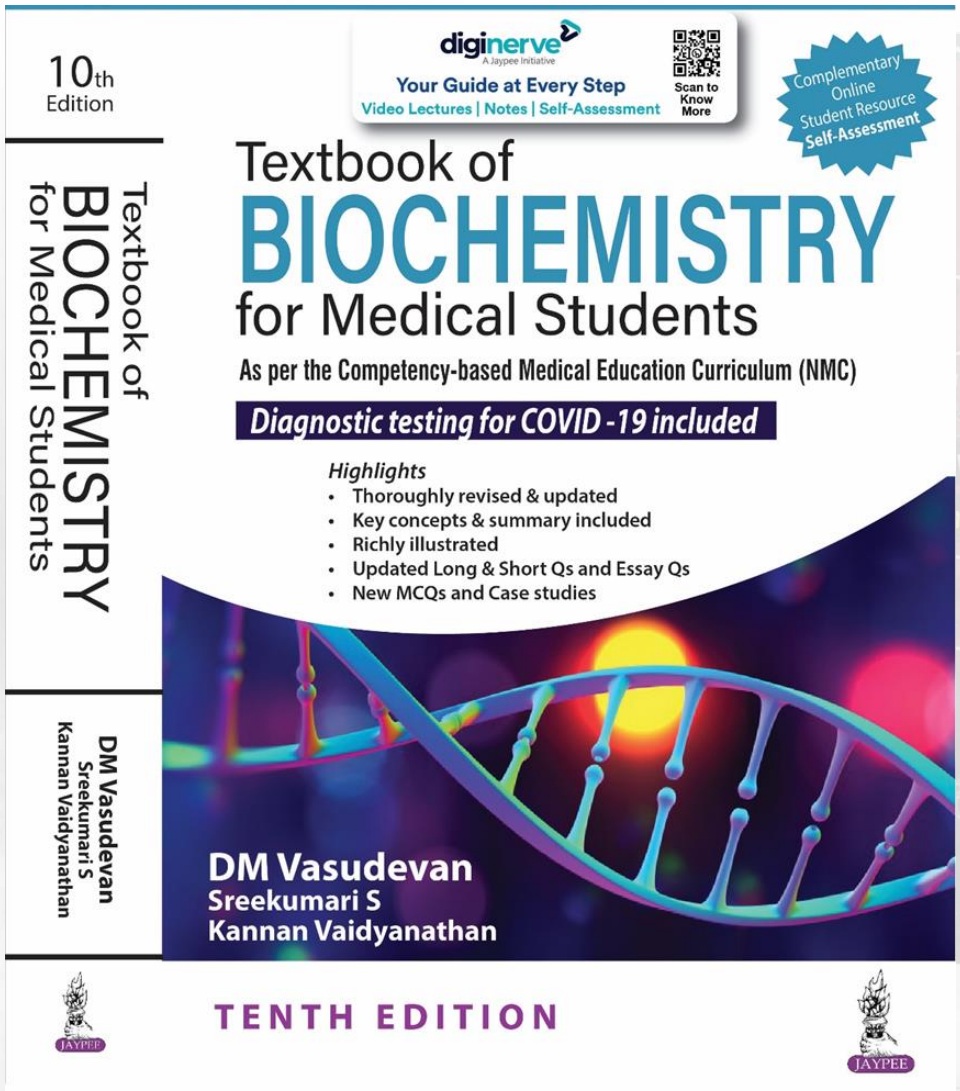


Chapter 30:

Mineral Metabolism. (Calcium, Phosphorus, Sulphur, Iron, Copper, Zinc, Selenium, Manganese, Lithium).

Textbook of
BIOCHEMISTRY
for Medical Students

By DM Vasudevan, *et al.*

TENTH EDITION

Important Minerals

Major elements (Essential)	Trace elements (Essential)
1. Calcium	1. Iron
2. Magnesium	2. Iodine
3. Phosphorus	3. Copper
4. Sodium	4. Manganese
5. Potassium	5. Zinc
6. Chloride	6. Molybdenum
7. Sulphur.	7. Selenium
	8. Fluoride.

Important Minerals

Minerals, (Necessary, but function unknown)	Minerals, (Nonessential, but present in tissues)	Minerals, (Toxic)
• Chromium • Nickel • Bromine • Lithium • Barium	• Rubidium • Silver • Gold • Bismuth	• Aluminium • Lead • Cadmium • Mercury

Calcium (Ca^{++})

Total calcium in the human body is about 1 to 1.5 kg, 99% of which is seen in bone and 1% in extracellular fluid.

Sources of Calcium

Milk is a good source for calcium. Calcium content of cow's milk is about 100 mg/100 mL. Egg, fish and vegetables are medium sources for calcium. Cereals (wheat, rice) contain only small amount of calcium. But cereals are the staple diet in India. Therefore, cereals form the major source of

calcium in Indian diet.

Daily Requirement of Calcium

An adult needs **1000 mg** per day and a child about 1200 mg/day. Requirement may be increased to 1500 mg/day during pregnancy and lactation. After the age of 50, there is a general tendency for osteoporosis, which may be prevented by increased calcium (1500 mg/day) plus vitamin D (20 μg/day).

Mechanism of Absorption of Calcium

Absorption is taking place from the first and second part of **duodenum**. Calcium is absorbed against a concentration gradient and requires energy. Absorption requires a carrier protein, helped by calcium-dependent ATPase. Out of the 1000 mg of calcium taken orally per day, 800 mg is excreted in stool and 200 mg is excreted through urine.

Factors Causing Increased Absorption

Vitamin D: Calcitriol induces the synthesis of the carrier protein **(Calbindin)** in the intestinal epithelial cells, and so facilitates the absorption of calcium.

Parathyroid hormone increases calcium transport from the intestinal cells.

Acidity favors the calcium absorption.

Amino acids: Lysine and arginine increase the calcium absorption.

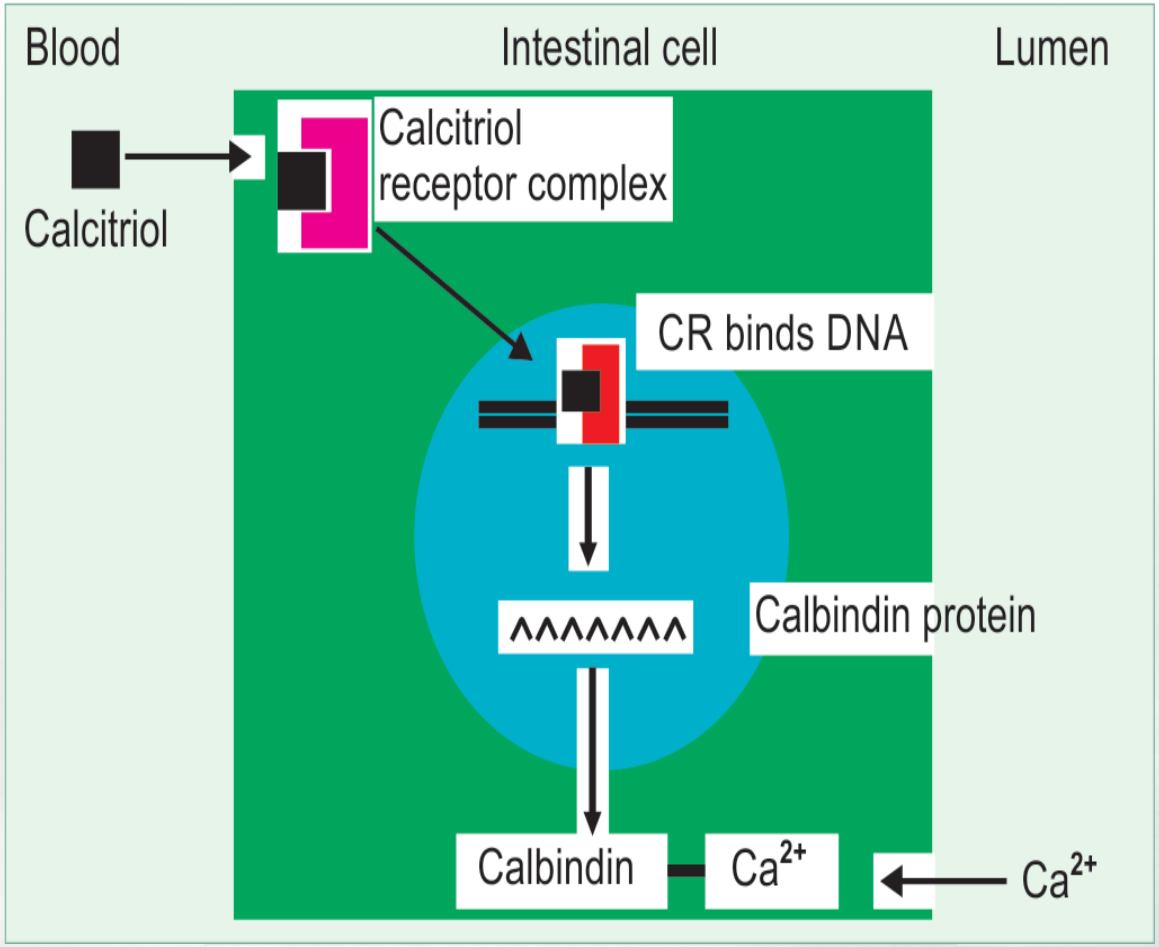
Factors Causing Decreased Absorption

Phytic acid: Hexaphosphate of inositol is present in cereals.

Oxalates: present in leafy vegetables, which cause formation of insoluble calcium oxalates.

Malabsorption syndromes: Here fatty acid is not absorbed, causing formation of insoluble calcium salt of fatty acid.

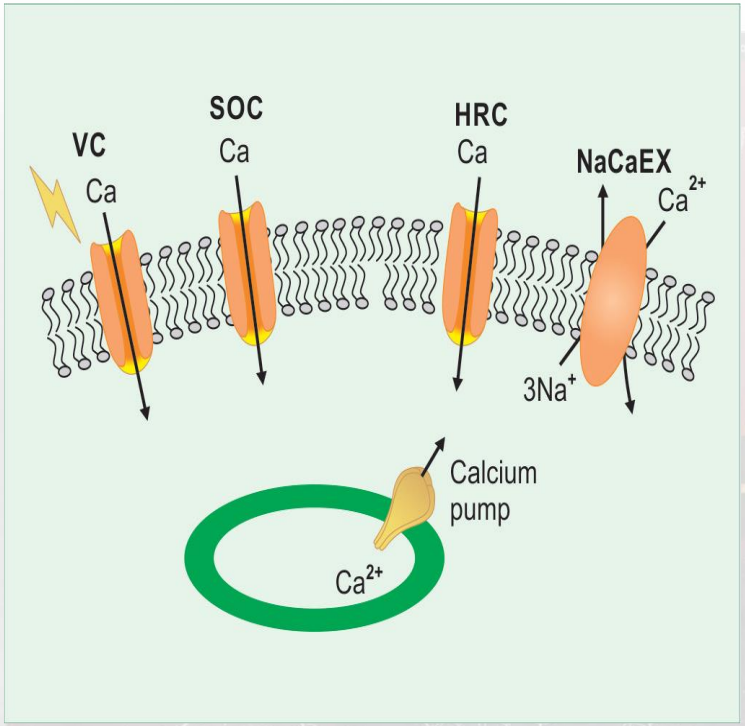
Phosphate: High phosphate content will cause precipitation as calcium phosphate. The optimum ratio of calcium to phosphorus which allows maximum absorption is 1:2–2:1. Milk contains this optimum ratio.



Calcitriol increases calcium absorption.
(C: calcitriol; CR: calcitriol receptor complex).

Calcium in Cells

- Calcium is mainly extracellular. The cell membrane is generally impermeable to calcium ions.
- Calcium influx into the cell is by Na+/Ca++ exchange mechanism. This mechanism is rapid, but has low affinity for calcium (NaCaEx).
- Entry of Ca++ into mitochondria is by a calcium uniport system.
- But calcium ions exit by a Na+-Ca++ antiport system, which in turn is dependent on the Na+-H+-ATPase pump, this is called calcium pump.
- Other calcium channels are voltage operated channels (VC) and second messenger operated channels (SOC).



Regulated calcium channels of different types. (VC: voltage operated channels, which are activated by membrane depolarization; SOC: second messenger operated channels, which are activated by inositol phosphate, cyclic nucleotides or diacylglycerol; HRC: hormone receptor operated channels, these are activated by hormones or neurotransmitters; NaCaEx: sodium-calcium exchanger. Moreover, calcium pump will remove calcium from subcellular organelles to cycosol of the cell).

Functions of Calcium

Activation of Enzymes

Calmodulin can bind with 4 calcium ions. Calcium binding leads to activation of enzymes. Calmodulin is part of various regulatory **kinases.**

Some other enzymes are activated directly by Ca++ without the intervention of calmodulin.

Muscles

Calcium mediates **excitation and contraction** of muscle fibers. Upon getting the neural signal, calcium is released from sarcoplasmic reticulum. Calcium activates ATPase; increases action of actin and myosin and facilitates excitation-contraction coupling. The trigger of muscle contraction is the interaction of calcium with troponin C. Calcium decreases neuromuscular irritability.

Nerve Conduction

Calcium is necessary for transmission of **nerve** impulses from presynaptic to postsynaptic region.

Functions of Calcium, Continued

Secretion of Hormones

Calcium mediates secretion of insulin, parathyroid hormone, calcitonin, vasopressin, etc. from the cells.

Second Messenger

Calcium and cyclic AMP are second messengers of different hormones. One example is glucagon.

Vascular Permeability

Calcium decreases the passage of serum through capillaries. Thus, calcium is clinically used to reduce allergic exudates.

