed from I) water 87% ii) Solids 13%, which include:
1- proteins 2-Lipids 3- Carbohydrates 4-Minerals 5- Vitamins

Percentage composition of milk of different species: -

SPECIES	WATER	TOTAL SOLID	PROTEINS gm%	FAT gm%	LACOSE gm%	ASH gm%
Cow	87	13	3- 4	2- 4	3.5- 5	0.7
Buffalo	85	15	3- 4	6- 7	4- 4.8	0.8
Woman	87.5	12.5	0.8- 1.5	2- 4	6- 7.5	0.25

1-Milk proteins: - *Characterized* by:

1-High biological value (contain all essential amino acids in a well balanced proportionate each other)
2- high coefficient of digestibility (85-95%).

$$\text{Coefficient of digestibility} = \frac{\text{Amount of } N_2 \text{ absorbed}}{\textbf{Total } N_2 \textbf{ present in such protein}} \times 100$$

3- Easily digested and easily absorbed.

- Milk proteins are synthesized in the mammary gland.

-The ***total amount*** of milk proteins differs according to the species e.g.: -

Human milk contains, 1.5 gm% -Cow milk 3.8 gm% -Buffalo milk, 4.5 gm%

Types of milk proteins

The main type of milk proteins is:
1- Casein 2-Lactalbumin 3-Lactglobulin
4-Milk enzymes (all enzyme are proteins in nature)

1- Casein

-It is the principal milk proteins
-It is about 65% of total proteins in human milk and 85% in cow and buffalo milk.
-It is a phosphoprotein (contain 0.7% P) and so it is a type of conjugated proteins.

-At the pH of fresh milk, casein exists as complex particles containing Calcium, inorganic phosphate, magnesium and citrate. Various names have been applied to such complex; the most common name is **Calcium Caseinate-phosphate complex.**
-The isoelectric point of casein is 4.6; at this pH protein is p.pt and does not move in an electric field

*Casein can be p.pt if calcium and phosphate attached to it are dissolved and separated from casein, this occur in ***souring*** *when* lactic acid is produced changing pH of fresh milk from 6.6 to 4.6, at this pH casein become free of bound salts and so it p.pted.
* The fluid remaining after removal of casein is called **whey** and the remaining milk proteins are called whey proteins.

-If fresh milk is boiled; a film is formed at its surface formed from calcium caseinate.
-Under the influence of milk clotting enzymes which are rennin, pepsin and chemotrypsine, casein is changed to soluble paracasein (leaving the calcium attached to the protein) which is the milk clot or *milk curd*, so this process is called **curdling** which is the process for preparation of cheese.
-Casein is not a homogenous protein, it consists of 3 fractions, and every one differs from the other in composition, in solubility and electrophoretic mobility.
It consists of:
α -Casein$\rightarrow$ (75 % of total casein). β- casein$\rightarrow$ (22 % of total casein)
γ- casein$\rightarrow$ (3 % of total casein).

Whey proteins, are milk proteins without casein, They are present in the whey, so it includes lactalbumin and lactglobulins and enzyme bound to such proteins. Whey proteins are about 35 % of total proteins for human milk and 15 % for cow and buffalo milk.

- **2-Lactalbumin:**
- It is about 87% of total whey proteins.
-It is an albumin and so belongs to simple proteins & consists of 2 different fractions:
a- α -lactalbumin (32 % of total whey proteins)
b- β-Lactglobulin (55 % of total whey proteins), it called globulin because it needs small amount of Nacl to be dissolved exactly as globulin but it is not p.pt by half saturated solution of ammonium sulfate and so it belongs to the lactalbumin fraction.
3- Lactglobulin: -
It is about 13 % of total whey proteins and includes,
a) Euglobulin (true globulin) b) Pseudoglobulin (false globulin)
They are of equal concentration in milk, and they carry the antibodies causing immunity so they called immunoglobulis, they increased in colostrum.

N.B. Both lactalbumin and globulin are, a)-simple proteins,
b)-coagulable by heat when isolated from milk, if present in milk they don't coagulate on heating because pH is no favorable c)-Rich in cysteine and cystine.

Milk enzymes: include

a- *Catalase :* it carried by fat globules and acting on hydrogen peroxide producing H_2O and O_2

Its importance increases when the breast is inflammed (mastitis) so it is a diagnostic method for mastitic breast.

b- *Peroxidase* It is carried by lactalbumin fraction acting on hydrogen peroxide in presence of reducing agent producing water and oxidized agent .

$H_2O_2 + 2\,H\,A \rightarrow 2\,H_2O + 2\,A$

Importance →it is so resistant to destruction by heat, Therefore if it is found destroyed indicated that milk was heated to high temperature and so is sterilized

N.B Sterilization of milk , heating of milk to 116 C for 15 minute, its purpose is to destroy all organisms (harmful and non-harmful).

c-Xanthine oxidase : It is carried by fat globules acting on hypoxanthine giving xanthine and acting also on xanthine giving uric acid.

$$\text{Hypoxanthin} \xrightarrow{\text{Enzyme}} \text{Xanthine} \xrightarrow{\text{Enzyme}} \text{uric acid}$$

D- Alkaline phosphatase :- It is carried by fat globules, catalyzing hydrolysis of phosphate esters.

Its importance is that it is destroyed by the same temperature which can destroy the harmful bacteria e.g. tubercle bacilli. So if this enzyme was found to be destroyed, this indicate that milk is well pasteurized.

N.B Pasteurization: Heating of milk to 60 C for 30 minutes or 70 C for 15 second. Its purpose is to destroy the harmful bacteria only

E- Amylase:- It is carried by lactalbumin fraction, catalyzing hydrolysis of α-,1,4-glycosidic linkage of starch, dextrin or glycogen.

-Its importance is that it increased in mastitis (inflammation of breast)

*F-Lipase :-*its carried by casein ,catalyzing hydrolysis of primary ester linkage of triglycerides

$$CH_2 — O\text{-}OC — R$$

-Its importance is that it acts on milk fats producing free fatty acids with production of undesirable taste (rancidity).

Enzyme	**Carrier**	**Action**	**Importance**
Catalase	fat globules	$H_2O_2 \rightarrow H_2O + O_2$	↑ in mastitis
Xanthine oxidase	fat globules	Hypoxanthin Xanthine to uric acid	
Peroxidase	Lactalbumin	$H_2O_2 + 2HA \rightarrow 2H_2O + 2A$	destroyed in sterilized
Alkaline	fat globules	Hydrolysis of (P) esters.	Destroyed in

