Estimation of serum calcium by trinder's method

Introduction

99% calcium -- Bones and teeth

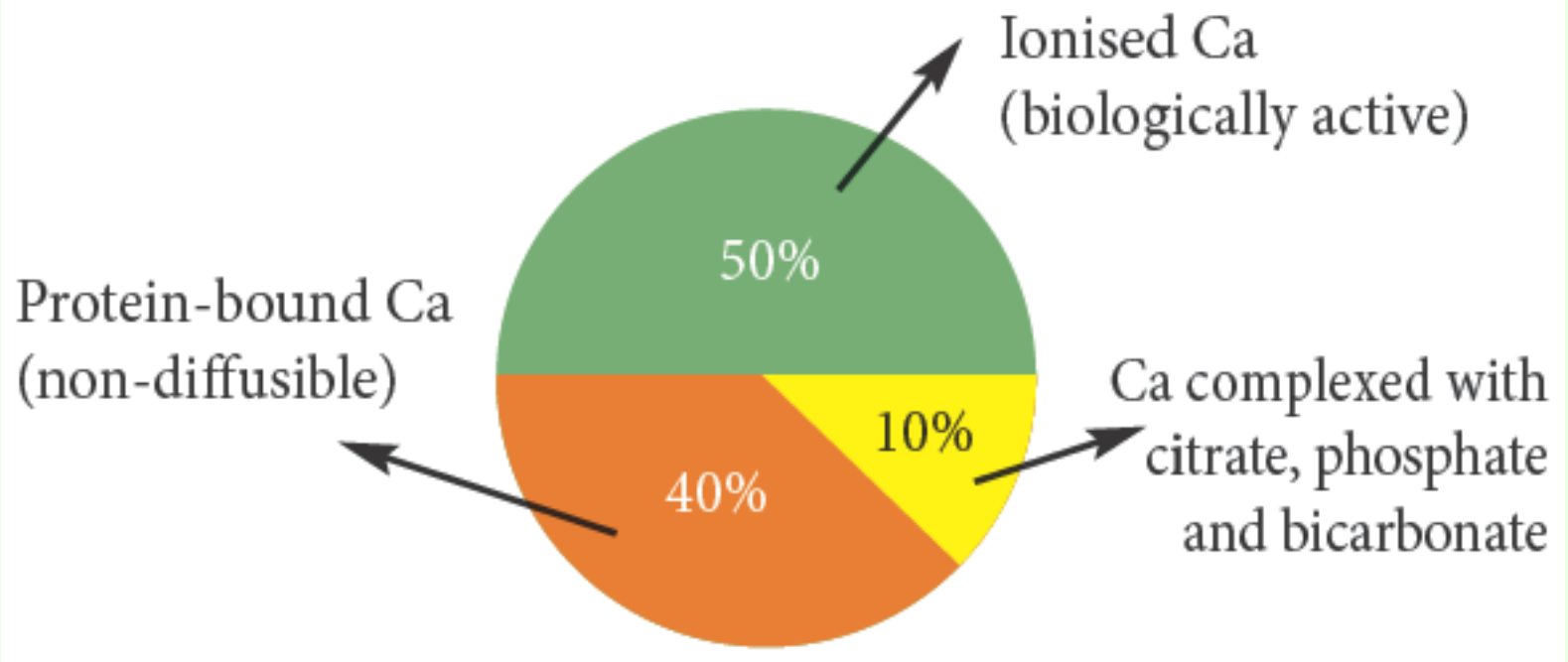
1% calcium – extracellular fluids

Normal blood level

Normal calcium level in plasma 9–11 mg/dl

Ionised calcium

About 5 mg/dl of calcium is in the ionised form and is metabolically active.



Different proportions of various forms of circulating calcium.

BIOCHEMICAL FUNCTIONS

1. Development of bones and teeth
2. Muscle contraction
3. Blood coagulation
4. Nerve impulse transmission
5. Activation of enzymes
6. Calcium as second messenger
7. Membrane permeability
8. Release of hormone

Factors promoting calcium absorption

i) Vitamin D
ii) Parathyroid hormone
iii) Acidity
iv) Amino acids
v) Lactose

Factors inhibiting calcium absorption

i) Phytic acid

ii) Oxalates

iii) Malabsorption syndrome

iv) Phosphate

v) Alkalinity

REGULATION OF BLOOD CALCIUM LEVELS

There are effective controls to maintain the narrow range (9–11 mg/dl) of blood-calcium. The *hormones–calcitriol, parathyroid hormone (PTH)* and *calcitonin* play a major role in blood calcium homeostasis.