Coagulation

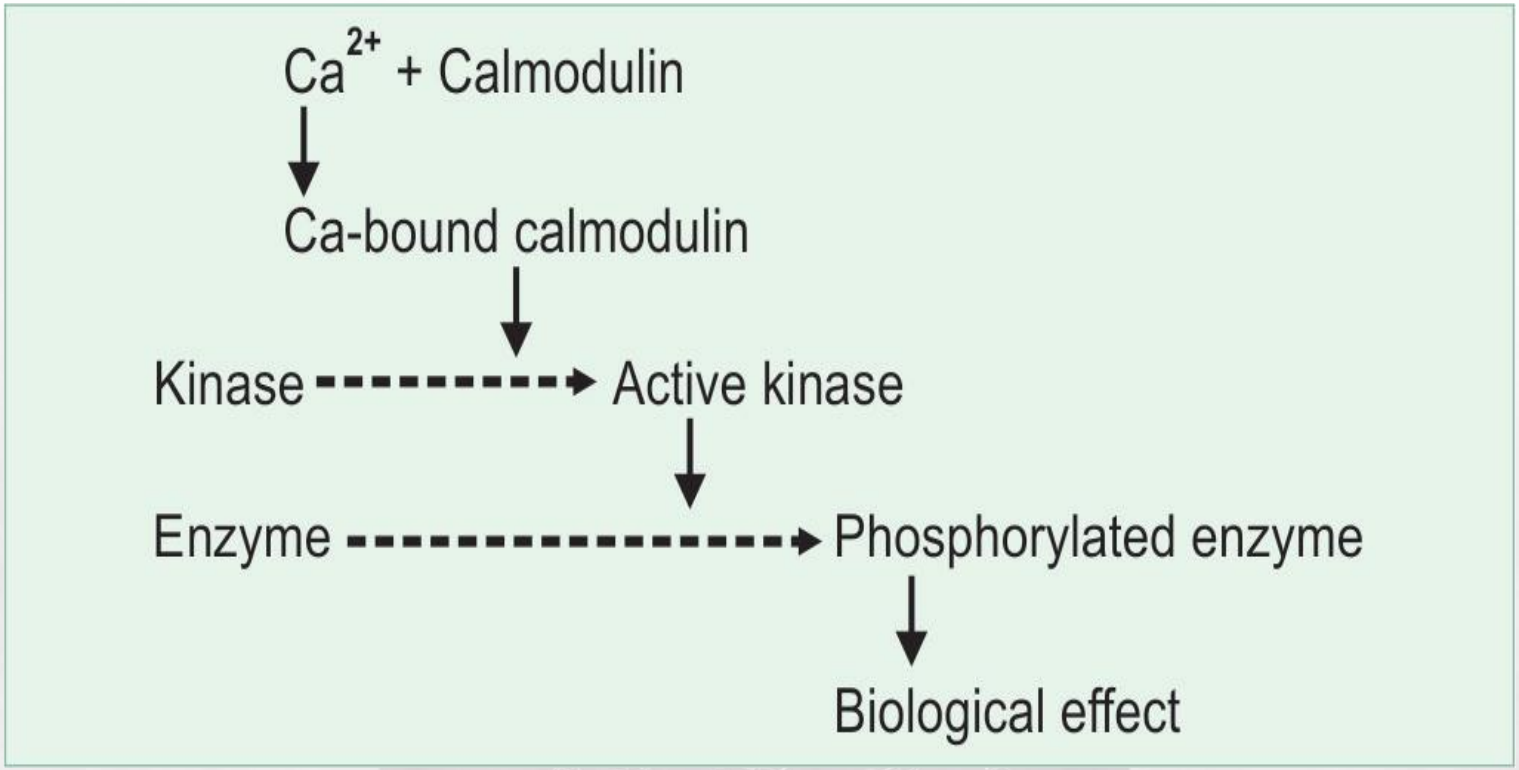
Calcium is known as factor IV in blood coagulation cascade.

Myocardium

Ca++ **prolongs systole**. In hypercalcemia, cardiac arrest is seen in systole.

Bone and Teeth

The bulk quantity of calcium is used for bone and teeth formation.



Mechanism of action of calmodulin.

Selected List of Enzymes Activated by Ca++ and Mediated by Calmodulin

Adenyl cyclase
Ca++ dependent protein kinases
Ca++ -Mg++ -ATPase
Glycerol-3-phosphate dehydrogenase
Glycogen synthase
Myosin kinase
Phospholipase C
Phosphorylase kinase
Pyruvate carboxylase
Pyruvate dehydrogenase
Pyruvate kinase

Calcium in Blood

Normal blood calcium level is **8.5 to 10.5 mg/dL** (10 mg/dL of Ca++ = 5 mEq/L). About 5 mg/dL of calcium is in **ionized** form and is metabolically active. Another 1 mg/ dL is complexed with phosphate, bicarbonate and citrate. These two forms are diffusible from blood to tissues.

About 4 mg/dL of calcium is **bound to proteins** in blood and is nondiffusible.

Homeostasis of Blood Calcium Level

There are effective controls to maintain this narrow range of blood calcium (**8.5 – 10.5 mg/dL**).

Vitamin D

Cholecalciferol is synthesized from 7-dehydro-cholesterol in skin under the influence of sunlight. It is then hydroxylated at 25th position in liver and further hydroxylated at the 1st position in kidney. The active derivative is called dihydroxycholecalciferol or **calcitriol.**

Vitamin D and Absorption of Calcium

Calcitriol promotes the absorption of calcium and phosphorus from the intestine. **Calcitriol** enters the intestinal cell and binds to a cytoplasmic receptor. The hormone-receptor complex interacts with DNA and causes **derepression** and consequent transcription of specific genes that code for calbindin. Due to the increased availability of calcium binding protein, the absorption of calcium is increased.

Vitamin D, continued

Vitamin D and Bone

Vitamin D is acting independently on bone. Vitamin D increases the number and activity of **osteoblasts**, the bone forming cells. Calcitriol stimulates osteoblasts to secrete **alkaline phosphatase**. Due to this enzyme, the local concentration of phosphate is increased. The ionic product of calcium and phosphorus increases, leading to mineralization.

Vitamin D and Renal Tubules

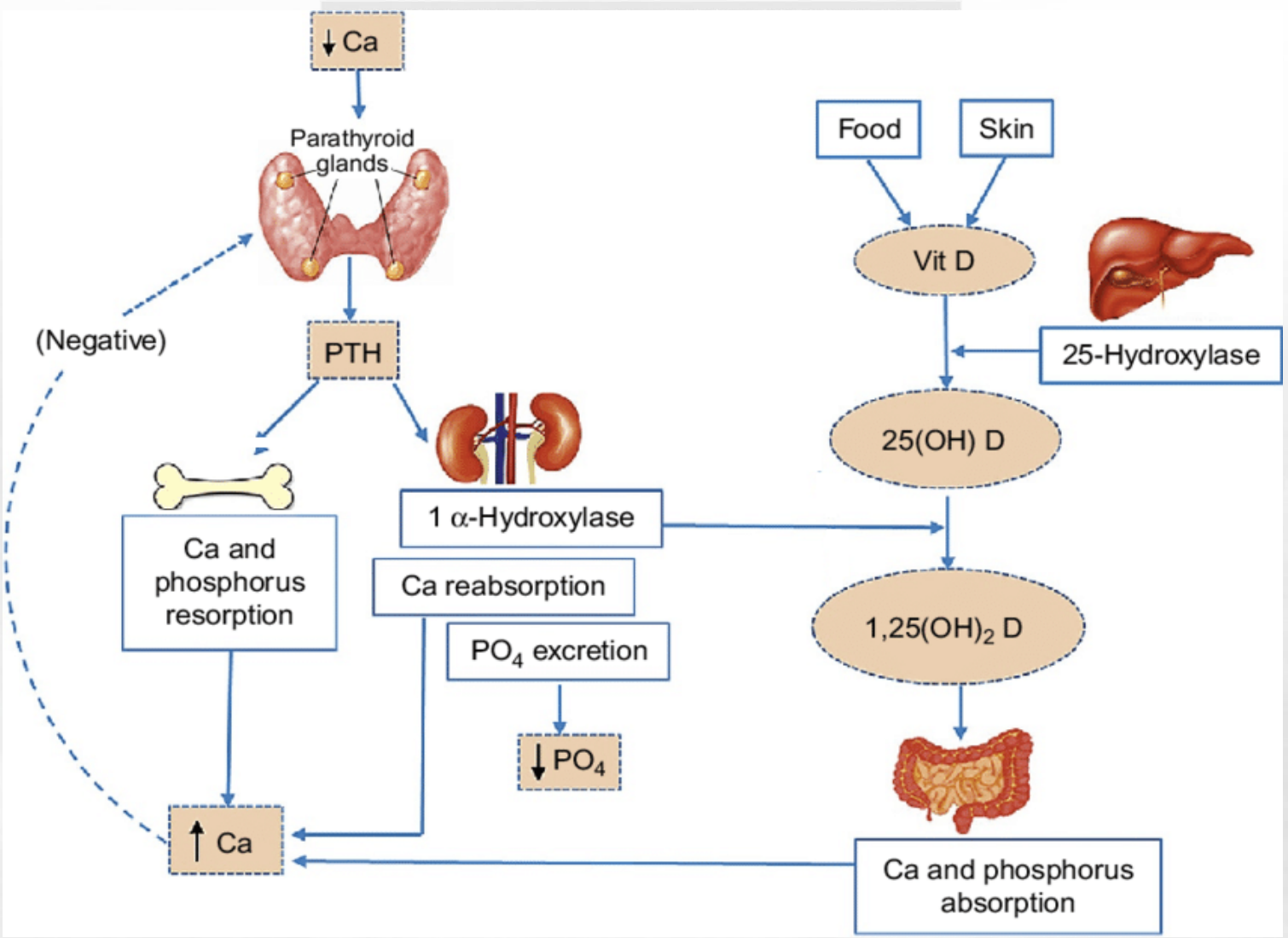
Calcitriol increases the reabsorption of calcium and phosphorus by renal tubules, therefore, both minerals are conserved (PTH conserves only calcium).

Calcitonin and calcitriol are different

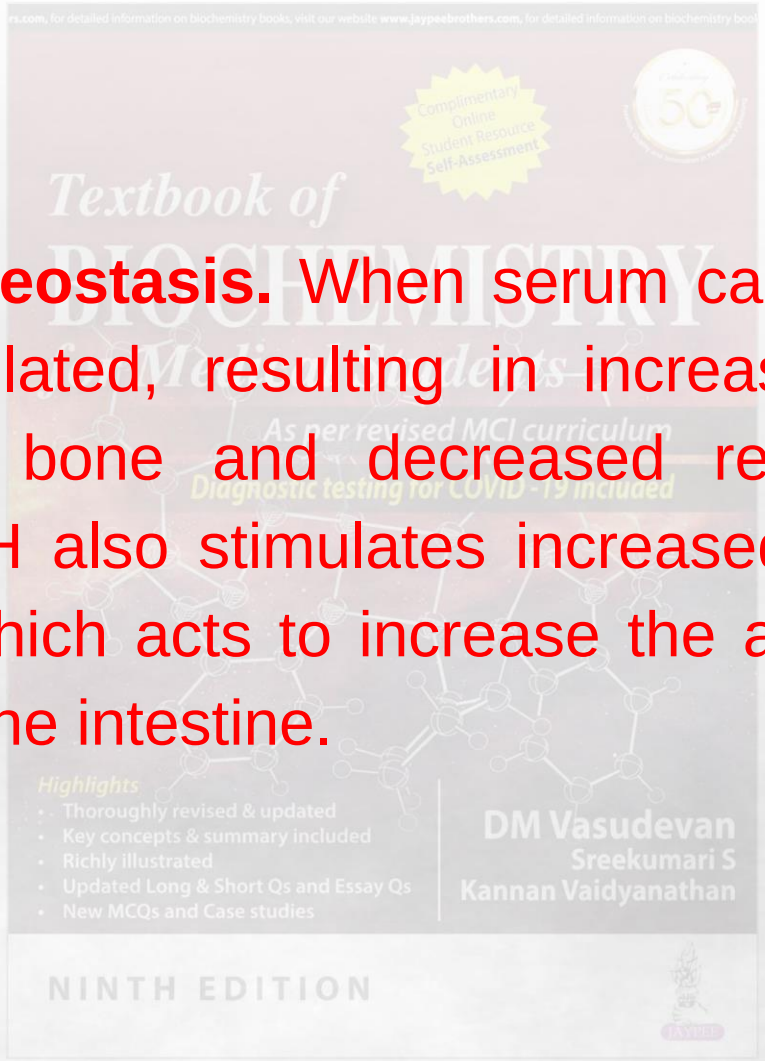
Calcitonin is the peptide hormone released from thyroid gland. It decreases blood calcium level.

Calcitriol is the active form of vitamin D. It increases the blood calcium.

Calcium Homeostasis



Calcium Homeostasis



Calcium homeostasis. When serum calcium is low, PTH is stimulated, resulting in increased calcium release from bone and decreased renal calcium excretion. PTH also stimulates increased production of calcitriol, which acts to increase the absorption of calcium from the intestine.

Parathyroid Hormone

The first 35 amino acids of PTH are biologically active. Control of release of the hormone is by negative feedback by the ionized calcium in serum. The release of hormone is mediated by cyclic AMP. The normal PTH level in serum is 10–60 ng/L. In primary hyperparathyroidism, this is increased to 100 ng/L.

Mechanism of Action of Parathyroid Hormone

PTH activates **adenylyl cyclase** with consequent increase in intracellular calcium concentration. A kinase is activated and enzyme systems are activated.

PTH and bones

In the bone, PTH causes demineralization**.** The numbers of osteoclasts are also increased. Osteoclasts release lactate which solubilizes calcium.

PTH and kidney

In kidney, PTH causes decreased renal excretion of calcium and increased excretion of phosphates.

PTH and intestines: PTH stimulates 1-hydroxylation of 25-hydroxycalciferol in kidney to produce calcitriol. This indirectly increases calcium absorption.

Calcitonin

It is secreted by the thyroid parafollicular or clear cells.

Calcitonin is a polypeptide. Calcitonin secretion is stimulated by serum calcium, gastrin, glucagon and biological amines. Calcitonin decreases serum calcium level. It **inhibits resorption of bone**. It decreases the activity of osteoclasts and increases that of osteoblasts.

Calcitonin and PTH are directly antagonistic. The PTH and alcitonin together promote the bone growth and remodeling.