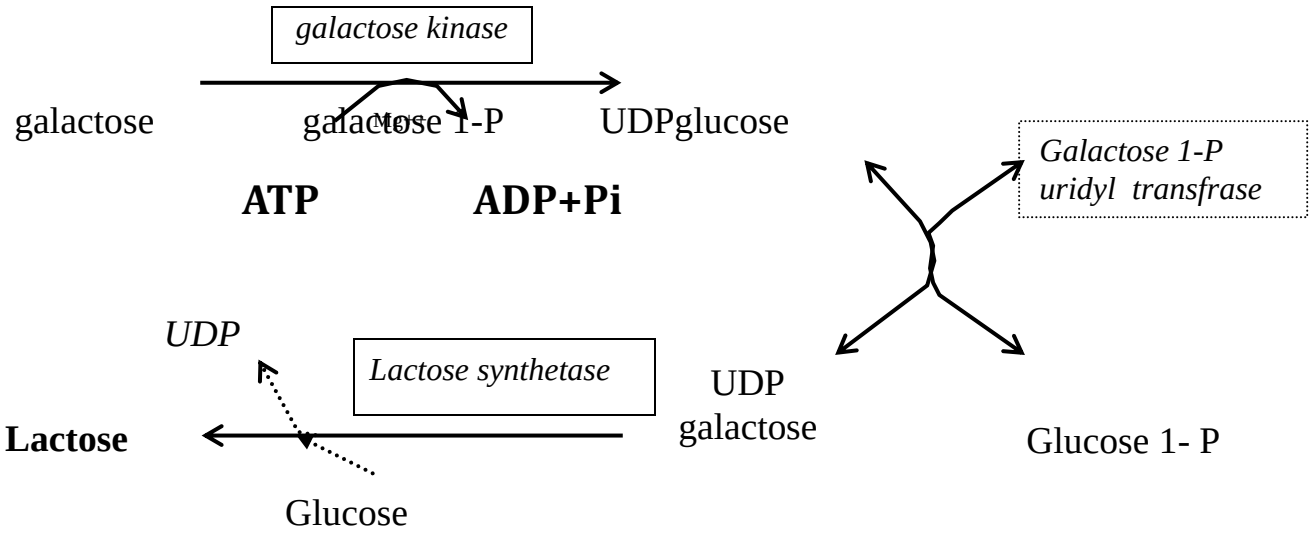
phosphatase			pasteurized
Amylase	Lactalbumin	Hydrolysis of α,1,4-glycosidic linkage of starch, dextrin or glycogen.	↑ in mastitis
Lipase	casein	Primary ester linkage of triglycerides	Rancidity

2) Milk carbohydrate

The main significant CHO in milk is lactose
-Its amount is higher in human 7 gm%, than in cow milk ; 5gm%
*Lactose is very suitable for baby and young nutrition **because: -**

1-It is less sweet than sucrose, enabling he baby o drink much milk without loss of appetite and prevent nausea and vomition.
2- lactose non-fermentable & so does not produce gases in the intestine, which may cause colic pain
3- It possess laxative effect, prevent constipation.
4- Lactose changed to lactic acid by certain bacteria, producing souring of milk, that help Ca & Phosphorus, iron absorption from the intestine
5- Lactose yields glucose and galactose, which enter in formation of cerebroside of brain, and both produce energy by oxidation.

-Biosynthesis of lactose: -



Synthesis of lactose occurs in the mammary gland and catalyzed by lactose synthetase. This enzyme has 2 subunits, a catalytic subunit (galactosyl transferase) and a modifier subunit (α-lactalbumin).

In mammary gland α-lactalbumin modify the activity of galactosyl transferase, so that galactose residueis transferred to glucose. The level of the modifier is under the control of prolactin. During pregnancy, only the catalytic subunit is synthesized in the mammary gland. While following birth, prolactin increase leading to synthesis of the modifier subunit too, resulting in formation of he complete enzyme, lactose synthase and consequent synthesis of lactose.

Congenital deficiency of galactos 1-phosphate uridyl transferase produce disease called **galactosaemia** in which there is marked accumulation of galacose and galactose 1-P in the blood and appear in urine.

3 - Lipids of Milk =butter

Composition: -

a- Mainly triglyceride , contain both saturated and unsaturated fatty acids , most of them palmitic , stearic and oleic.

- Human milk contain highly unsaturated fatty acids (10% of total) , with *no short* chain F.As., while in cow milk, the highly unsaturated F.As comprise about (1/2%) only, while short chain F.As 5-10%, which is mainly butyric short chain. However, human milk is richer in essential F.As

b- Trace of cholesterol, mainly in free state in cow's milk and mainly estrified in human's milk.

c- Phospholipids, present in small amount. Cow's milk has double the amount present in human's milk.

d-Fat soluble vitamins

4- Mineral of milk

Milk is rich in Ca –P, K and chloride necessary for stability of casein, but contain moderate amount of Na and Mag.

Milk is poor in iron and copper so, it is not very perfect food.

-Cow's milk contain more mineral than Human's milk as the rate of growth of calves is more than hat of babies.

-Infant after the 4h month of age must be given iron and copper in vegetable soup, otherwise anemia may occur.

Milk Vitamins

Milk Rich in vitamin A & β-carotene, low in vit B & Pantothenic acids.

Poor source for vitamin C and D

Moderate amount of other vitamin

B- Variation in milk composition: -

1) Inherited variation:- Marked in fat content ,and less in protein and lactose.
2)Nutritional variation:- *Overfeeding* has no effect on milk composition , while *underfeeding* lead to a) reduction of milk volume b) increase fat content
c) may reduce protein lactose d) fluctuation of inorganic constituent.
3) Seasonal variation and effect of temperature:- fat content, proteins and minerals are more during winter than summer.
4) Age of cow:- fat content decline with increase of age.
5) Stage of lactation, at the beginning of lactation, colostrum is different from ordinary milk
6- Variation due o milking procedure:-AT the beginning of the procedure fat is low and increase at the end of the process.
7- infection of the mammary glands:- mastitis decrease fat, lactose and casein concentration and increase whey proteins and chlorides.

IV- Nutritive value of milk

Milk is considered as ((The most nearly perfect food)) because it contain (Carbohydrate, protein, fat, inorganic salts and vitamins) necessary for growth, maintenance of life and reproduction.. It is no very perfect food because its deficient in iron, copper and vitamin D

Milk is a diet of high nutritive value because;-
1) Good source for ca, P and riboflavin
2) Good source for protein of high biological value
3) Supply young by antibodies from the mother.

Colostrum,

It is the fluid secreted in the 1st week after labor. It differs from ordinary milk in that,
1- It is yellow in color due to excess carotene
2- It contain more mineral elements and proteins, the greater amount of proteins is due to the presence of high amount of globulin (α, β and γ -globulin). Especially γ -globulin present in excess in colostrum, which is responsible for providing the infant with antibodies for production of immunity against diseases.

Since globulin are heat coagulable, so colostrum has the property of being coagulated on boiling, ordinary milk does not coagulate on boiling due to the small amount of globulin.
3- It contains a factor called "Trypsin inhibitor" which inhibits trypsin enzyme of pancreatic juice.
4-It contain less fat and less carbohydrates
5- It contains more fat-soluble vitamins e.g. A, D, E and K

Humanization of milk:-

It the modification of the cow's milk composition o be more or less resembling to human milk, this can be occur as follow:-

1} 1 litre of milk is pasteurized and left in cool place for sometime until h cream isolated on milk surface.

-Transfer the upper half litre, with the whole cream part and added to it half litre of water, this correct the protein and mineral contents (lipid is constant)

-then added 40 gm lactose its concentration.

-Humanized milk still differ from human milk in a-Ratio of casein to other proteins

b-Its mineral still poor in Fe and Cu.