VITAMIN D

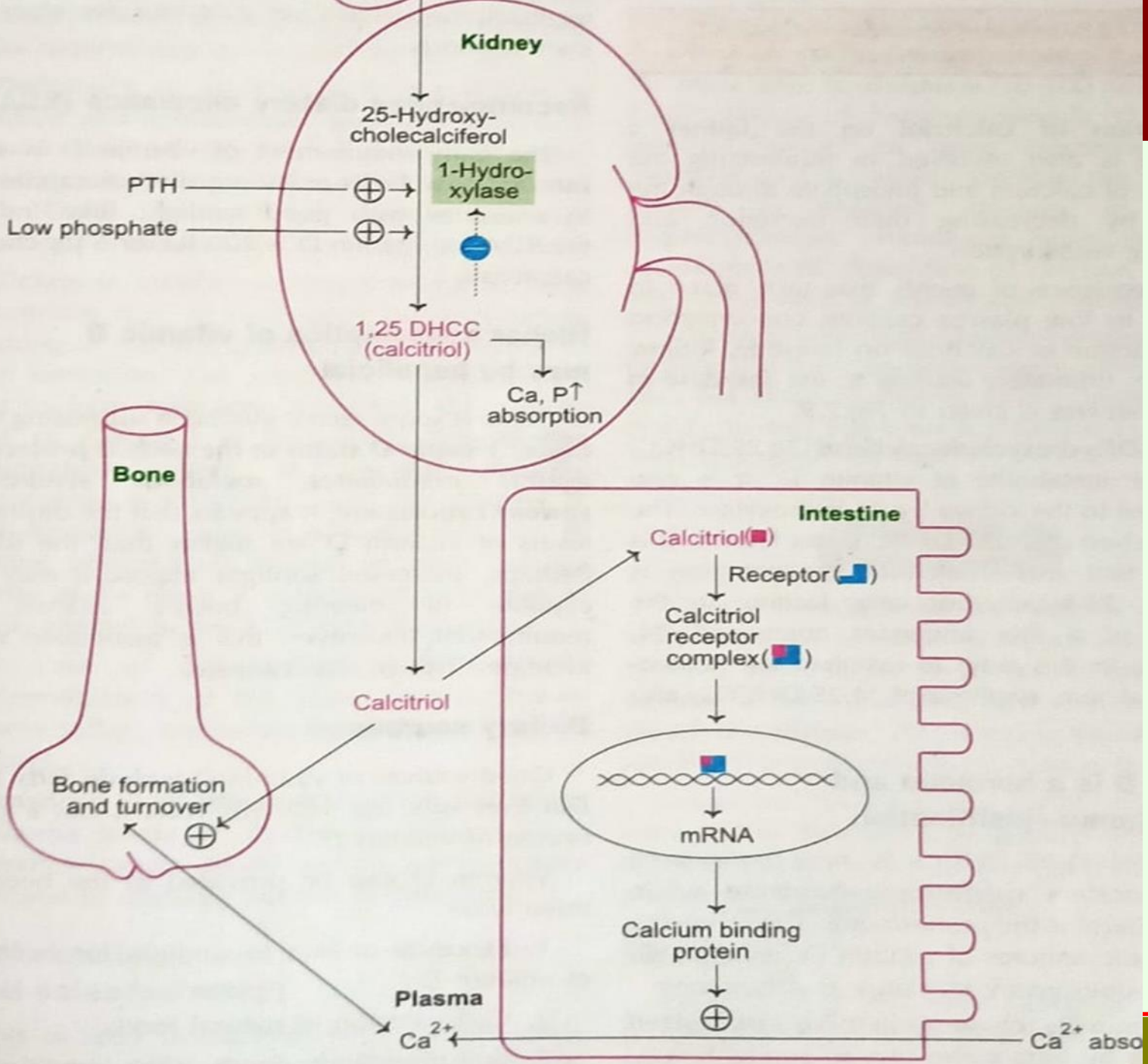
- Vitamin D and intestinal absorption of calcium
- Vitamin D and bone
- Vitamin D and renal tubules

PARATHYROID HORMONE (PTH)

- PTH on bones
- PTH on kidney
- PTH on intestine

Calcitonin

- Calcitonin, calcitriol and PTH act together to achieve calcium homeostasis


Kidney
25-Hydroxy-
cholecalciferol
PTH
1-Hydro-
xylase
Low phosphate
1,25 DHCC
(calcitriol)
Ca, P↑
absorption
Bone
Intestine
Calcitriol
Receptor
Calcitriol
receptor
complex
Calcitriol
mRNA
Bone formation
and turnover
Calcium binding
protein
Plasma
Ca2+
Ca2+ abso

DISEASE STATES

Hypercalcemia

Increased serum Ca level is associated with

Hyperparthyroidism

Hypervitaminosis D

Multiple myeloma

Metastasis of bone

Padgets disease

Hypocalcemia

Hypoparathyroidism

⇩ *vit D (Rickets ,Osteomalecia)*

Malnutrition, malabsorption

Advanced renal failure

Pancreatitis

Level below 7mg % –Tetany

Severe convulsions –could be fatal

Treated by 10% calcium gluconate –IV (10 min)

Principle

Naphthol hydroxamic acid + calcium (RT-30min) → (centrifuge) → calcium napthtyl hydroxamate (PPT)

↓ EDTA (boining waterbath)

Dissolved ppt + ferric nitrite → Reddish brown coloured complex

↓

OD at 470nm

PROCEDURE

Label 3 test tube as blank ,standerd and test /unknown

Reagents	Blank	Std	Unknown
Disttiled Water	0.2 ml		
Calcium STD		0.2 ml	
Serum			0.2 ml
Calcium reagent	5ml	5ml	5ml
Mix well and stand at RT for 30 min then centrifuge and decant the supernatant by inverting tubes.			
EDTA	1ml	1ml	1ml
Shake well and keep it in boiling water bath for 10 min To dissolve the ppt .cool the tubes			
Colour reagent	3ml	3ml	3ml
Mix well and read OD at 470 nm			

CALCULATION

Concentration of calcium in sample =

$$\frac{\text{O.D of unknown -O.D. of blank}}{\text{O.D of Std -O.D. of blank}} \times \text{conc of Std/ml} \times \text{vol of Std} \times \frac{100}{\text{vol of serum}}$$

$$\frac{U-B}{S-B} \times 0.1 \times 0.2 \times \frac{100}{0.2}$$

$$\frac{U-B}{S-B} \times 10$$

Otrher methods

1.Arsenazo method

2.OCPC –O-cresolphtalein complexone method