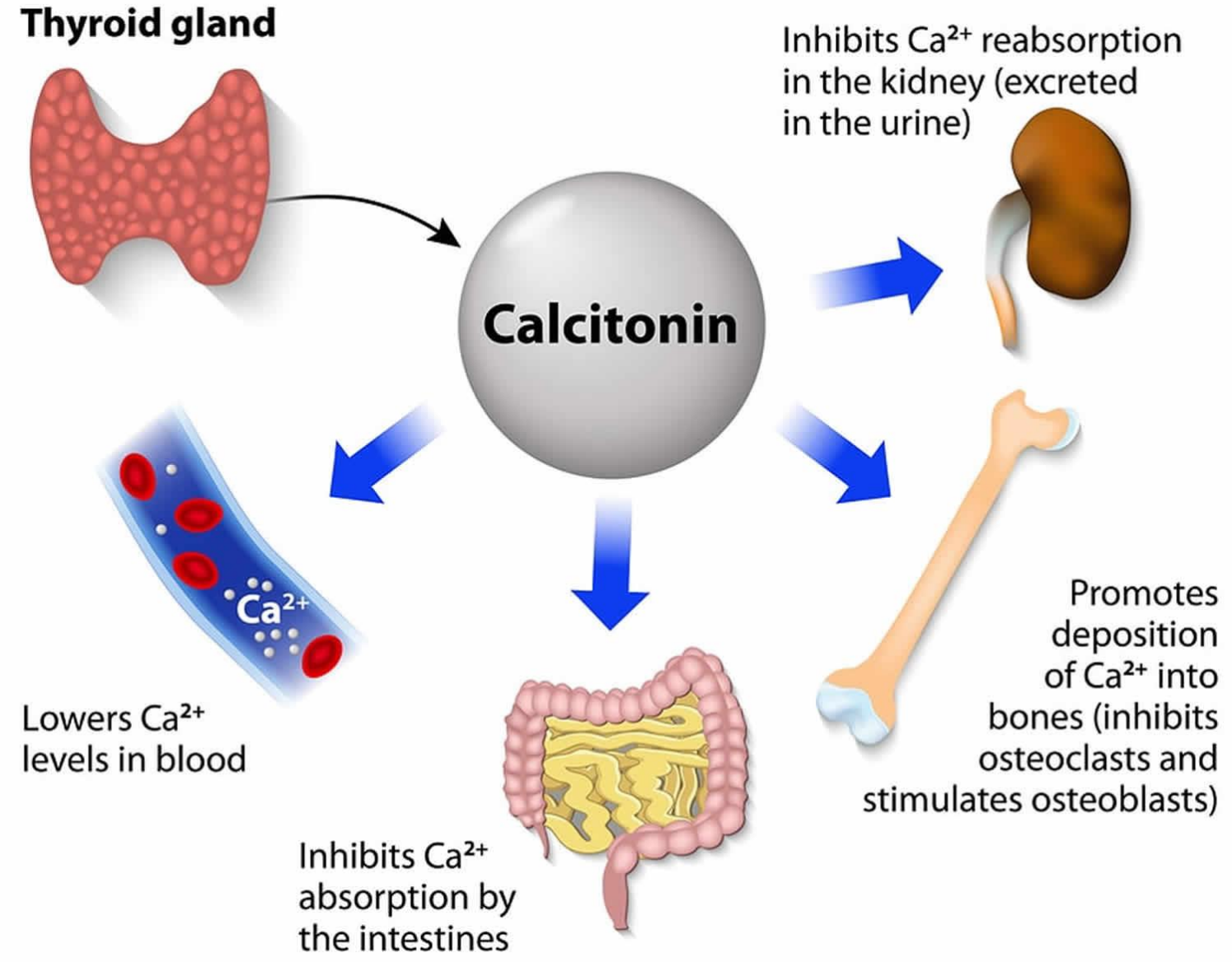
Calcitonin level is increased in medullary carcinoma of thyroid and therefore, is a **tumor marker**.

Procalcitonin

It is the precursor to calcitonin. The levels have been shown to rise with severity of **sepsis**. Procalcitonin (PCT) is markedly elevated in acute bacterial infections; but not in viral infections.

Comparison of action of three major factors affecting serum calcium			
	Vitamin D	PTH	Calcitonin
Blood calcium	increased	drastically increased	decreased
Main action	absorption from gut	demineralisation	opposes demineralisation
Calcium absorption from gut	increased	increased (indirect)	
Bone resorption	decreased	Increased	decreased
Deficiency manifestation	rickets	tetany	--
Use in rickets	drug of choice	contraindicated	theoretically beneficial
Effect of excess	Hypercalcemia+	Hypercalcemia++	hypocalcemia

Actions of Calcitonin



Effect of Phosphorus on calcium

There is a **reciprocal** relationship of calcium with phosphorus. The ionic product of calcium and phosphorus in serum is kept as a constant. (In normal adults, calcium = 10 mg/dL × phosphorus 4 mg/dL; so ionic product is 40). In children, ionic product of calcium and phosphorus in blood is about 50 (instead of 40 in normal adults).

Serum Proteins

In hypoalbuminemia (e.g. nephrosis, malnutrition), the total calcium is decreased. In such cases, the metabolically active ionized form is normal, and so there will be no deficiency manifestations.

Alkalosis and Acidosis

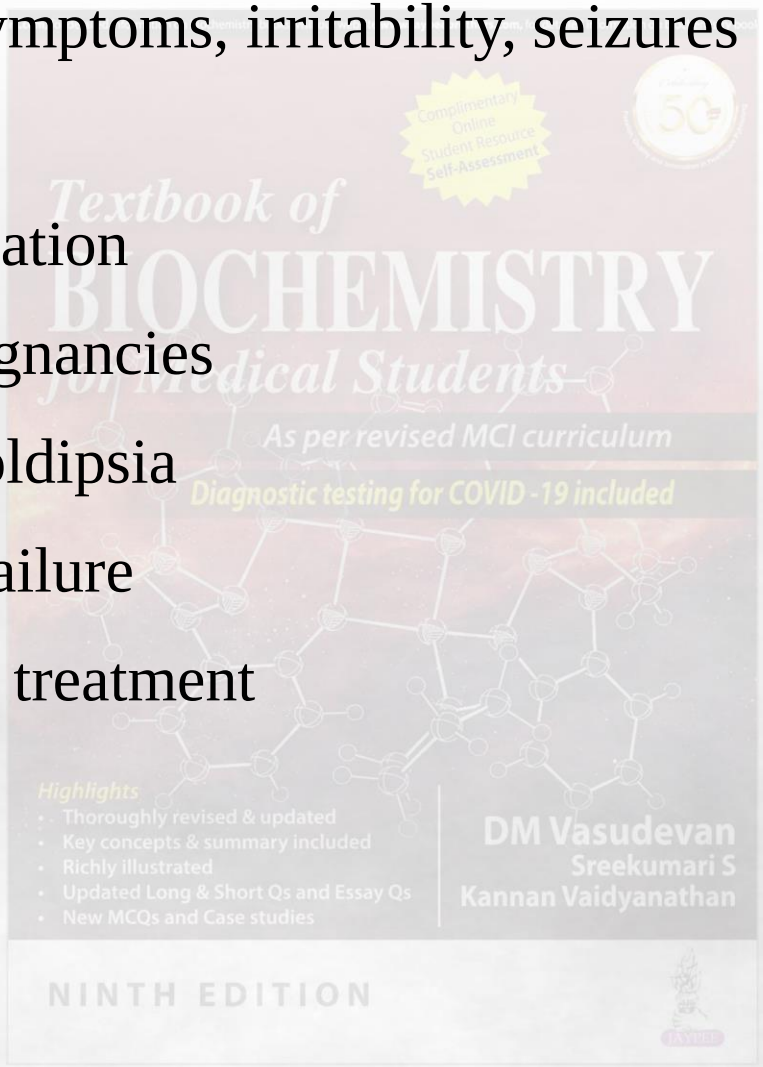
Alkalosis favors binding of more calcium with proteins, with consequent lowering of ionized calcium. Here total calcium level is normal, but calcium deficiency may be manifested. Acidosis favors ionization of calcium.

Renal Threshold

The renal threshold for calcium in blood is 10 mg/dL.

When to Check Calcium Level?

1. Neurological symptoms, irritability, seizures
2. Renal calculi
3. Ectopic calcification
4. Suspected malignancies
5. Polyuria and poldipsia
6. Chronic renal failure
7. Prolonged drug treatment



Causes of hypercalcemia

1. Hyperparathyroidism
2. Multiple myeloma
3. Paget's disease
4. Metastatic carcinoma of bone
5. Thyrotoxicosis, Addison's disease
6. Benign familial hypercalcemia
7. Dehydration
8. Prolonged immobilization
9. Tuberculosis, leprosy, sarcoidosis
10. Milk-alkali syndrome
11. Drugs
    Thiazide diuretics
    Excess vitamin D or vitamin A
    Excess calcium given IV
    Lithium, Theophylline

Symptoms of hypercalcemia

1. Polyuria and poldipsia (ADH antagonism)
2. Confusion, depression, psychosis
3. Osteoporosis and pathological fracture
4. Renal stones
5. Ectopic calcification and pancreatitis
6. Serum alkaline phosphatase may be increased

Management of hypercalcemia

- Adequate hydration, IV normal saline
- Furosemide IV to promote calcium excretion
- Steroids, if there is calcitriol excess
- Definitive treatment for the underlying disorder

Causes of hypocalcemia

1. **Deficiency of vitamin D**

Decreased exposure to sunlight
Malabsorption, dietary deficiency
Hepatic diseases
Decreased renal synthesis of calcitriol
Nephrotic syndrome (binding protein lost)
Anticonvulsant therapy

2. **Deficiency of parathyroid**

Hypoparathyroidism (primary, secondary)

3. **Increased calcitonin**

Medullary carcinoma of thyroid

Causes of hypocalcemia

4. **Deficiency of calcium**

Intestinal malabsorption
Acute pancreatitis
Alkalosis decreasing ionized calcium

5. **Deficiency of magnesium**

6. **Increase in phosphorus level**

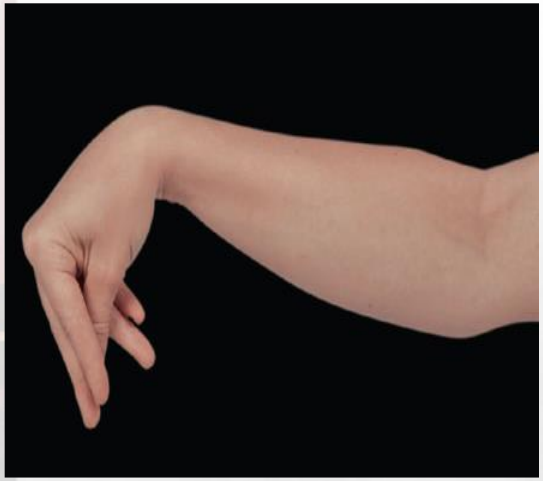
Renal failure
Phosphate infusion
Renal tubular acidos

Symptoms of hypocalcemia

1. Muscle cramps
2. Paresthesia, especially in fingers
3. Neuromuscular irritability, muscle twitchings
4. Tetany (Chvostek's sign, Trousseau's sign)
5. Seizures
6. Bradycardia
7. Prolonged QT interva

Treatment of hypocalcemia

1. Oral calcium, with vitamin D supplementation
2. Underlying cause should be treated
3. Tetany needs IV calcium (usually 10 mL 10% calcium gluconate over 10 minutes, followed by slow IV infusion). IV calcium should be given only very slowly



Carpopedal spasm in tetany.

Chronic deficiency of calcium leads to white patches in nails.

Knock-knee defect in deficiency of calcium

Requirements for growth of bone

1. Calcium
2. Phosphorus
3. Vitamin D and calcitriol
4. Parathyroid hormone
5. Calcitonin
6. Vitamin A (for ground substance)
7. Vitamin C
8. Sex steroids
9. Amino acids

Bone Mineralization

It is the process by which inorganic calcium and phosphate are deposited on the organic matrix, **osteoid.** The osteoblasts synthesize and secrete organic matrix, which is then mineralized. Osteoclasts are involved in bone resorption. The osteoblasts are under the effect of hormones PTH and calcitriol.

Secretion of **alkaline phosphatase** by osteoblasts is increased by vitamin D. The enzyme liberates phosphate from substrates. So the ionic concentration of [calcium × phosphate] is increased to supersaturation level.

Calcium phosphate is deposited as **hydroxyapatite** crystals over the matrix of triple stranded quarter staggered collagen molecules. Calcium in the bone is in dynamic equilibrium with serum calcium; hydroxyl apatite intrabecular bone acts as a reservoir.

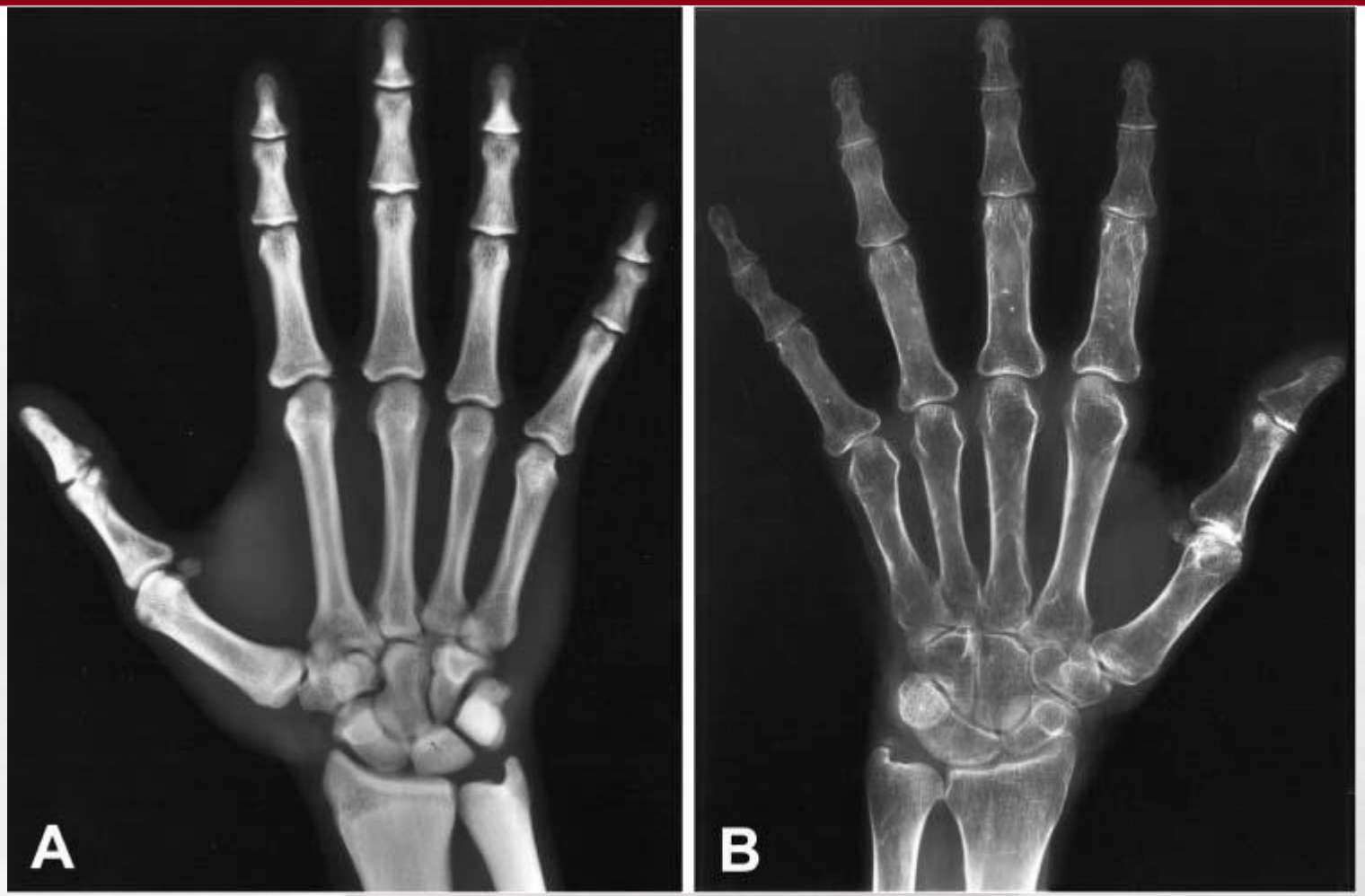
Compact bone ←	Trabecular bone ←→	Serum calcium
$Ca_3(PO_4)_2$	$Ca_{10}(PO_4)_6(OH)_2$	Ca^{++}
(Total 1 kg)	(Total about 5 g)	(Total 500 mg)

Osteoporosis

It is associated with an increased risk for fractures (vertebra, hip and forearm). The basic abnormality is decrease in bone mass, which attains a peak by the age of 30 and starts declining by 35–45 years of age in both men and women. After the age of 40–45, calcium absorption is reduced and calcium excretion is increased; so, there is a net negative balance for calcium. This is reflected in demineralization.