V- Dairy product:

Milk clotting (Cheese formation):-

This occurs by rennin enzyme and also by pepsin and chymotrypsin, milk clotting done by 2 steps

1) Caseinogen $\xrightarrow[\text{Enzymaic setps}]{\text{Rennin}}$ Casein (soluble in water) + Peptide

2) Casein $\xrightarrow[\text{Chemical steps}]{\text{Ca++ ions}}$ Ca Caseinate ↓

Precipitated entangling fat, fat soluble vitamin and carotene ,this is the milk clot (Cheese)

-When the clot separated, the fluid left is called whey, that, formed of water, lactalbumin, lactglobulins, water-soluble vitamins, , lactose minerals very small amount of Ca.

-whey is yellow-green in color due to is high content of vitamin B1 riboflavin

N.B:- -If rennin is added o boiled milk, clot no formed as heating ppt Ca ions as calcium phosphate.

-Rennin is secreted from fourth stomach of calf as inactive prorennin , it activated by low pH of young gastric juice , while in adult it is not activated due to unfavorable pH.

-Milk Clotting is important for slow evacuation of stomach, and offer better medium from digestive enzymes.

Milk curdling (Zabadi- formation- youghort);-

Curdling is the pptation of caseinogen at its I.E.P. (4.6)

* It occur either by adding acetic acid or adding bacteria that change lactose into lactic acid (Souring).

Casinogn when ppt.d entangles the fat, fat soluble vitamins7 carotene (milk curd). Also the fluid remain is called whey where it is composed of; water, lactalbumin, Lactglobulin, lactose, water soluble vitamins all minerals and lactic acid, so it has a bitter taste.

URINE ANALYSIS

Urine formation:

-Urine is formed by filtration of the blood plasma through the glomerular capillaries into Bowman`s capsule

-This process is chiefly governed by capillary blood pressure & the osmotic pressure of plasma proteins.

-Urine volume related to the number of osmotically active solute particles remaining in the renal tubules.

FUNCTION OF THE KIDNEY

1-Regulation of water electrolyte balance & osmotic pressure.

2-Regulation of pH through excretion of

Sulfuric acid ⟶ Sulfur containing Amino Acid

Phosphoric acid ⟶ from nucleoproteins and Amino acid.

3-Elimination of waste product as urea and uric acid.

4-*Glomerulus function:*

Filtration = Efferent filtration pressure - Renal blood pressure (osmotic pressure of plasma proteins + pressure of Bowman`s capsule)

= 45 or 65 - (24 + 15) = 6 or 26 mmHg

-The glomerular filtrate is 150 ml /Minute

5-Tubular function include A)- Reabsorption of 99% of water,

Obligatory reabsorption of about 80-85% in proximal convoluted tubules with Nacl

Facultative reabsorption of about 12% in distal convoluted tubules under the control of antidiuretic hormone

B) Absorption of Na^+ ions :-About 80-85% absorbed by proximal C.T under the influence of aldosterone and the remaining absorbed by distal convoluted tubules.

C) Absorption of K^+ ions, nearly all K pass through proximal C.T. together with $chloride^-$

D) Reabsorbtion of glucose; all glucose absorbed by proximal C.T. after phosphorylation by hexokinase, such process is inhibited by phlorizine. Renal threshold for glucose is 180-mg%.

Threshold capacity of kidney: -

It means capacity of the kidney to reabsorb back the substance to the blood stream.

* Substance of high threshold capacity: - as glucose, amino acids, vitamins, lipids, Minerals.
* Substance of low threshold capacity :- urea, uric acid, creatin.
* Substance of no threshold capacity :- hippuric acid

How can you take urine sample :-

1- By the way of catheter which must be carefully sterilized, if the sample used for bacteriological examinations (in female use metal catheter while in male use elastic catheter)

2- Urine specimens collected in clean container , the best time for collection is first thing in the morning when the urine is most concentrated.

3- The specimen should be analyzed without delay. If it not possible the urine should be stored refrigerator to inhibit decomposition by bacteria, which decompose urea into ammonia.

Urine preservation: - urine could be preserved by using

1- **Toluene:-**which form a layer on the surface of urine prevents putrefaction - not interfere with ketone bodies.
2- **Formaline :-** Drop of 40% to 30 ml urine prevents urine cells & casts in urine (specific for sediment).
3- **Phenol: -** 1 drop/ 30-ml urine.
4- **Boric acid**: - 1 gm/30 ml urine -interfere with urinary glucose.
5- **Sodium florid: -**specialized for urinary and blood glucose.
6- **Thymol** :- one crystal/100 ml urine. Excess interferes with proteins & bile salts.

The ordinary urine is examined under the following heads: -

A) Physical examination: - **III) Microscopical Exam:-**
II) -Chemical examination of urine

I)-Physical examination:

1] Volume:-

The normal volume of urine , in adult man is 1-1.5 litre/ day. In cow 14 litre/day
In horse 4.7 litre/day In sheep and goat 1 litre/day .
The volume varies under normal and abnormal condition.-

**Polyuria:* - it is the increase in excretion of urine volume, this may indicate the loss of concentrating ability of kidney
**Oliguria:*- decrease in urine volume 9urinary out put is ½ litre/day.
**Anuria:*- no urine excretion or total loss of urine as in complete obstruction of urinary pathway.
The ratio of day urine (i.e 8 a.m to 8 p.m.) to night urine (i.e. 8 p.m. to 8 a.m.) should be at least 2:1 and sometime 3:1 or more in healthier individuals. In renal disease this ratio is reduced and may be reversed.

**The urine volume vary under normal and abnormal pathological conditions:-*

Physiological variations:- as in polyuria occur in case of :-

1- In winter more than summer
2- During the day more than the night.
3- In children more than adult in relation to body weight.
4- Volume increase with high protein diet , as excess urea cause diuresis.
5- excessive fluid drinking , also by drinking tea, coffee & ingestion of diuretic drugs

Oliguria occur in case of , Sweeting, less fluid drinking.

Pathological variations:-

Polyuria occur in case of :-
1- Chronic nephritis
2- Diabetes mellitus
3- Diabetes insepidus
4- increased blood pressure
5- contracted kidney

Oliguria occur in case of
1- Acute nephritis
2- Cardiac failure
3- chest disease and shock
4 low blood pressure
5- Excessive fluid loss due to ; vomition, diarrhea, fever

2] Color:-

The normal urine color is straw yellow in color, which is due to the presence of urochrome and urobilin.

Abnormal color:-

A-Due to physiological variations:-

1-Pale yellow or light color, this happen in all physiological condition that cause poluria as excessive protein eating.
2- Deeper or intense yellow color as in summer
3- Red color as in case of administration of some drugs or food e.g. urisept
4- Blue color due o methylene blue dyes

B -Due to pathological variations:-

1-Pale yellow or nearly colorless, this occur in all pathological condition that cause polyuria as D.M
2- Dark yellow as in pathological conditions that cause oliguria as fever & acute nephritis.
3-Milky yellow due to presence of fat called chyluria and purulent inflammation.
4-Red color or smoky dark brown in haematuria and Hb uria
5- Greenish yellow as in jaundice
6-black upon standing in case of Alkaponuria and malignant melanoma.
7- Orange brown in presence of urobilinogen
8-Cloudy appearance in presence of insoluble Ca , magnesium & phosphate.

N.B.

Alkaponuria :-In born error due to deficiency of homogentisic acid oxidase in tyrosine metabolism, so transformation of homogentisic acid into malleyl aceto acetic acid stop, So homogentisic acid excreted in urine which upon exposure to air give black color

Chyluria: - Condition in which the lymph may be excreted in urine due to rupture in lymphatic vessels of urinary tract. It may be parasitic in case of filariasis, or non-parasitic in case of thoracic duct obstruction and tumor.