Osteoporosis is a silent disease with no symptoms, but its clinical consequences can be debilitating. Common sites for osteoporotic fractures include the spine, wrist, and hip. Bone mineral density (BMD) is measured by the dual energy X-ray absorptiometer (DXA).

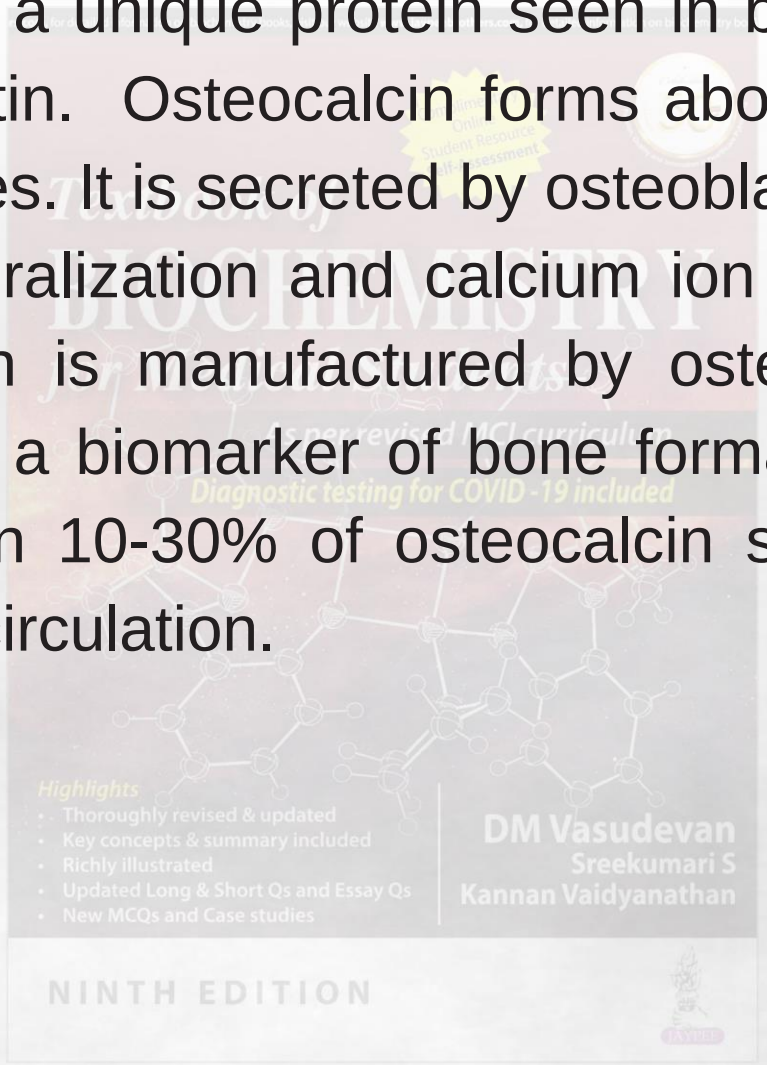
Osteoporosis



A= normal x-ray. B= osteoporotic bone.

Osteocalcin

Osteocalcin is a unique protein seen in bone, found in bone and dentin. Osteocalcin forms about 1% of total protein in bones. It is secreted by osteoblasts and plays a role in mineralization and calcium ion homeostasis. As osteocalcin is manufactured by osteoblasts, it is often used as a biomarker of bone formation. During bone formation 10-30% of osteocalcin synthesized is released into circulation.



Osteonectin

It is a glycoprotein in the bone that binds calcium. It is secreted by osteoblasts during bone formation, initiating mineralization and promoting mineral crystal formation. This protein also shows an affinity for collagen in addition to bone mineral calcium. Osteonectin also increases the production of matrix metalloproteinases, a function important to invading cancer cells within bone. Overexpression of osteonectin is reported in chronic pancreatitis and in many human cancers such as breast, prostate and colon.

Biomarkers of Bone Diseases

- Serum calcium
- Serum inorganic phosphorus
- Serum magnesium
- Urinary excretion of calcium and phosphorus
- Total alkaline phosphatase and bone isozyme of alkaline phosphatase (BAP)
- Serum 25-hydroxy vitamin D
- PTH measurement, if serum calcium is abnormal
- Osteocalcin which increases in metabolic bone disease
- Type I collagen telopeptides; markers of bone resorption
- N-telopeptides and C telopeptides

Phosphorus

Total body phosphate is about 1 kg; 80% of which is seen in bone and teeth and 10% in muscles. Phosphate is mainly an intracellular ion and is seen in all cells.

Functions of phosphate ions

1. Formation of bone and teeth
2. Production of high energy phosphate compounds such as ATP, CTP, GTP
3. Synthesis of nucleoside coenzymes such as NAD and NADP
4. DNA and RNA synthesis, (phosphodiester linkages)
5. Formation of phosphate esters, such as glucose-6-phosphate
6. Formation of phosphoproteins, e.g. casein
7. Activation of enzymes by phosphorylation
8. Phosphate buffer system in blood. The ratio of Na2HPO4: NaH2PO4 in blood is 4:1 at pH of 7.4

Requirement and Source

Requirement is about 500 mg/day. Milk is a good source. Cereals, nuts and meat are moderate sources.

Calcitriol increases phosphate absorption.

Serum Level of Phosphorus

Serum level of phosphate is **3.4 – 4.5 mg/dL** in normal adults and is 5–6 mg/dL in children. Fasting levels are higher. The postprandial decrease of phosphorus is due to the utilization of phosphate for metabolism.

Monovalent and divalent phosphate ions are present in plasma at a ratio of 1:9 in alkalosis, at a ratio of 1:4 at pH 7.4, and at a ratio of 100:1 at pH of 4.5 in urine.

Causes of hyperphosphatemia

1. **Increased absorption of phosphate**

Excess vitamin D

Phosphate infusion

2. **Increased cell lysis**

Chemotherapy for cancer

Bone secondaries

3. **Decreased excretion of phosphorus**

Renal impairment

Hypoparathyroidism

4. **Hypocalcemia**

5. **Massive blood transfusions**

6. **Thyrotoxicosis**

7. **Drugs**

Chlorothiazide, Nifedipine, Furosemide

Causes of hypophosphatemia

1. **Decreased absorption of phosphate**

Malnutrition

Malabsorption

Chronic diarrhea

Vitamin D deficiency

2. **Intracellular shift**

Insulin therapy

Respiratory alkalosis

3. **Increased urinary excretion**

Hyperparathyroidism

Fanconi's syndrome

Hypophosphatemic rickets

4. **Hereditary hypophosphatemia**

5. **Drugs:** Antacids, diuretics, salicylate

Important Combinations of Serum Calcium and Phosphate Levels in Blood

1. **Increased P with decreased Ca**

Hypoparathyroidism

Renal disease

2. **Increased P with normal or increased Ca**

Milk alkali syndrome

Hypervitaminosis D

3. **Decreased P with increased Ca**

Hyperparathyroidism

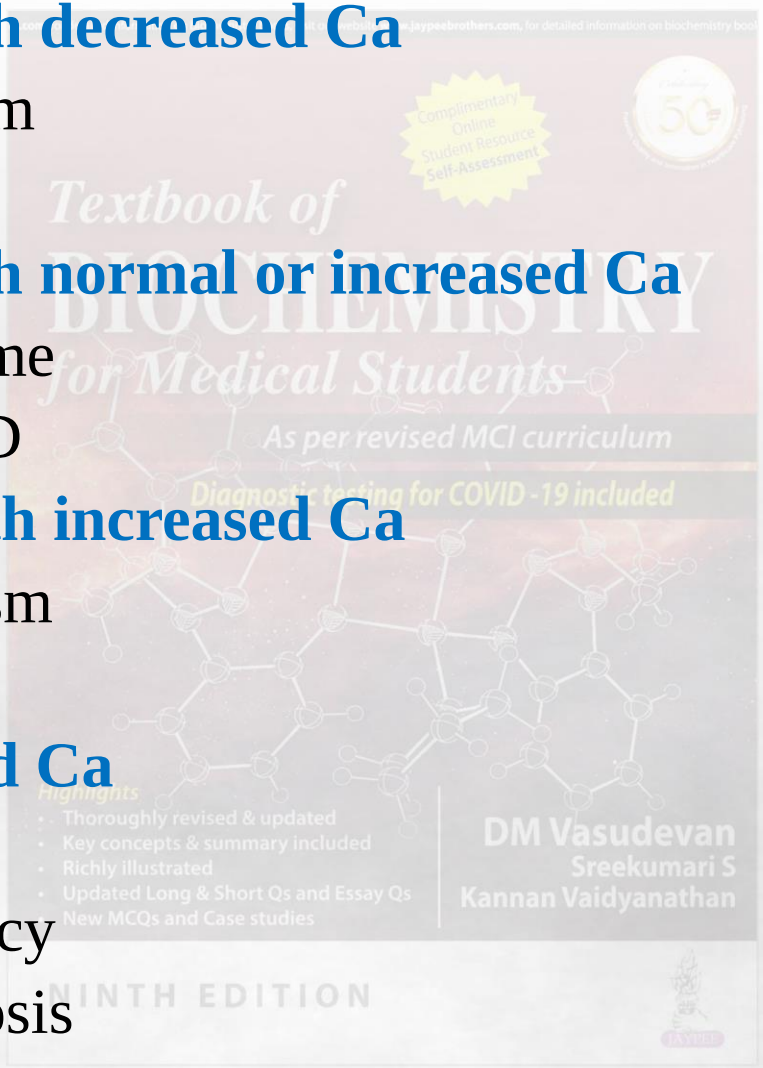
Sarcoidosis

4. **Decreased P and Ca**

Malabsorption

Vitamin D deficiency

Renal tubular acidosis



Sulfur

Source of sulfates is mainly amino acids cysteine and methionine.

Functions of Sulfur

- The disulfide bridges keep polypeptide units together, e.g. insulin, immuno-globulins. Chondroitin sulfates are seen in cartilage and bone. Keratin is rich in sulfur, and is present in hair and nail.
- Many enzymes and peptides contain -SH group at the active site, e.g. glutathione. Coenzymes derived from thiamine, biotin, pantothenic acid and lipoic acid also contain sulfur.
- If sulfate is to be introduced in glycosaminoglycans or in phenols for detoxification, it can be done only by phosphoadenosine phosphosulphate (PAPS).
- Sulfates are also important in detoxification mechanisms, e.g. production of indoxyl sulfate.

Excretion

The total quantity of sulfur in urine is about 1 g/day. This contains 3 categories:

1. Inorganic Sulfates

It is about 80% of the total excretion. This is proportional to the protein intake.

2. Organic Sulfate or Ethereal Sulfate

It is also called conjugated sulfate. It constitutes 10% of urinary sulfates. Tryptophan is converted to indoxyl by intestinal bacteria. These are absorbed and conjugated with sulfates and excreted through urine. Therefore, this represents the putrefactive activity in intestine, and this fraction is increased in intestinal stasis. This part is also proportional to protein intake.

3. Neutral Sulfur or Unoxidized Sulfur

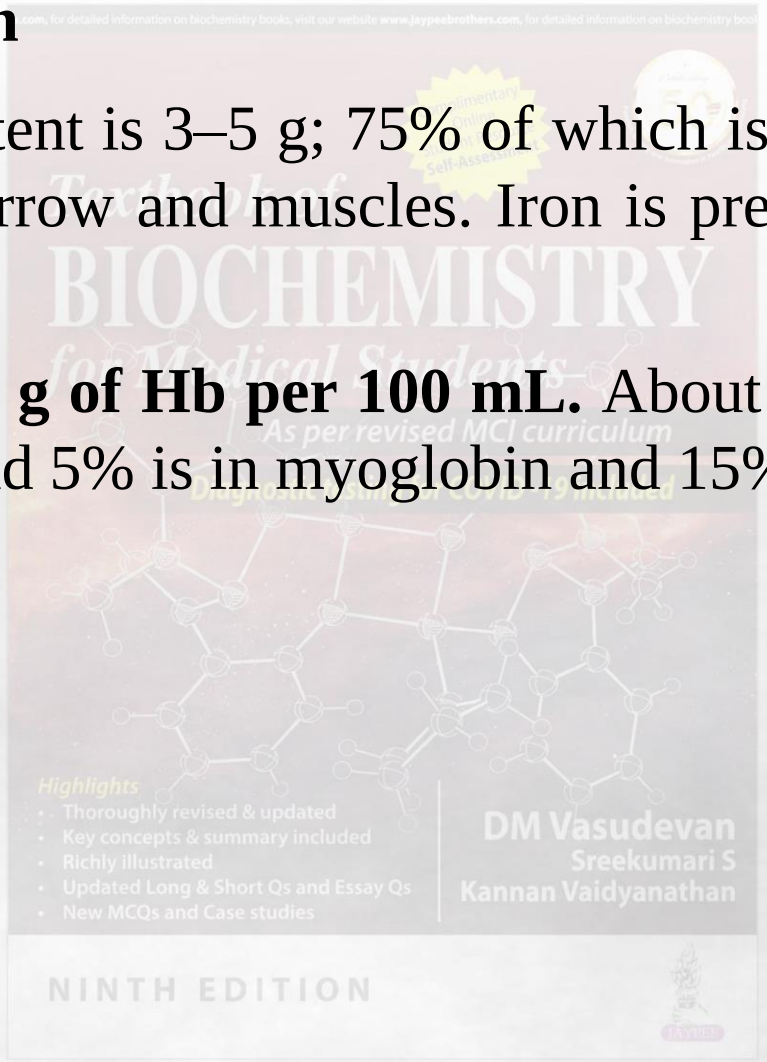
This fraction constitutes 10% of total sulfates. Sulfur containing organic compounds such as amino acids, thiocyanates and urochrome constitute this fraction. In obstructive jaundice, taurocholic acid is excreted in urine, and hence this fraction is increased. It is also increased in aminoacidurias.

Iron (Fe)

Distribution of Iron

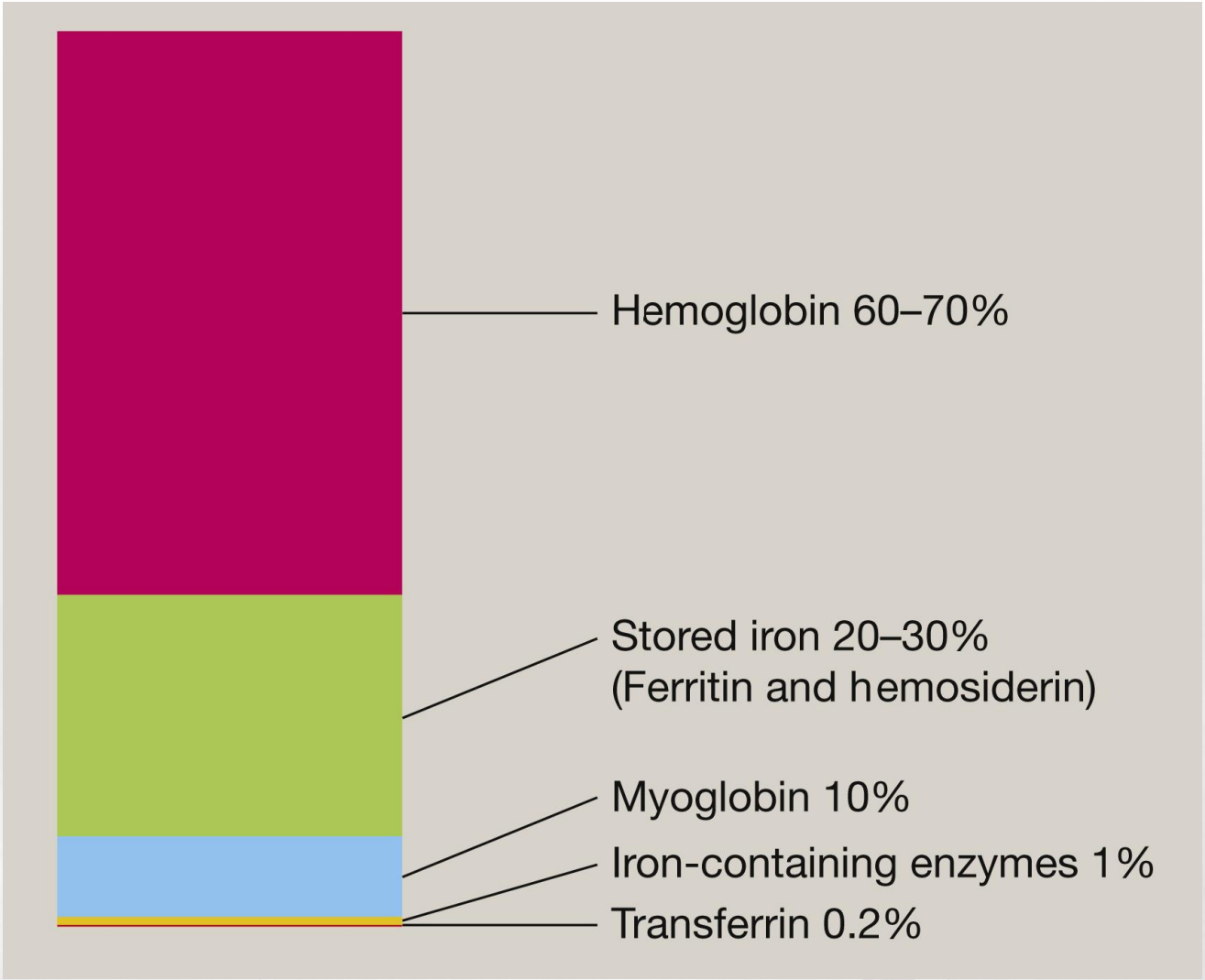
Total body iron content is 3–5 g; 75% of which is in blood, the rest is in liver, bone marrow and muscles. Iron is present in almost all cells.

Blood contains **14.5 g of Hb per 100 mL.** About 75% of total iron is in hemoglobin, and 5% is in myoglobin and 15% in ferritin.

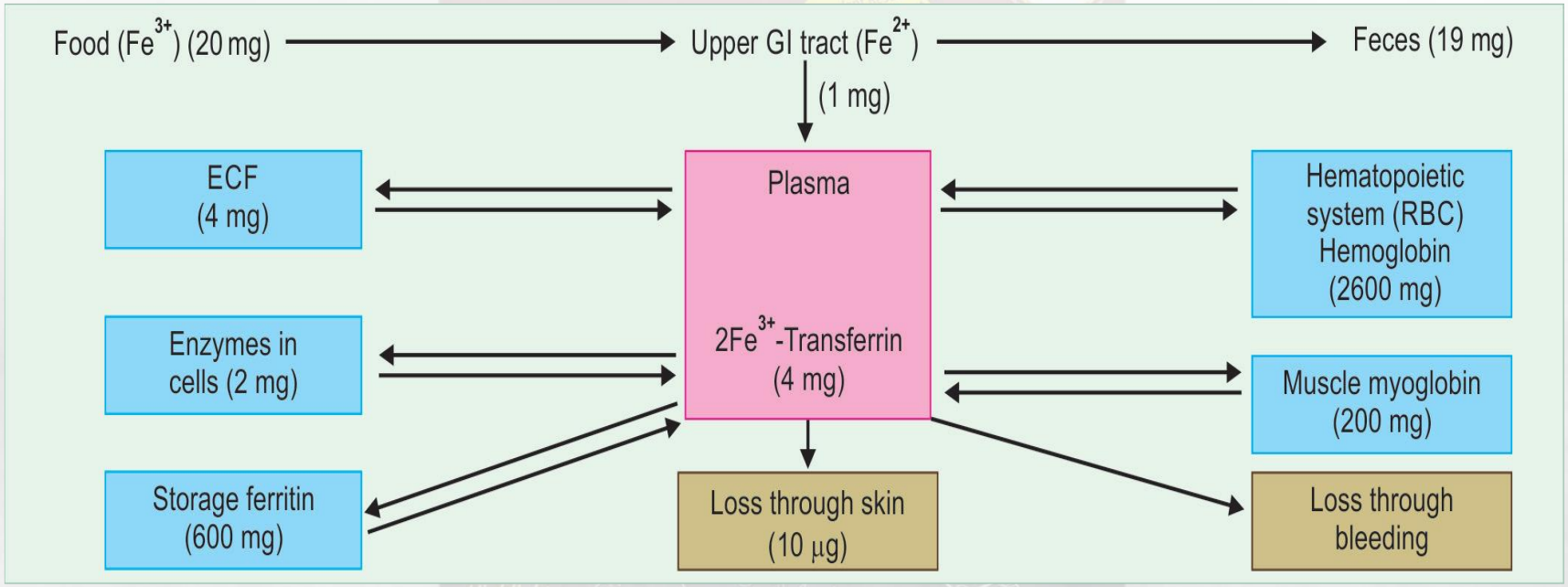


Iron-containing proteins			
Name	**Mol.wt.**	**No. of iron atom**	**Site**
Heme-containing proteins			
Hemoglobin	65,000	4	RBC
Myoglobin	17,000	1	Muscle
Cytochrome oxidase	180,000	2	Mitochondria
Cytochrome b	30,000	1	do
Cytochrome c1	37,000	1	do
Cytochrome c	12,000	1	do
Cytochrome b5	15,000	1	ER
Cytochrome p-450	55,000	1	ER, Mitochondria
Catalase	240,000	4	RBC
Lactoperoxidase	93,000	1	Milk
Tryptophan pyrrolase		4	Cytosol
Nitric oxide synthase		1	Endothelium

Iron-containing proteins			
Name	**Mol.wt.**	**No. of iron atom**	**Site**
Iron-Sulphur Complexes			
Complex III Fe-S	30,000	2	Mitochondria
Succinate DH	27,000	4	Mitochondria
Xanthine oxidase	275,000	8	Liver
Nonheme Iron-containing Proteins			
Aconitase	66,000	2	TCA cycle
Phe-hydroxylase	110,000	2	Liver
Transferrin	77,000	2	Plasma
Ferritin	450,000	4,000	Tissues
Hemosiderin		Many	Liver



Compartmentalization of total body iron.



Normal iron kinetics.

Requirement of Iron

Daily allowance of iron for an adult Indian is **20 mg**, out of which about 1–2 mg is absorbed. Children between 13–15 years need 20–30 mg/day. Pregnant women need 40 mg/day. Transfer of iron and calcium from mother to fetus occurs mainly in the last trimester of pregnancy.

In the first 3 months of life, iron intake is negligible because milk is a poor source of iron. During this time, child is dependent on the iron reserve received from mother during pregnancy. After 3 months of life, diet supplementation with cereals is essential for supplying the iron requirement.

Sources of Iron

Leafy vegetables are good sources. Pulses and cereals contain lesser quantity of iron. In a typical Indian diet, the major quantity of iron is received from cereals because of the bulk quantity taken. Liver and meat contains good quantity of iron. **Jaggery** is a good source of iron. Cooking in iron utensils will improve the iron content of the diet. Milk is a **very poor source**.

Factors Influencing Absorption of Iron

Iron is absorbed by upper part of duodenum.

Reduced Form of Iron

Only Fe++ (**ferrous**) form (reduced form) is absorbed. Fe+++ (ferric) form is not absorbed.

Ascorbic Acid

Ferric ions are reduced with the help of gastric HCl, ascorbic acid, cysteine and-SH groups of proteins.

Interfering Substances

Iron absorption is decreased by **phytic acid** (in cereals) and **oxalic acid** (in leafy vegetables).

Other Minerals

Calcium, copper, lead and phosphates will inhibit iron absorption. One atom of lead will inhibit absorption of 1,000 atoms of iron.

Mucosal Block Theory

Major regulation of body iron pool is at the level of absorption. When iron stores in the body are depleted, absorption is enhanced. When an adequate quantity of iron is stored, absorption is decreased. This is referred to as the mucosal block of absorption of iron.

Mucosal cells absorb only about 1% of the dietary iron and the unabsorbed iron is excreted in feces.

Iron metabolism is unique because homeostasis is maintained by regulation at the level of absorption and not by excretion. No other nutrient is regulated in this manner. Iron is said to be a **one-way element** because the body pool is mainly regulated by absorption and utilization.

Absorption of Iron in Three Phases

About 1-2 mg of iron enters the body daily from dietary absorption. Iron absorption takes place in 3 steps – luminal phase, mucosal cellular phase and basolateral phase (release into the portal blood stream).

Luminal Phase of Iron Absorption

Only ferrous (and not ferric) form of iron is absorbed. The trivalent ferric iron present in the food is converted into bivalent ferrous iron by duodenal **ferric reductase**. Ferrous iron in the intestinal lumen binds to mucosal brush border protein, called **divalent metal transporter-1 (DMT-1).** **(**Therefore, all other divalent ions, including calcium, copper and lead will competitively inhibit the iron absorption). The bound iron is then transported into the mucosal cell.

Absorption of Iron in Three Phases

Mucosal Cellular Phase

In the next step, iron is binding to apoferritin and temporarily stored in the cell as **ferritin** or transported across the mucosal cell depending on body iron status. If there is anemia, the iron is further absorbed into the bloodstream. If body stores are saturated with iron, any iron accumulated in the mucosal cell is lost when the cell is desquamated. Thus the fraction of iron absorbed is decided by the iron status. When iron is in excess, absorption is reduced; this is the basis of "mucosal block".