3] Specific gravity: -

-It is inversely proportion to the volume of urine
-The chief factors influencing specific gravity are total solid as urea, Nacl and phosphate.
-Normal specific gravity in man is 1.015-1.025.
-Specific gravity indicate the relative proportion s of dissolved components to the total volume of the urine, it also reflect the relative degree of concentration or dilution of urine sample.

Variation in specific gravity: -
Specific gravity of urine varies according to renal function
-Concentrated urine has high specific gravity & intense yellow color while; diluted urine has low specific gravity & pale yellow color

Physiological variations:

Low specific gravity	***High specific gravity***
High fluid intake	Sweating

Pathological variations: -

-Low specific gravity	***-High specific gravity***
-D.I, glumerulonephritis Pyelonephritis chronic nephritis	-D.M. , acute nephritis, hepatic - disease , albuminuria

Fixed specific gravity of urine is an indication of several renal damages with disturbance of both concentrating and diluting ability of kidney.

Determination of specific gravity: -

It measured by urinometer as follow: -
1-Fill proper cylinder with urine and remove froath from the surface by filter paper.
2-The urinometer should be floated not touch the sides or bottom of the cylinder

3- The depth to which the urinometer is sinking in the urine indicates specific gravity of urine.

4-Urinometer is calibrated with respect to distilled water at 1.00 at specific temperature indicated at the instrument itself (15ċ) – If temp. of urine above or below that temp. a correction of ± 0.001 for each 3C should be made.
-Addition of (0.001) for every 3 ċ above 15ċ
and subtraction of 0.001 for every 3ċ below 15ċ.

For example: -

If room temp. 24ċ and urinometer reading 5 so observed specific gravity = 1.005

Correction = $\frac{24-15}{3}$ = 9/3 =3

So corrected Specific gravity at 15ċ = 1.005 – 0.003 =1.002

*Calculation of the total solids on the basis of specific gravity, through multiply last 2 figures by 2.66 (Long's coefficient).

So if corrected specific gravity = 1.005 So T.S. = 5X 2.66 = 13.30 gm/litre

-From that calculation the urine output during 24 hours can be measured.

4] Odor: -

Normal odor, urineferous odor due to the presence of volatile organic acids.

Abnormal odors as follow: -

1- Rotten apple odor indicates acetonurea. Ketosis.
2- Putrid odor, in case of pus, putrefaction. (Pyuria).
3- Ammoniacal odor; in case of fermentation due to bacterial decomposition of urine.
4- Charactaristic odor, in some aromatic compounds.

5] Reaction (PH):-

Depend mainly on the *type of diet*: -

A-In carnivorous animals: The urine normaly acidic (5.5-6.5) this is chiefly due to protein rich diets which contain amino acids where sulfur group of sulfur containing amino acids oxidized to sulfuric acid.

Similarly phospholipids, nucleic acid all yield phosphorus group, which is oxidized to phosphoric acid.

B-In Herbivorous animals (ruminant):

The urine is normally alkaline due to citrate, tartarate and oxalate.

C-In Omnivorous animals (man & dogs):

PH of urine depends on type of diet. Grass, vegetable and fruits give alkaline urine, while eating meat allow urine to be acidic

Reaction can be measured by different types of pH papers where red appear after immersion of it in urine sample indicate acidic urine
- Blue indicate alkaline and no changes in case of neutral urine.
PH normally in man is 5-7

Urinary acidity increases in: -

1) Diet rich in protein as meat.
2) Ingestion of acidic salts e.g. NH_4cl
3) Ketosis (Starvation, diet rich in fat and low in carbohydrate)
4) Lactic acid in prolonged muscular excersis.
5) Uric acid in leukemia.
6) Respiratory acidosis and diseases (blood CO_2 accumulate leading to increase H_2CO_3).

Urinary acidity decreases (alkaline urine) in :-

1) Diet rich in alkaline ash e.g. fruits and vegetable
2) Ingestion of alkaline salts e.g. HCO3 and lactate.
3) Vomition continuously leading to loss of stomach Hcl
4) Respiratory alkalosis.
5) After meal, because H^+ ion is secreted in gastric juice but HCO_3^- excreted in urine (Alkaline tide).

6] Aspect (Appearance):-

* *Normally urine* is clear, except in horse due to presence of glands in urinary tract secrete calcium carbonate, calcium phosphate and mucous secretion make urine normally turbid.

- *Abnormal turbidity* may be due to bacteria, pus, blood, chyle, epithelial cells, mucous, phosphate (in alkaline urine) and urate (in acidic urine).
- These substances usually p.pt in normal urine on standing because it become alkaline and urate p.pt. This deposit may appear by centrifugation.

7] Sediment or deposit: -

Normal urine has no deposits (particularly of normal female urine deposit a slight whitish mucoidal deposit).
Abnormal urine contain deposit present as in albuminuria

II) -Chemical constituents of urine

Normal constituents of urine: -
Normal urine composed of the following
{1} Water $\rightarrow$ 1-2 Litter /day
{2} Inorganic constituents
{3} Organic compound (2 and 3 are total solid --- 50 gm /day)
A- Non protein nitrogenous constituents
B-Non nitrogenous constituents

Inorganic constituents: - 15 gm/day mainly Nacl

1- Chlorides: cl^-

-Chloride beside urea affects the specific gravity of urine.
-It present in urine in the form of Nacl. Daily output about 10-15 gm.
-Its amount decrease in excessive respiration, fever, fasting, diarrhea, and chronic nephritis.

2- Phosphate: - $PO4^{--}$

- Inorganic phosphate is about 96% of total phosphate in urine, while organic phosphate is 4%.
- Phosphates are held dissolved in solution in acid urine and p.pt in alkaline urine. The turbidity of urine in ammoniacal fermentation is due to triple phosphate.
- -There is 2 form in urine, monobasic phosphate NaH_2PO_4 and dibasic Na_2HPO_4. The ratio of these 2 salts determine pH of urine.

3-Bicarbonate:- HCO_3^-

Present in herbivorous urine and in small amount in carnivorous urine.

5- Sulfate SO_4 :-

From sulfur containing amino acids during its metabolism most of its sulfur is oxidized to sulfate.

Urinary sulfate includes:

a-Inorganic sulfate:- about 90% of total sulfate and is excreted with Na, K, and NH2 This sulfur is precipitated by Barium chloride.
b-Ethereal sulfate (conjugated):- conjugated mostly with phenol (detoxification of phenol in liver) and indoxyl sulfate and indican.
c- Neutral sulfate: - It includes cysteine, cystine (increase in cysteinuria) methionine , taurine, H_2S, thiocyanate(increased excretion in case of poisoning by cyanid & nitrile)
-It is constant and arises only from endogenous catabolism.

B; Cations:-

1- Sodium & potassium:- 2 gm/day

-Their amount in urine varies with Na intake, the ratio of K to Na is about 3:5
-Their excretion are regulated and controlled by the adrenal cortical hormones
. An excess loss of Nacl through urine occur in adrenocortical insufficiency occur in Addison's disease.