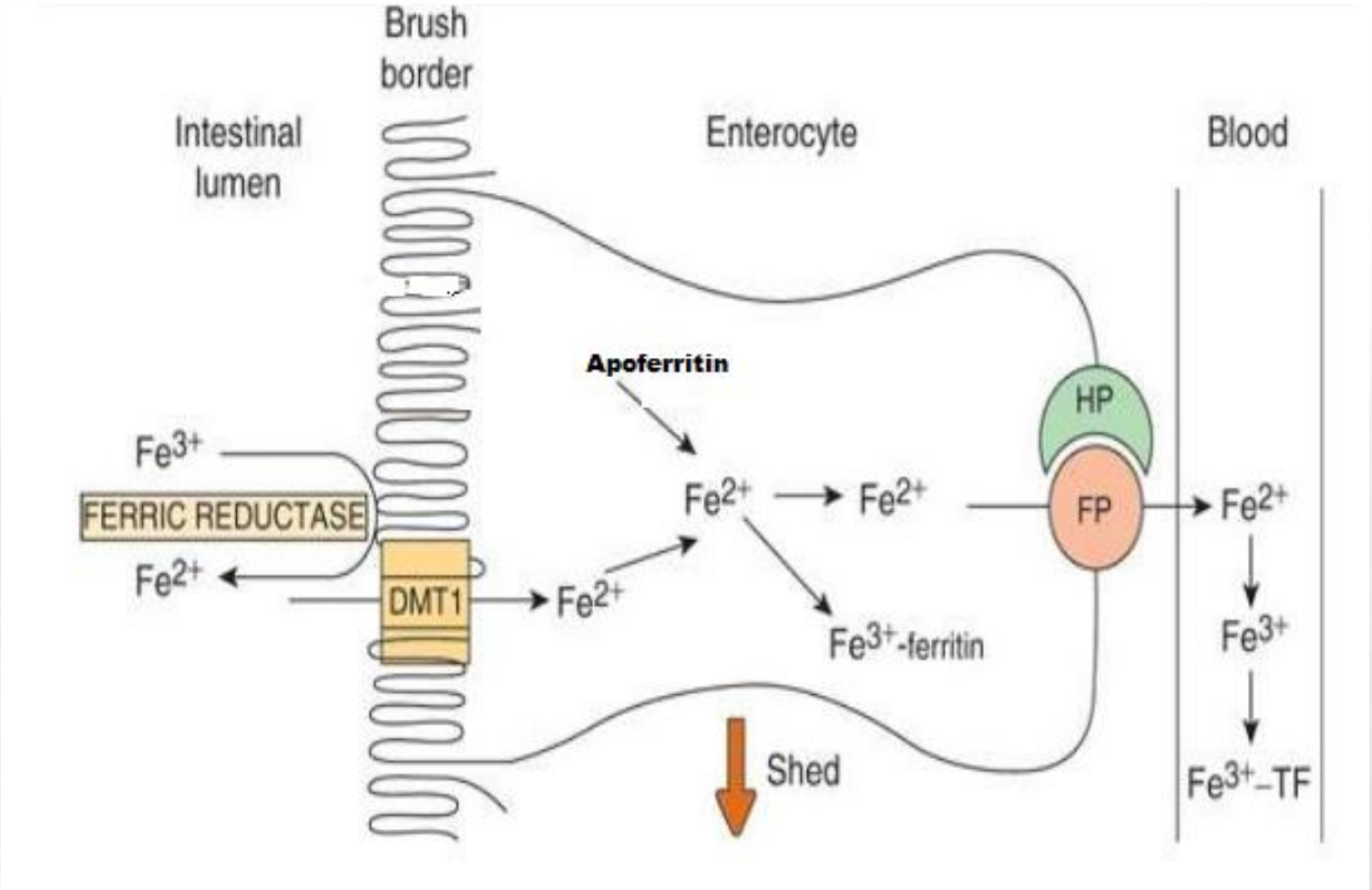
Absorption of Iron in Three Phases

Basolateral Phase

The mechanism of iron absorption from the intestinal lumen to the mucosal cell is different from the iron release from the intestinal cell to the blood stream. During this third phase, ferrous iron is released into the portal circulation by a basolateral iron transporter called **Ferroportin.**

This export requires **Hephestin,** a copper-containing protein. It oxidizes the ferrous iron to ferric form to load on to the transferrin in blood. This export can happen only when there is free transferrin in plasma to bind the iron. Iron crosses the cell membrane as ferrous form. In the blood, it is re-oxidized to ferric state by the ferroxidase activity of ceruloplasmin, and then transported by **transferrin.**

Absorption of Iron



Iron Absorption.
DMT1 = Divalent metal transporter.
FP = Ferroportin. TF = transferrin.

Summary of Absorption of Iron

In the food, iron is in Fe^{+++} (ferric) form. This is reduced to Fe^{++} (ferrous) state by ferric reductase. This ferrous iron is internalized with the help of the divalent metal transporter. Later, the ferrous iron is oxidized to the ferric state which binds with ferritin for temporary storage. According to the requirement, the ferric iron is released, reduced to the ferrous state, and crosses the cell membrane with the help of Ferroportin. Then re-oxidized to the ferric state by hephestin. This ferric iron is bound with transferrin and transported in the blood.

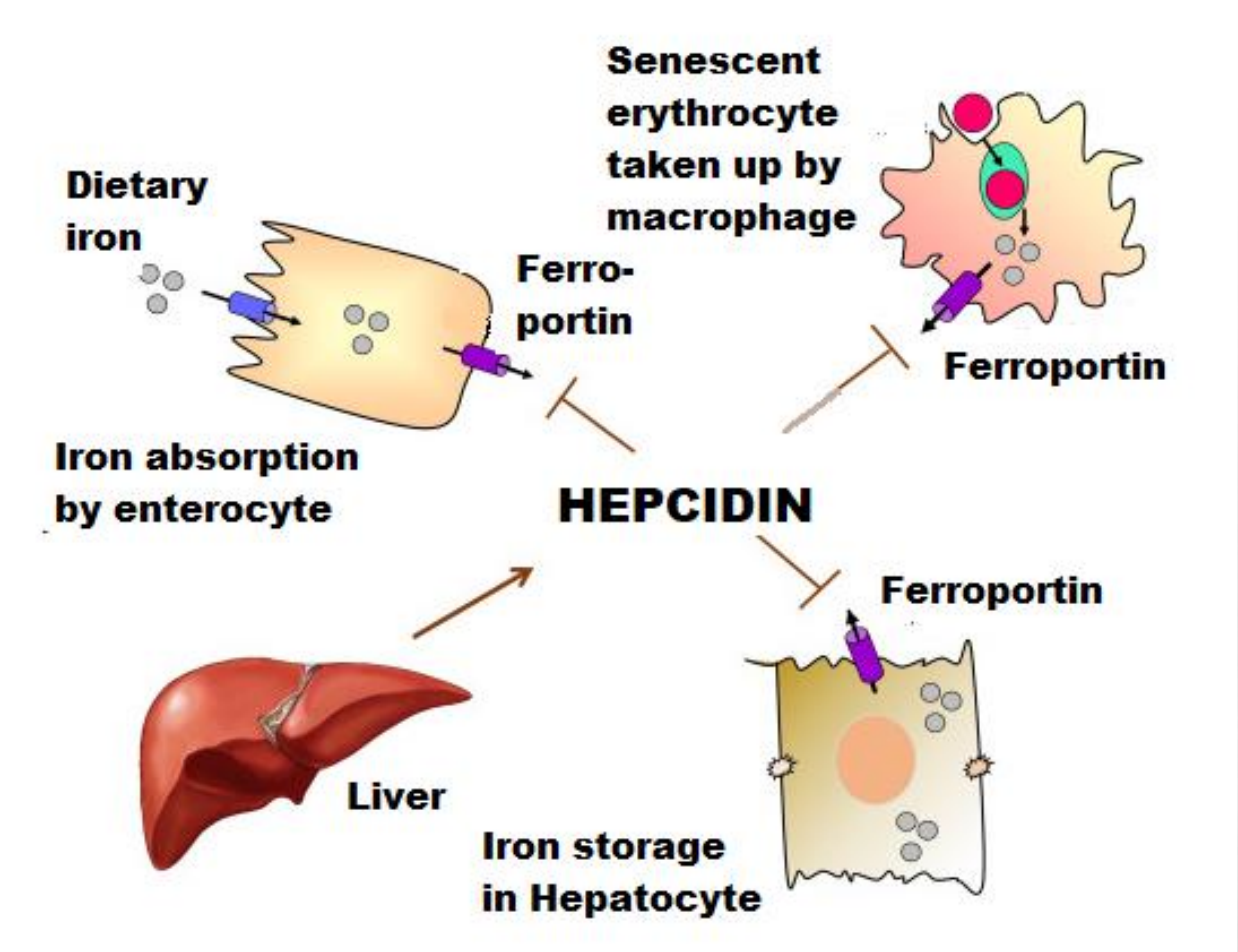
Regulation of Absorption by Four Mechanisms

Iron is absorbed from the duodenal enterocytes; while erythroid precursors are consuming the iron, hepatocytes are storing the excess iron. These three organs are well coordinated by the following four mechanisms.

Regulation by Hepcidin

Hepcidin is a small peptide with 25 amino acids. It is produced by liver cells and is involved in killing bacteria and hence the name. Hepcidin is synthesized in response to iron needs. Its production decreases in anemia, hypoxia and inflammation. Its level is increased by high iron stores and by inflammation. Hepcidin reduces the synthesis of both divalent metal transporter and ferroportin. Moreover, hepcidin binds to ferroportin and the Hepcidin-ferroportin complex breaks down quickly

Regulation of Absorption by Hepcidin



The action of Hepcidin in Iron Metabolism

Regulation of Absorption by Four Mechanisms

Storage Regulation

As body iron stores fall, the mucosa is getting the signals to increase absorption. This signal transduction is carried out by Transferrin receptors.

Erythropoietic Regulation

In response to anemia, the erythroid cells will send signals to the mucosa to increase iron absorption. This signal molecule may be **erythropoietin** from the kidney. Anemia and increased erythropoietic activity give signals for lowering hepcidin levels and consequently increased iron absorption. But when there is infection or inflammation, hepcidin level increases and iron availability for erythropoietic activity decreases causing the iron deficiency anemia related to infection.

Regulation of Absorption by Four Mechanisms

Regulation by Transferrin Receptor

There is a reciprocal relationship between the synthesis of ferritin and **transferrin receptor** (TfR). The expressions of proteins involved in uptake, storage and transport of iron are regulated at the transcriptional level by the IRP (Iron responsive protein) and IRE (iron responsive element). In the case of high concentration of iron, the mRNA for ferritin is translated and ferritin is synthesized. But mRNA for TfR is degraded, resulting in reduced TfR protein synthesis. Thus, when iron levels are high, ferritin is synthesized to store iron. At the same time, there is no requirement for further uptake of iron, so the TfR is not synthesized.

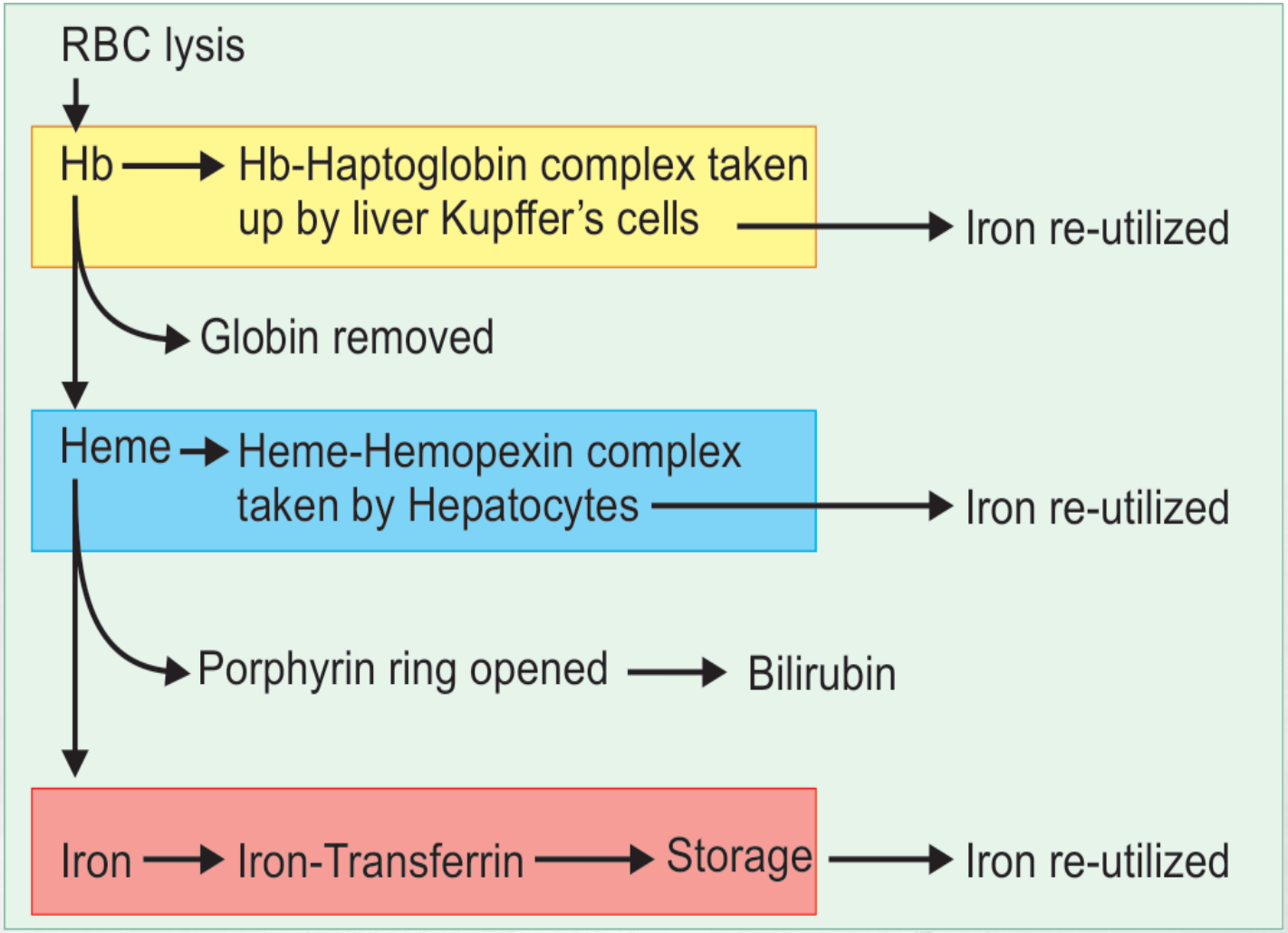
Iron Transport in Blood and Uptake by Cells

- Transport form of iron is **transferrin**. Normal plasma level of transferrin is 250 mg/100 mL. In iron deficiency, this level is increased. One molecule of transferrin can transport 2 ferric atoms.
- **Total iron binding capacity** (TIBC) in plasma is 400 μg/100 mL; this is provided by the transferrin. One-third of this capacity is saturated with iron. In iron deficiency anemia, TIBC is increased (transferrin level is increased); but serum iron level is reduced. One molecule of transferrin can bind two ferric ions.
- In blood, **ceruloplasmin** is the ferroxidase, which oxidizes ferrous to ferric state.
- **Transferrin receptors** (TfR) are present on cells which synthesize heme. The iron-transferrin complex is taken up by the body cells by the receptor mechanism. The transferrin receptor binds two molecules of transferrin. The iron-transferrin-receptor is internalized. Iron is taken in by the cells.

Storage of Iron

- The storage form is **ferritin**. It is seen in intestinal mucosal cells, liver, spleen and bone marrow. The **apoferritin** can take up to 4,000 iron atoms per molecule. Ferritin contains about 23% iron.
- Normal plasma contains very little ferritin. Ferritin in plasma is elevated in iron overload. Thus ferritin level in blood is an index of body iron stores.
- Synthesis of TfR and ferritin are reciprocally controlled. When iron levels are high, ferritin is synthesized to store the iron; but the TfR synthesis is blocked.
- In iron deficiency anemia, ferritin content is reduced.
- **Hemosiderin** is also a storage form of iron, but it is formed by partial deproteinization of ferritin by lysosomes and are found as aggregates in tissues like liver, spleen and bone marrow. It is more insoluble than ferritin, and iron is more slowly released.

Ferritin is decreased in iron deficiency anemia. When iron is given in anemia, apoferritin production is induced within a few hours. Estimation of ferritin in chronic kidney disease (CKD) is of prognostic significance since ferritin level less than 100 mcg/dL indicates iron deficiency. In order to treat the anemia in CKD, the iron stores should be adequate and this is denoted by serial ferritin estimations in a patient on treatment to check the efficacy of treatment with recombinant erythropoietin.



Conservation of iron in the body.

Excretion of Iron

- Iron is a one-way element. That is, very little of it is excreted.
- *The regulation of homeostasis is done at the absorption level.*
- Women up to menopause will lose iron at a rate of about 1 mg/day. The loss in male is <0.5 mg/day.
- Almost no iron is excreted through urine.
- Feces contain unabsorbed iron as well as iron trapped in the intestinal cells, which are then desquamated. About 30% of cells in the intestinal lining are replenished everyday, and so this loss is considerable.
- All the cells in skin contain iron. The upper layers of skin cells are constantly being lost, and this is another route for iron loss from the body.

Iron Deficiency Anemia

Causes of iron deficiency

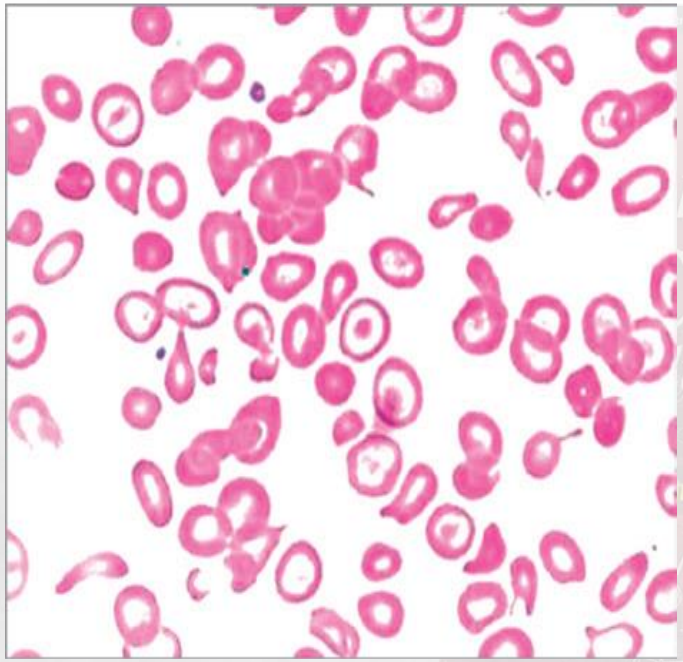
1. Nutritional deficiency of iron.
2. **Lack of absorption:** Subtotal gastrectomy and hypochlorhydria.
3. **Hookworm infection:** One hookworm will cause the loss of about 0.3 mL of blood per day. Calculation shows that about 300 worms can produce a loss of 1% of total body iron per day.
4. **Repeated pregnancies:** About 1 g of iron is lost from the mother during one delivery.
5. **Chronic blood loss:** Hemorrhoids (piles), peptic ulcer, menorrhagia.
6. **Nephrosis:** Haptoglobin, hemopexin and transferrin are lost in urine, along with loss of iron.
7. **Lead poisoning:** Iron absorption and hemoglobin synthesis are reduced. In turn, iron deficiency causes more lead absorption. It is a vicious cycle.

Iron Deficiency Anemia

- It is the most common nutritional deficiency disease. About 30% of world population is anemic. About 85% of pregnant women suffer from anemia.
- Maternal anemia contributes to increase in perinatal mortality.
- Iron deficiency is characterized by **microcytic hypochromic** anemia. Anemia is diagnosed when **hemoglobin level is <12 g/dL for women and <13 g/dL for men** and/or ferritin level is below 12 μg/dL.

Clinical Manifestations

- When the level is lower than 10 g, body cells lack oxygen and patient becomes uninterested in surroundings (**apathy**).
- Prolonged iron deficiency causes atrophy of gastric epithelium leading to **achlorhydria**, which in turn causes lesser absorption of iron.
- Very chronic iron deficiency anemia will lead to impaired attention, irritability, lowered memory and poor scholastic performance.
- Chronic iron deficiency is manifested as **koilonychia** or "spoon nail"



Peripheral blood smear.
Iron deficiency manifests as microcytic hypochromic anemia.

Koilonychia or “spoon nail” in chronic iron deficiency anemia.

Classification of Anemias

1. **Impaired production of RBCs**

a. **Defect in heme synthesis:** Deficiency of iron, copper, pyridoxalphosphate, folic acid, vitamin B12 or vitamin C. Lead will inhibit heme synthesis.

b. **Defect in regulators:** Lack of erythropoietin due to chronic renal failure.

c. **Defect in stem cells:** Aplastic anemia due to drugs, infections

2. **Intracorpuscular defects**

a. **Hemoglobinopathies:** HbS, HbC, HbM

b. **Thalassemias,** Spherocytosis, glucose-6-phosphate dehydrogenase deficiency.

3. **Extracorpuscular causes**

a. **Infections:** Malaria, *streptococcus*

b. **Autoimmune hemolysis,** Isoimmune hemolysis, Rh incompatibility

c. **Hemolysis due to drug sensitization:** Alpha-methyldopa, quinine, etc.

4. **Hemorrhage:** Hematuria, hematemesis, hemoptysis, menorrhagia, hemophilia (absence of AHG), thrombocytopenia.

Parameters indicating the iron status of the body

Status of Iron	Serum Iron mcg/dL	Ferritin mcg/L	TIBC mcg/dL
Normal	60 - 170	15-300	300-400
Pregnancy	Low	Low or N	High
Iron deficiency (Absolute)	Low	Low	High
iron deficiency (Functional)	Low	N or high	Low
Hemolytic anemia	High	High	N or high
Infections	Low	N or low	N
Hemosiderosis	High	High	Low

Treatment of Iron Deficiency

- Oral iron supplementation is the treatment of choice. 100 mg of **iron** + 500 μg of **folic acid** are given to pregnant women, while 20 mg of iron + 100 μg folic acid to children.
- Iron tablets are usually given along with **vitamin C,** to convert it into ferrous form, for easy absorption.
- Administration of iron-zinc combinations are beneficial to correct deficiency of both.
- There are different pharmaceutical preparations. Ferrous sulfate is the first choice, as it is easily absorbed and has maximum bioavailability. If that is not tolerated, ferrous fumarate or ferrous gluconate may be tried. If that is also not tolerated, then iron polymaltose or iron bisglycinate may be tried.

Iron Toxicity

Hemosiderosis

Iron excess is called hemosiderosis. It occurs in persons receiving repeated blood **transfusions**. So, the regulation at the level of intestine is circumvented leading to iron overload.

Primary Hemosiderosis

It is also called hereditary hemochromatosis. Iron absorption is increased and transferrin level in serum is elevated. Excess iron deposits are seen.

Iron Vessels

Cooking in iron vessels increases the availability of iron.

Bantu Siderosis

Bantu tribe in Africa is prone to hemosiderosis because the staple diet, corn, is low in phosphate content.

Iron Toxicity

Hemosiderosis, continued

Hemochromatosis

When total body iron is >25–30 g, hemosiderosis is manifested. In the liver, hemosiderin deposit leads to death of cells and cirrhosis. Pancreatic cell death leads to diabetes. Deposits under the skin cause yellow-brown discoloration, which is called hemochromatosis. The triad of cirrhosis, hemochromatosis and diabetes are referred to as **bronze diabetes**. Acquired hemochromatosis occurs in chronic alcoholics.

Treatment of Hemosiderosis

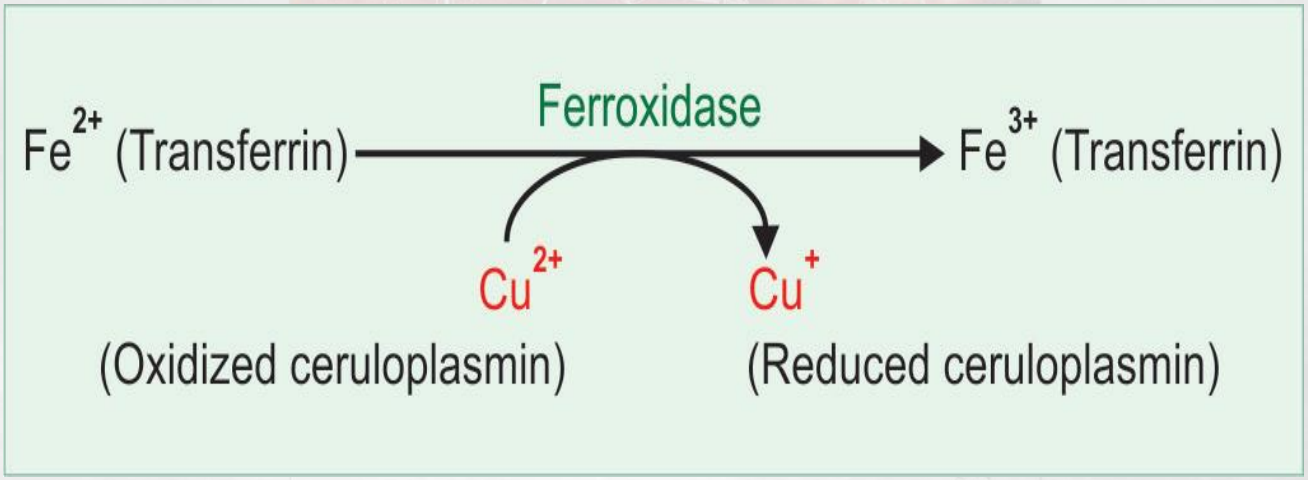
Repeated phlebotomy every week, till serum iron, and ferritin reach near normal levels. Desferroxamine, a chelating agent, forms an iron chelate with Fe+++ to form ferroxamine which is excreted in urine.

Copper (Cu)

- Total body copper is about 100 mg. It is seen in muscles, liver, bone marrow, brain, kidney, heart and in hair.
- Copper containing enzymes are ceruloplasmin, cytochrome oxidase, cytochrome c, tyrosinase, lysyl oxidase, ALA synthase, monoamine oxidase, superoxide dismutase and phenol oxidase.
- Copper containing nonenzymatic proteins are hepatocuprein in liver (storage form), cuprothionine in liver, cerebrocuprein in brain, hemocuprein in RBC and erythrocuprein in bone marrow.
- Copper **requirement** for an adult is 1.5–3 mg per day.
- Major dietary sources are cereals, meat, liver, nuts and green leafy vegetables.
- Milk is very poor in copper content.
- Excretion is mainly through bile.
- Urine contains very small quantities of copper under normal conditions.

Functions of Copper

- It is necessary for mobilization of iron from mucosal, reticuloendothelial, and hepatic parenchymal cells through the action of ceruloplasmin. Thus incorporation of iron into hemoglobin needs copper.
- It is necessary for tyrosinase activity.
- It is a cofactor for vitamin C requiring hydroxylations.
- It increases HDL and so protects the heart.



Function of ceruloplasmin.

Wilson's Disease

Ceruloplasmin level in blood is drastically reduced in Wilson's hepatolenticular degeneration. The basic defect is in a gene encoding a **copper binding ATPase** in cells (*ATP7B* gene in liver cells). This is required for normal excretion of copper from liver cells; in its absence, copper is accumulated in cells, leading to copper deposits in liver and brain.

Copper Deficiency Anemia

- Copper is essential for the formation of hemoglobin.
- Copper containing ceruloplasmin helps in iron transport.
- Copper is an integral part of ALA synthase.
- Copper helps the uptake of iron by normoblasts.
- Copper deficiency is manifested as anemia.
- RBC count is reduced; cell size is small; but hemoglobin concentration is more or less normal. Copper deficiency thus results in **microcytic normochromic anemia**.

Cardiovascular Diseases

In copper deficiency, elastin becomes abnormal, leading to **weakening of walls** of major blood vessels. This favors aneurysm and fatal rupture of the wall of aorta.

Menke's Kinky Hair Syndrome

It is an X-linked defect (affects only male children). It is a condition in which dietary copper is absorbed from GI tract; but cannot be transported to blood due to absence of an intracellular **copper binding ATPase** (mutation in *ATP7A* gene). The copper that has entered into the cell is not able to get out of the cells, and so it accumulates. Hence copper is not available for metabolism, resulting in defective cross-linking of connective tissue.

Melanin

Copper is present in tyrosinase which is necessary for melanin formation. Copper deficiency thus leads to hypopigmentation.

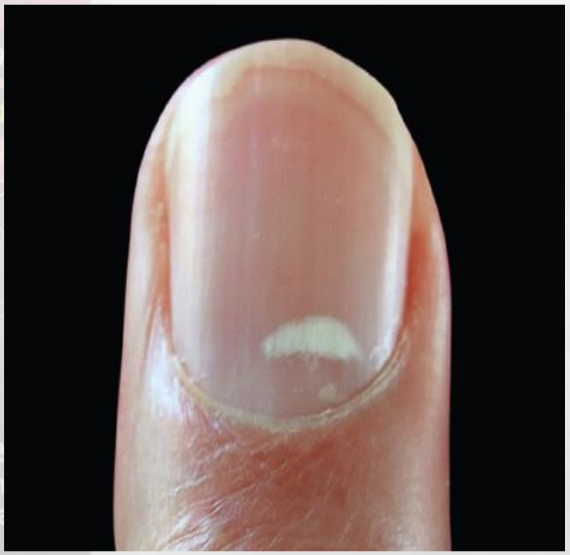
Zinc (Zn)

- Total zinc content of body is about 2 g, out of which 60% is in skeletal muscles and 30% in bones.
- Rich dietary sources are grains, beans, nuts, cheese, meat and shellfish. Copper, calcium, cadmium, iron and phytate will interfere with the absorption of zinc.
- In liver, zinc is stored in combination with a specific protein, **metallothionein**. Zinc is excreted through pancreatic juice and to a lesser extent through sweat.
- More than **300 enzymes** are zinc-dependent. Some important ones are carboxypeptidase, carbonic anhydrase, alkaline phosphatase, lactate dehydrogenase, alcohol dehydrogenase and glutamate dehydrogenase.
- RNA polymerase contains zinc and so it is required for **protein** biosynthesis. Extracellular superoxide dismutase is zinc dependent and so, zinc has **antioxidant** activity.