2- Calcium and Magnesium:- 1/2 gm/day

Their excretion take part in feces, urinary calcium is 0.2 gm, Mag. About 0.15 gm daily and depend on the intake in the diet.

{2} Organic constituents: 35 gm/day

1st) Non protein nitrogenous constituents: NPN

1) UREA: -

Urea is the main solids of urine, it represent about 85- 92% of total nitrogen in urine
-The amount of urinary urea is about 20- 40 gm/day
It is diuretic substance in urine.

-All urea in urine is of exogenous source.
-If urine is lifted for long time; ammoniacal odor is produce due to ammonia produced from decomposition of urea by bacteria.
Urea is synthesized in liver through ammonia detoxification (ammonia result from deamination of the amino acids & excreted by the kidney in the urine).
How to prove that urea is formed in liver not in kidney & excreted by the kidney?
* Nephrectomy in dog ⟶ urea increase in blood
* Hepatic-ectomy ⟶ urea decrease in blood
* Both Nephrectomy & Hepatic-ectomy ⟶ urea remain constant in blood.

Urea as kidney function test:
15 gm of urea are dissolved in 100 c. C water and taken per Os & let animal fast, then measure urea in urine: -
2.5 gm% or more ⟶ very good 2- 2.5 gm % ⟶ sufficient
Less than 2 gm % ⟶ insufficient Less than 1 gm% ⟶ poor.

2) Uric acid: -

1- Uric acid is the end product of purin catabolism
2- The level of uric acid in urine 1s 0.7 gm/day, one halve is of exogenous source & other halve is of endogenous source. This amount increase in case of leukemia (increase nuclear metabolism and uric acid excretion markedly increased) , gout and uricouric drugs as allopurinol and this amount decrease in case of chronic nephritis.
3- The uric acid is one of the normal reducing substance in urine.
3- In all domestic animals except man & Dalmatian dog the uric acid is oxidized into allantoin.

3) Creatine: -

Its amount is traces 60- 150 mg/day. Its inconstant constituent of urine. Formed by connection between glycin, methionin and argenine

4) Creatinine :

-It is the end product of creatine & creatine P.
Its amount in urine is constant whatever the volume of urine -in female 0.7- 1 gm/day.
- in male 1- 1.8 gm/day due to muscular bulk.
It is one of the normal reducing substance in urine
-It is mainly of endogenous source only.

Creatinine coefficient: - It is the number of mgs of creatinine excreted per kg body weight per day. In male its amount 20- 24 but in female 18- 22 mg/kg/day.
*Conditions in which creatinine in urine are; Muscle destruction, fever, after labor, hyperthyroidism, in children, D.M. and starvation.

5) Hippuric acid :- or benzoyl glycine.

1- Hippuric acid produced as result of detoxification of benzoic acid, which present in vegetable foods & canned food.
2- Its amount in urine is about 0.7 gm/day & vary according to precursor in food.
3- such conjugation process occurs mainly in liver.

6) Allantoin :-

In mammals other than man Apes it is the end product of purine metabolism.

7) Ammonia:-

1- The urinary ammonia is synthesized in the distal convoluted tubule by
a) Direct: (40%) from deamination of amino acids in the kidney
b) Indirect: - about (60%) produced by the action of glutaminase on the glutamine received by kidney from other tissues.
2- Its amount about 0.7 gm/day (0.5-0.8 gm/day), this amount
* Markedly increases in acidosis (up to 10 gm/day) and through hydrolysis by bacteria as in cystitis or sample without preservative.

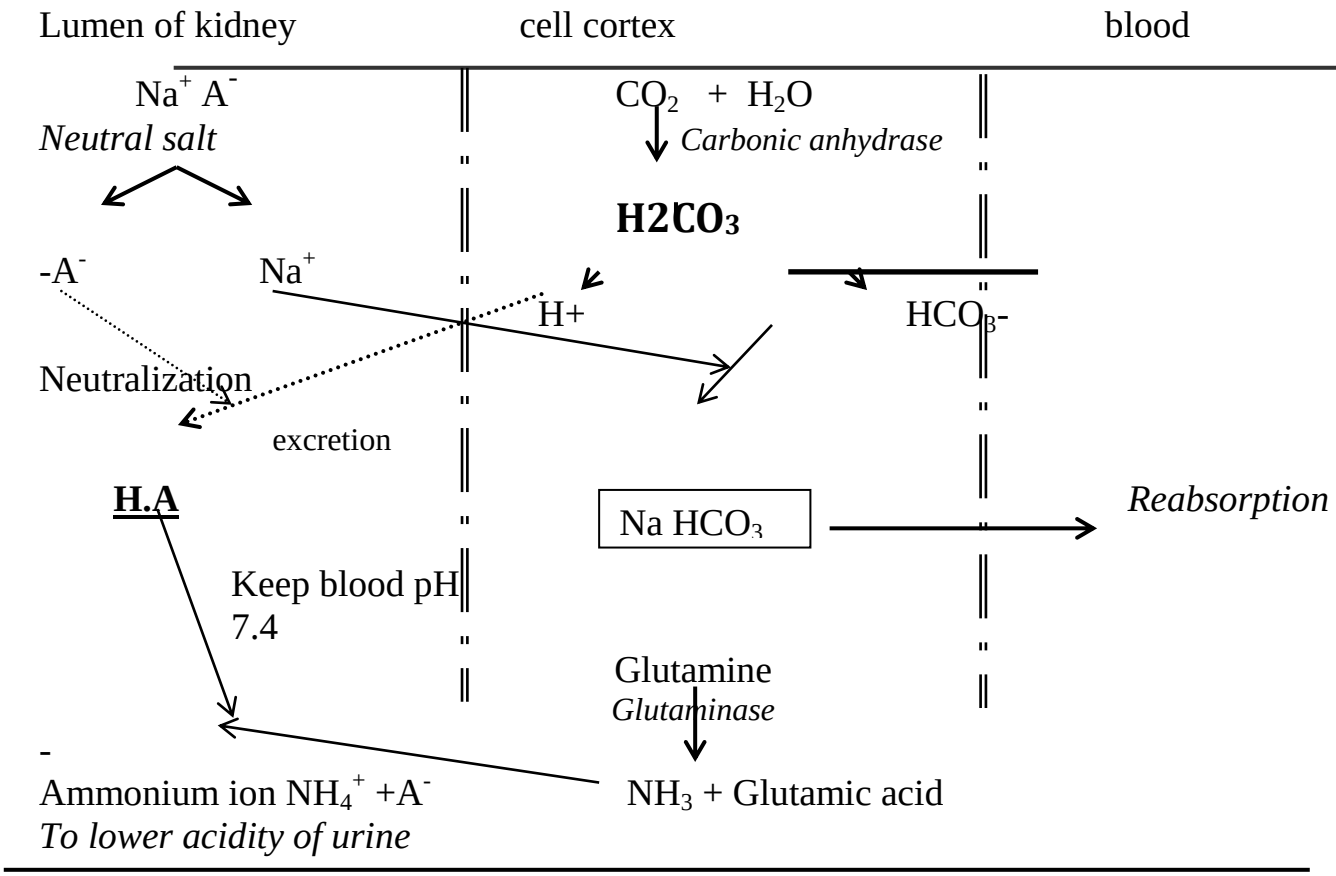
* Almost absent in alkalosis.
* It decrease in sever nephritis due to decrease capacity of kidney to form it.