Zinc Deficiency Manifestations

Poor wound healing, lesions of skin, impaired spermatogenesis, hyperkeratosis, dermatitis and alopecia are deficiency manifestations of zinc. There is reduction in number of T and B lymphocytes. Macrophage function is retarded. Zinc deficiency leads to depression, dementia and other psychiatric disorders. Zinc binds with amyloid to form a plaque in **Alzheimer's** disease.

Acrodermatitis enteropathica is a recessive condition where zinc absorption is defective and is characterized by acrodermatitis (inflammation around mouth, nose, fingers, etc.), diarrhea, alopecia (loss of hair in discrete areas), ophthalmoplegia and hypogonadism. Chronic zinc deficiency is manifested as leukonychia punctata or "white spots" in finger nails.



White spots in finger nails due to zinc deficiency.

Requirement of Zinc

Requirement for adult male 5–10 mg/day and for adult female is 4–7 mg/day; in pregnancy and lactation 15–20 mg/day. Iron inhibits absorption of zinc.

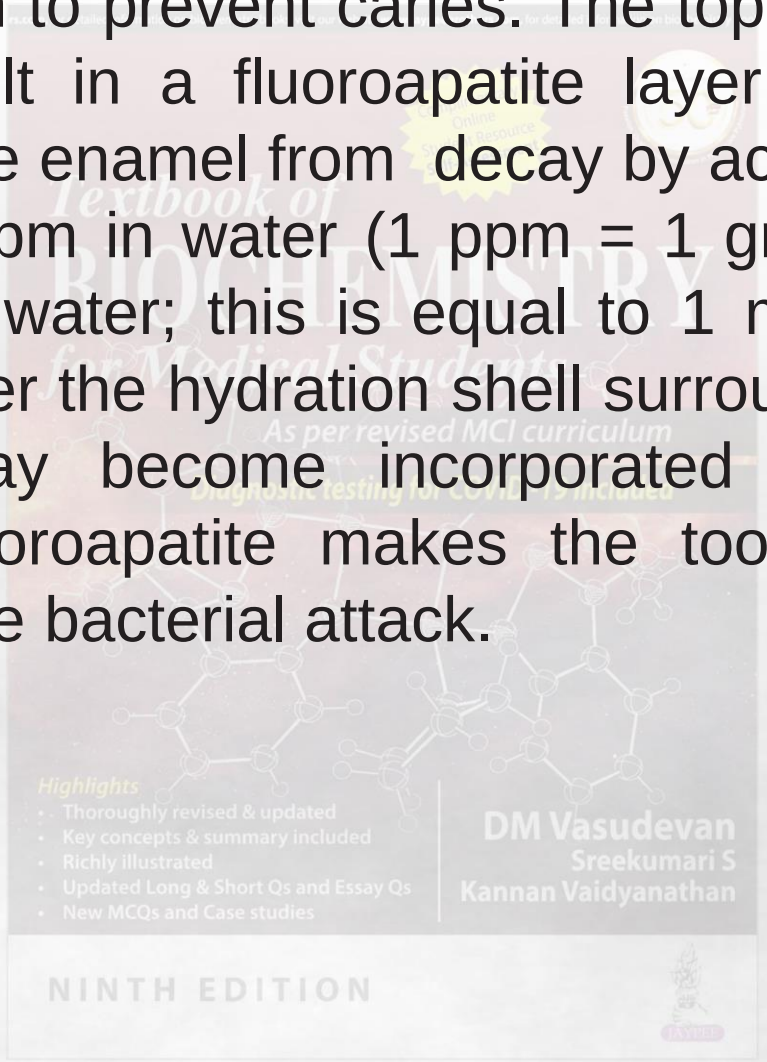
Zinc Toxicity

Toxic manifestations are seen when intake is >1,000 mg/day. Toxicity of zinc is usually seen in welders due to inhalation of zinc oxide fumes. Many rat poisons contain zinc compounds, which lead to accidental poisoning. Acute toxicity is manifested as fever, excessive salivation, headache and anemia.

Chronic toxicity may produce gastric ulcer, pancreatitis, anemia, nausea, vomiting and pulmonary fibrosis.

Fluoride

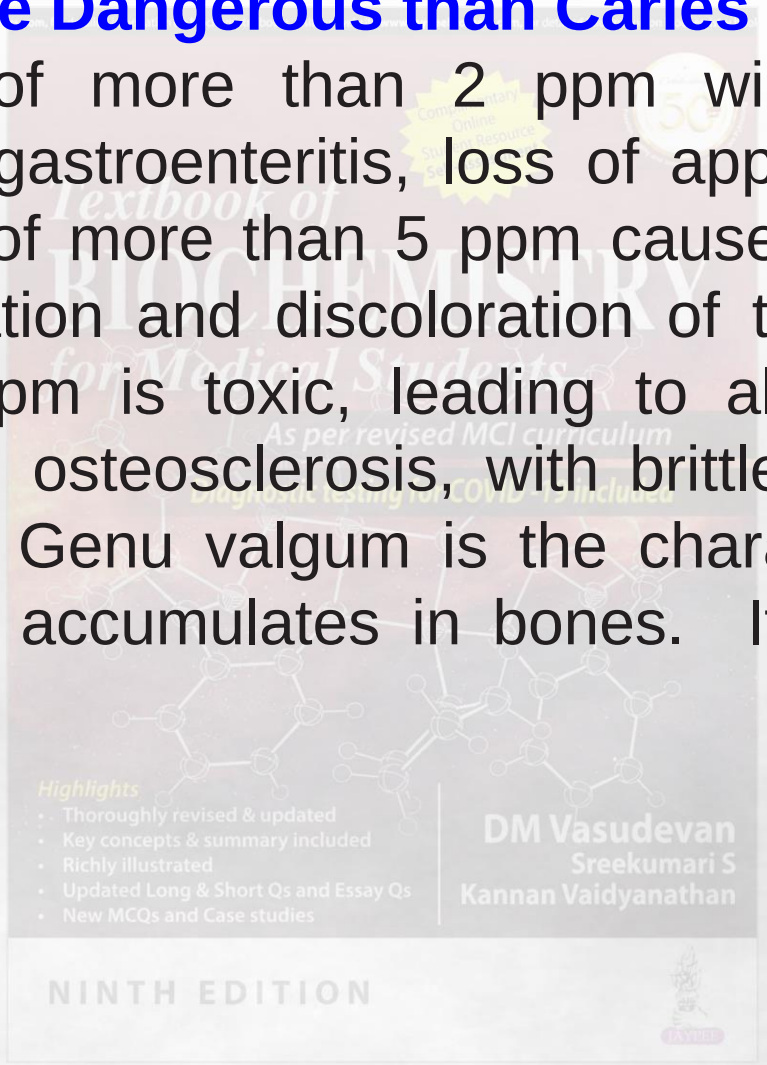
Fluoride is known to prevent caries. The topical application of fluoride will result in a fluoroapatite layer on the enamel, which protects the enamel from decay by acid. The safe limit of fluorine is 1 ppm in water (1 ppm = 1 gram of fluoride in million grams of water; this is equal to 1 mg per 1000 ml). Fluoride ions enter the hydration shell surrounding the apatite crystals and may become incorporated into the crystal surface. The fluoroapatite makes the tooth surface more resistant to plaque bacterial attack.



Fluoride

Fluorosis is More Dangerous than Caries

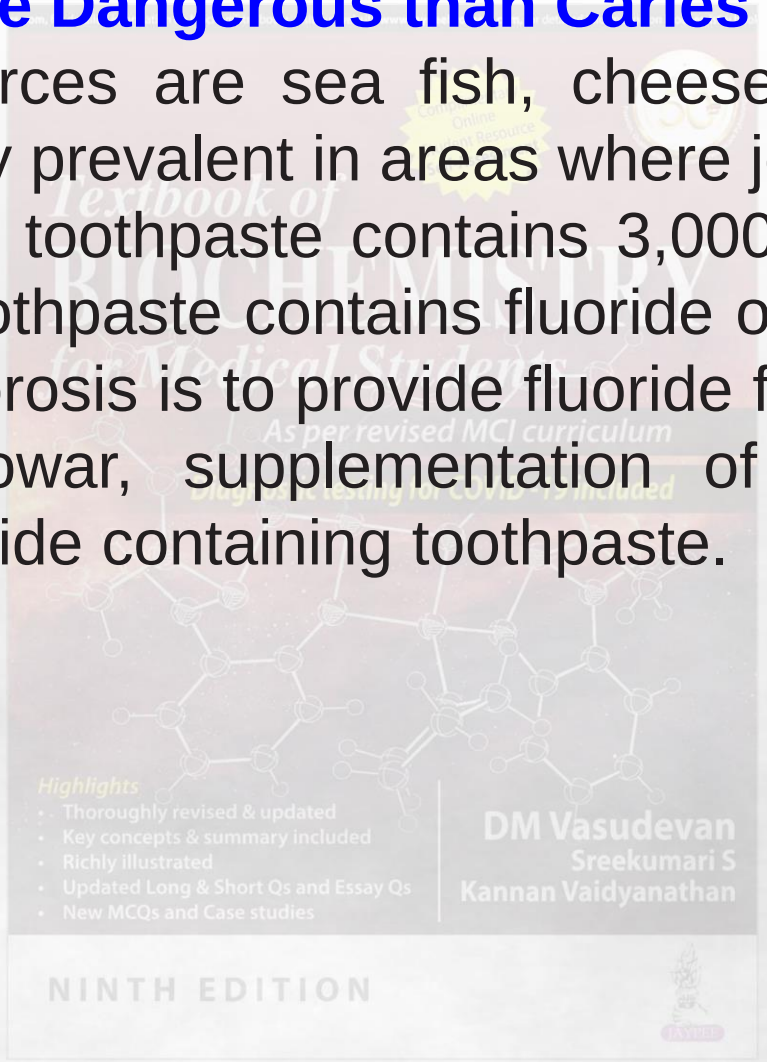
Fluoride levels of more than 2 ppm will cause chronic intestinal upset, gastroenteritis, loss of appetite and loss of weight. A level of more than 5 ppm causes the mottling of enamel, stratification and discoloration of teeth. A level of more than 20 ppm is toxic, leading to alternate areas of osteoporosis and osteosclerosis, with brittle bones. This is called fluorosis. Genu valgum is the characteristic feature. Ingested fluoride accumulates in bones. It is a cumulative toxin.



Fluoride

Fluorosis is More Dangerous than Caries

Fluoride-rich sources are sea fish, cheese, tea and jowar. Fluorosis is highly prevalent in areas where jowar is the staple diet. Fluorinated toothpaste contains 3,000 ppm of fluoride. Even ordinary toothpaste contains fluoride of about 700 ppm. Prevention of fluorosis is to provide fluoride free water, restrict the intake of jowar, supplementation of vitamin C and regulation of fluoride containing toothpaste.



Selenium (Se)

The UGA codon is acting as the codon for direct insertion of **seleno-cysteine** into selenium containing enzymes. Seleno-cysteine is directly incorporated into the protein during biosynthesis.

The requirement is 50–100 μg/day.

Normal serum level is 50–100 μg/dL.

Functions of Selenium

- In mammals, **glutathione peroxidase** (GP) is the important selenium containing enzyme. RBC contains good quantity of glutathione peroxidase.
- Thyroxin is converted to T3 by 5'**-de-iodinase** which is a selenium containing enzyme. In Se deficiency, this enzyme becomes less active, leading to hypothyroidism.
- Selenium acts as a nonspecific intracellular **antioxidant**. This action of Se is complementary to vitamin E. Availability of vitamin E reduces the selenium requirement. In Se deficiency, tissue vitamin E content is depleted.

Selenium (Se)

- In Keshan province in China, the soil is deficient in selenium. This leads to prevalence of **Keshan disease**. It is characterized by multifocal myocardial necrosis, cardiac arrhythmias and cardiac enlargement. Selenium is known to cure the disease.
- Isolated selenium deficiency in other parts of the world caused liver necrosis, cirrhosis, **cardiomyopathy** and muscular dystrophy.
- Selenium toxicity is called **selenosis**. Selenium is present in metal polishes and anti-rust compounds. The toxicity symptoms include hair loss, falling of nails, diarrhea, weight loss, and garlic breath (due to the presence of dimethyl selenide in expired air).

Manganese (Mn)

Total body manganese is 15 mg. Maximum concentration is in liver (1.5 ppm). **The requirement** of manganese is 5 mg/day.

Sources: Nuts are good sources and tea leaves are exceptionally rich in manganese.

Metabolism

The absorption is inhibited by iron. In blood, manganese is bound to the specific carrier protein, **transmanganin**. Manganese is excreted through bile.

Functions of Manganese

The following enzymes r are activated by manganese: Hexokinase, phosphoglucomutase, pyruvate carboxylase, isocitrate dehydrogenase, succinate dehydrogenase, arginase, glutamine synthetase and Mn dependent superoxide dismutase. Manganese is an integral part of glycosyl transferases. Mn is also required for RNA polymerase activity.

Lithium (Li)

- Lithium is used in treating manic depressive psychosis (bipolar disorders), the dose being 25–500 mg/day. Lithium will counteract both mania and depression.
- Therapeutically optimum concentration of Li in plasma is 7–10 µg/mL, while 12 µg is toxic.
- Since margin of safety is narrow, the treatment requires constant monitoring of blood level.
- Li causes inhibition of inositol phosphatase, leading to increased concentration of inositol phosphate in brain.
- Li elevates serotonin levels and reduces catecholamines in brain tissue.
- Lithium toxicity leads to hypothyroidism, hyperparathyroidism and kidney damage.

	Requirement for adult male / day	Blood level
Calcium	1000 mg	8.5-10.5 mg/dL
Phosphorus	500 mg	3.4-4.5 mg/dL
Magnesium	400 mg	1.8-2.2 mg/dL
Sodium	5-10 g	136-145 mEq/L
Potassium	3-4 g	3.5-5 mEq/L
Chloride		96-106 mEq/L
Iron (plasma)	20 mg	120 microg/dL
Copper	1.5-3 mg	100 microg/dL
Iodide	200 microg	10 microg/dL
Zinc	10 mg	100 microg/dL
Chromium	50 microg	25 nanogram/dL
Selenium	100 microg	100 microg/dL