3- Normally the urinary ammonia nitrogen is about 2.5 % to 4.5 % of the total nitrogen.

-Symptom of ammonia intoxication; include tremor, thirst, Slurring in speech, and high concentration lead to comma and death.

4- *Importance of urinary ammonia*
1- Conservation of sodium and potassium which are essential for important physiological activities.
2- Neutralization of high acidity of urine about 75% of H^+ excreted in urine as ammonium.
4- Help in keeping pH of blood within 7.4.

Buffer action of ammonia: -



8- Indican:

-It is the salt K-indoxyl sulfate, secreted normally in trace amount 4-20 mg/day.
-Its amount increase after excess putrefaction and constipation.

-Indican is derived from indol which inturne arises from the action of putrefied bacteria on tryptophan or protein containing it, this occur in large intestine.
When indol is absorbed it undergoes a series of detoxification in liver & indoxyl is formed, which conjugated with sulfate and neutralized to yield salt.

9-Amino acid: - certain amino acid present in small amount

B- Non nitrogenous Organic constituents:

1- Carbohydrate:-

1-Small amount of glucose undetectable by the ordinary test may be present
2-Arabinose may appear in the urine (non threshold) after eating large quantities of pentose rich foods(alimentary pentosuria)
3-Lactose may be present in urine of high lactating cow

2- Organic acid:-

1-oxalic acid -from food
2-volatile fatty acid
3-aromatic hydroxy acid
4-Glucuronic acid-it found in urine as glucuronate or glucuronides.coniugated with other compounds (putrefaction conjugation) there are 2 type of conjugated glucuronate
a-The glycoside type involve –OH group of aliphatic or aromatic alcohol (phenol naphthol)
b-While the ester type involve reaction with-COOH group of compound as benzoic acid phevylacitic
5-Lactic acid :it is the end product of glycolysis. urine is a way of disposal of lactic acid
6-Keton bodies
7-Six member of citric acid cycle, -citric, aconitic α- ketoglutaric -succinic-fumaric.

Abnormal constituents of urine: -

1-Sugar----- glucosuria
2-albumin ---------albuminuria
3-keton bodies -----ketonuria
4-lipids----- chyluria
5-blood ----- haematuria-Hb-uria
6-Indican-Indicanuria
7-bile uria

1}-Reducing sugars (usually glucose)

-Glycosuria: -

Means the presence of any kind of sugar in urine and include:-

Glucose- glucosuria -(Renal glucosuria-D.M) -(differentiate by-blood glucose)
-Fructose fructosuria-
-Galactose Galactosuria--- after ingestion of large amount of vegetable or fruits
-pentose pentosuria- Essential pentosuria
-Lactose (lactosuria) -in lactating female

1} Glucosuria

Causes:-

A-physiological causes:

1-Alimentary:- due to high carbohydrate intake
2-Emotional:- due to high level of blood adrenaline (fear)
3-(pregnancy and- lactation)-decrease carbohydrate tolerance

B-Pathological causes:-

Glucosuria with hyperglycaemia:-

1-diabetes mallitus-deficiency of insulin

2-hyperthyroidism 3-hyperpituitrism 4-hyperadrenal corticism

Glucosuria with normoglycemic cause:-
1-Renal diabetes, congenital condition where the renal threshold low about 160 mg %
2-Nephritis & nephrosis

C-Experimental glucosuria :-

1- Surgical pancreatectomy
2- Injection of phlorizine-which inhibit phosphorylation of glucose in renal tubule so inhibit its reabsorption
3-Injection of alloxan which cause destruction of β cell of langerhans of pancreas.

2} Proteinuria:-

-Types of protein in urine: -
1-albuminuria
2- Globulinuria (bence jones protein) as in case of multiple myeloma – leukemia.
It can be detected as follow;-globulin p.pt in warming to 50 : 60 ċ &dissolved at 100c then reprecipitated by cooling
3- Proteoses & peptones 4-nucleoprotein

-Albuminuria (proteinuria)

Causes:-
1-Physiological cause
1-High protein diet 2-Sever muscular excersise
3-Long standing 4-Common cold
5-Pregnancy 6-Normally proteinuria occur in horse

2-Pathological causes:-
a- Prerenal (no primary disease in kidney) as in:-
1- Heart disease (affect filtration)
2- Liver disease- so the deamination , transamination decrease & A.As increase
3- Hypertension 4- Drugs & chemical poisoning
5- Bence jones proteins (protienuria)
6- Haemogloin - present in plasma in case of hemolysis (protienuria)

b-Renal (primary disease of kidney)
Nephritis & Nephrosis (increase permeability or glomeruli)
c-Post renal: - (false)
Due to suppurative inflammation or tumor in urinary tract below the kidney (urethra and -urinary bladder)
-Because pus is protein in nature it give false result

3- Ketonuria (acetonuria):-

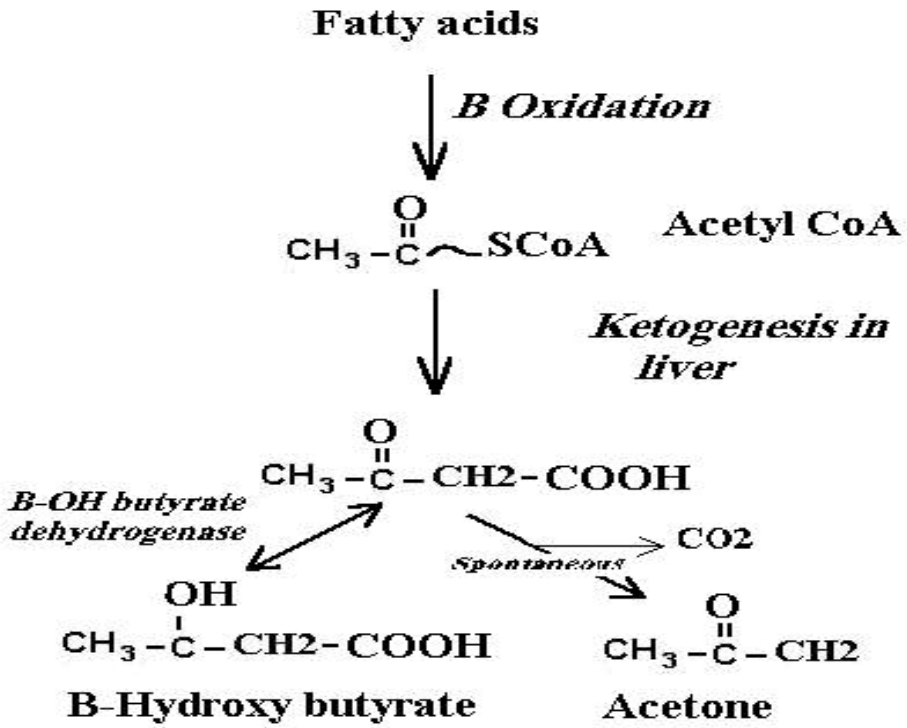
Ketone bodies are acetone, aceto acetate & B hydroxybutyrate, synthesized in liver from active acetate & catabolized in extrahepatic tissue.

-If ketone bodies increase in blood than 1.2 mg% -leads to ketonemia & excreted in urine called ketonuria (more than 0.1 gm/24 hours)
-ketoses means the presence of both-(ketonauria & ketonuria).

-Cause of ketosis:-

1-diabetes mellitus 2-Starvation
3-In high lactating animal 4-Low carbohydrate & high fat diet
5-Pregnancy toxemia in sheep.

Detected by Rothera s nitroprusside test:-



4} -Blood in urine:-

-A- Haemoglobinuria:

Causes as in
1-blood parasite (+) thieleria 2-hypophosphatemia
Urine is transparent red due to presence of Hb (renal threshold 150 mg%)

-B- Haematuria:-

Causes as in
1-tumor in urinary trait or kidney 2-Belhariziasis
3-physical injuries (as catheter) 4-urinary calculi
-presence of intact R B Cs in urine (has characteristic smoky appearance always associated with albuminuria)

Differentiation between Haemoglobinuria and haematuria:

Centrifugation of urine sample . then microscopical examination for sediment .
if R B Cs present indicate haematuria
Not present indicate, haemoglobinuria
N.B: in ammniacal urine Hb - hamaturia are mistaken
-blood detected in urine by Benzidine test:-

principals:-the Hb play peroxidase like activity which split H2O2 into H2 O &(o) nascent oxygen which oxidize benzidine according to amount of blood

5-Indican uria:-

Principals: - this test depends on that when urine mixed with conc . HCL the indoxyl is liberated from indican then oxidized into indigo blue pigment or dye.

6-Bileuria:-

Constituents of the bile are found in urine in bile du to obstructive hepatitis (yellowish green, when shaken froth readily)

1- Bile salts:-

- formation:- it formed in liver include: Na or K glycocholate or taurocholate .

-Glycine (non essential A.A)

-Taurin - formed from non-essential A.As cysteine |

Cholesterol in liver give cholic acid which react with CoASH yield cholyl CoA

Cholyl CoA + Glycine ⟶ Glycocholate + Na or k ↘
Na or K Glycocholate

Cholyl CoA + Taurin ⟶ Taurocholate . + Na or K ↘
Na or K Taurocholate

-Bile salt decreases the surface tension as soap and detected by hey sulfur test. bile salts present in urine in case of obstructive jaundice

2-Bile pigments:-

Degradation of haem normally formation of bilirubin

Bilirubin is produced from biliverdin in catabolism of the heme of hemoglobin. These reactions, which occur in the spleen, yield bilirubin, which is quite insoluble in water, and must be removed by several organ systems.

First, bilirubin complexes with serum albumin for transport to the liver. There, it is solubilized by conjugation with two molecules of glucuronic acid.

This solubilized compound, bilirubin diglucuronide, is secreted into the bile and ultimately excreted via the intestine. Defects in metabolizing bilirubin properly give rise to jaundice.

The basic pathway of heme breakdown is the following:

Heme -> Biliverdin -> Bilirubin -> (passage through blood to liver as bilirubin-albumin complex) -> Bilirubin Diglucuronide -> excretion.

-*Differentiation between plasma bilirubin (uncongugoted) bile bilirubin (conjugated)*:-

1-Plasma bilirubin cannot pass through urine (because of its large size of molecules they are water insoluble) but bile bilirubin can pass through glomerulus so appear in urine.

2-van den bergh reaction (on serum)

-*Direct*:- give color within one minute in case of bile bilirubin - obstructive jaundice (steatarrhes-clay)

-*Indirect reaction* - produce only after addition of alcohol for making plasma bilirubine is water soluble in case of haemolytic jaundice

III) Microscopical Exam of urinary Sediment :-

1- General Consideration :-

1- Micro-Exam of urine is of great importance and should never be omitted

2-Important Structures to identify include casts .RBCs WBCs & bacteria-Disregard object that have no significance as urine may be contaminated by some debris

3-If the urine sample is small; it should be centrifuged at low speed for 3 min

First to collect adequate amount of sediment & the supernatant used for chemical test

4-Examination ,on fresh Specimens because casts & RBCs disappear upon standing

2)-Method:-

-Agitate urine to suspend any sediment -fill centrifuge tub with urine
-centrifuge it for 3 min - pour most urine out of the tub
-Mix urine with sediment -pour a drop on glass slide & cover with clean cover glass
-Examine under Microscope with L P then H p to identify smaller object
-Finding reported as average number per L P or H P or few-many -abundant
-The constituent of urinary Sediment divided into:-

a:-Organized sediment:-

1-Epithelial cells

i-Types a-squamous epithelial cells
1-The largest of cells
2-Have irregular outline contain small nucleus
3-Derived from the superficial layer of urethra & vagina
b-Transitional epithelial cells:-
-various forms include round oval -Intermediate in size
-Derived from urethra bladder ureter
3-Renal epithelial cells:- small round cells
-usually can be identified when renal cells incorporated in epithelial cast

ii-Significance:-

1-a certain number of epithelial cells in urine is normal
2- greatly increased in No in pathological condition
-Renal cells present in case of interstitial nephritis
-Transitional cells increase in cystitis

2-Erythrocytes:-

Normally few small amount present increase in different renal diseases
-indicate haemorrhage somewhere in genitourinary tract- -haematuria

-3-Pus cells or WBCs:-

Appear as granular cells –a few cells may present normally
If increase indicate, infection in genitourinary tract (nephritis - cystitis -urethritis)

4-Castes

a-formation:-

-Casts are cylindrical bodies as their shape represent an actual cast of the tubular lumen
-They formed in distal &collecting tubules of kidney where urine reach maximum concentration & acidity favoring protein p. ptation
-When cells & cellular debris are present in the tubules they included in the hyaline matrix at the time of its formation as casts , giving a variety of types

b-types of casts:-

1-Hyaline cast:

1-Made of protein alone
2-colourless, homegeneous, semitransparent, usually rounded ends
3-soluble in alkaline urine (uncommon in large animals)
4-seen only in a well-darkened field

2-Granular cast:-

Hyaline casts containing fine or coarse granules derived from disintegrate of tubular epithelial cells
Significance: of renal changes; a-renal irritation ,b- renal inflammation , c-renal degeneration

Granular cast - indicate more severe type of renal disease than the hyaline cast , for it represents the disintegration of renal tubular epithelium

3-Epithelial cast:-

-formed from desquamated cells from the epithelial lining of the renal tubule
-often appears as 2 rows of epithelial cells, large nucleoli
significance:- indicate a cut nephritis-degeneration of tubular epithelium

4-Waxy cast:-

yellow or gray in color , broader than hyaline casts
significance:-advanced sever nephritis

5-Fatty cast:-

-contain numerous fat globules that are very refractive
-colorless, or will stain or any to red with Sudan 1 stain
sigin: Degenerative tubular disease occasionally seen in cats with renal disease

6-Erythrocyte cast:-

found after long retention of large numbers of R B Cs in renal tubules

7-Leucocytic cast:-

W B Cs agglomerated into casts -significant pyelonephritis
5-animal parasite e.g. Egyptian bilhuriziasis
6-spermatozoa
7-urethral filament

b-unorganized sediment:-

1-*The more common are:-*

-calcium phosphate - found in alkaline urine
-calcium oxalate - found in acid urine & neutral triph phosphate
uric acid & sodium urate, ammonium urates - found in acid urine
2-the less common are :- ca-carbonate & tyrosine are only excreted in urine in the presenc of sever hepatic parenchymal damage whereas cystine is excreted in cystinuria.

Tumor marker

Def. Several biochemical tests are useful, either as primary tumor markers or as secondary tests for invasion or metastases or cancer.

Classification of tumour markers:

1. Proteins: a. Enzymes b. Hormones c. Other proteins

i. Monoclonal Immunoglobulins

ii. β2-microglobulin

iii. C-peptide

iv. Hormone receptors (estrogen and progesterone receptors)

2. Oncofoetal antigens

3. Carbohydrate markers

4. Genetic markers a. Oncogenes b. Tumour suppressor genes

5. Newer —markers‖ a. Microarray b. Proteomics c. Cell free RNA

Proteins

A. Enzymes

i. Alkaline phosphatase

 Useful in assessment of bone or liver metastases.

 Correlates better with extent of liver metastases than other liver enzymes.

 Elevated GGT confirms liver origin.

 Placental ALP elevated in malignancies such as ovarian, lung and GIT cancers.

ii. Lactate Dehydrogenase

 Glycolytic enzyme released following cell damage.

 Usually tumours that primarily utilise glycolysis rather than aerobic respiration for their energy requirements. Elevated with many large tumours, including breast, colon and stomach tumours; also in lymphoma, leukaemia and neuroblastoma.

 Levels correlate well with tumour burden.

iii. Creatine kinase

 Occasionally elevated in tumours such as prostate, prostate and small cell carcinoma of the lung.

 Usually BB iso-enzyme. May cause false elevation in CK-MB level in certain CK-MB assays.

iv. Neuron specific enolase

 Glycolytic enzyme found in neuronal tissue and neuroendocrine system. Elevated in small cell carcinoma of the lung, neuroblastoma, phaechromocytoma, carcinoid, medullary carcinoma of the thyroid and pancreatic endocrine tumours. Associated with poor prognosis.

Markers of prostate cancer

vii. Prostatic acid phosphatase

 Tartrate sensitive acid phosphatase produced by epithelial cells of prostate gland.

 No longer in routine use, replaced by PSA.

 Clinical use restricted to confirmation of metastatic prostate cancer and prostate cancer staging.

viii. Prostate Specific Antigen (PSA)

 Sensitive and stable

 Expressed by normal, benign, hyperplastic and malignant prostatic tissue.

 Useful in detection, staging and monitoring of prostate cancer.

 Not specific for prostate cancer. Also elevated in benign conditions such as:

Prostatitis – returns to normal within 6 weeks, urethral catheterisation, acute urinary retention.

B. Hormones

Can be elevated either due to:

• Overproduction by endocrine tissue

• Ectopic production (non-endocrine tissue)

Thus elevation of a specific hormone is not specific for a particular tumor.

Hormones used as tumour markers include:

1. ACTH

• Produced by corticotrophic cells of anterior pituitary.

• Can be pituitary or ectopic production. Level >200ug/L suggestive of ectopic production.

• Usually small cell carcinoma of lung (also pancreatic, beast, stomach and colon cancer).

2. Calcitonin

• Produced by C-cells of thyroid. Marker for medullary carcinoma of thyroid.

• Valuable in screening, diagnosis, prognosticating and monitoring treatment

• Correlates with tumour volume, invasion and metastases.

3. ADH: small cell carcinoma lung; adrenal cortex, pancreatic and duodenal cancer

4. Gastrin: gastrinoma

5. Growth Hormone: pituitary adenoma; renal, lung cancer

6. Human placental: trophoblastic, gonads, lung, breast cancer

7. PTHrP: breast, lung cancer.

C. Other proteins

i. Paraproteins – see protein notes

ii. β2 microglobulin – see protein notes

iii. C-peptide – useful in insulinomas

Oncofoetal Antigens

Produced normally in foetal life. High concentrations in foetal serum, decreasing to low levels or disappearing after birth.

i. α –Foetoprotein (AFP)

• Synthesized by foetal liver; predominant plasma protein in early foetal life

• Elevated in neural tube defects (maternal serum and amniotic fluid). Protein crosses from foetal plasma into amniotic fluid via defect.

• Mild elevations (<200ug/L) in benign liver conditions such as hepatitis (recovery) and cirrhosis. Very high levels in primary hepatoma

• Used to detect, prognosticate and monitor treatment in 50% of hepatocellular carcinomas.

ii. Carcinoembryonic antigen (CEA)

• Part of large family of relate glycoproteins

• Elevated in various cancers incl. 70% of colorectal cancers, stomach (50%), pancreas (50%) cancers,

• Not sensitive screening test, useful in clinical staging prognosticating and monitoring therapeutic response.

Carbohydrate antigen (ca) markers

High molecular weight mucins / blood group antigens found on tumour cell surface or secreted by tumour cells. Useful as tumour markers, more specific than naturally secreted markers such as enzymes and hormones.

i. CA 15-3: Marker for **breast** carcinoma; also elevated in other malignancies such as pancreatic, lung, ovarian, colorectal and liver cancer.

ii. CA 125: Glycoprotein, mw >200kDa. Marker for **ovarian** cancer.

iv. **CA 19-9:**. Marker for **adenocarcinoma of pancreas** (↑ in 80% of cases), but rise is too late to be useful in early detection.

<u>Newer "Tumour Markers"</u>

Microarrays (DNA "chip"):

Allows for the rapid identification of sequence polymorphisms and mutations, and quantification of gene expression in thousands of genes simultaneously (using mRNA). Expression patterns vary between healthy and diseased tissue.

Proteomics (protein "chip"):

Is the study of the protein expression (vs mRNA expression in DNA microarrays) in a given cell or tissue at a given time. Cells, healthy or diseased, have specific protein signatures.

Cell-free nucleic acids: Increased circulating levels of tumor-derived cell free nucleic acids (DNA or RNA) have been detected in the serum / plasma of patients with malignancies.

The body's response to malignancy

1. The "acute phase response" occurs in malignancy. The changes are cytokine-mediated and are nonspecific: same as in any chronic inflammatory process.

 ↑ erythrocyte sedimentation rate (ESR)

 ↑ CRP, haptoglobin, α1-antitrypsin, fibrinogen, ferritin, ceruloplasmin

2. Cachexia: Wasting and ↓ albumin:

 ↓ intake of food (anorexia, vomiting, depression)

 Catabolic state: probably mediated by cytokines especially tumour necrosis factor (TNF)

3. Tumour lysis syndrome.

<u>Acute tumour lysis syndrome</u>

Features:

• Hyperkalemia: 12-24h after chemotherapy

• Hyperuricemia: 2 - 4 days after chemotherapy

• Hyperphosphatemia: 2 - 4 days

• Hypocalcemia: 2 - 4 days

• Complications and Management:

Hyperkalemia → cardiac arrhythmias. Treat with:

• K-losing (loop) diuretic + fluids

• Glucose + insulin

• Dialysis

Hyperphosphatemia and hypocalcemia → metastatic calcification, nephrocalcinosis, renal failure:

• Fluids + loop diuretic

• Calcium gluconate I.V. infusion if tetany or convulsions.

• **Hyperuricemia** → renal failure: acute urate nephropathy due to urate crystal deposition in renal tubules, and acute gout.

• ↑ hydration & alkalinize urine (sodium bicarbonate), if no hyperphosphatemia is present.

• Allopurinol (xanthine oxidase inhibitor).