Calcium and phosphate ions

Biochemistry II
Lecture 11

2008 (J.S.)

Calcium distribution and turnover

Daily intake
about. **25 - 30 mmol** / d

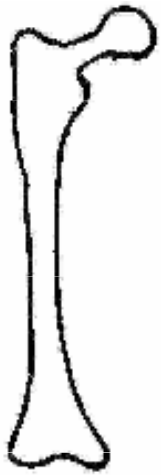
phosphates,
oxalates,
phytates

absorption
< 15 mmol / d

secretion
up to 7 mmol / d

17 – 25 mmol / d

Mineral deposits
25 mol Ca
(rapidly exchangeable
100 mmol)



bone remodelation
7.5 mmol / d

rapid exchange
500 mmol / d

ECT
2.5 mmol / l
total only 35 mmol

ICT
signaling function
0.1 µmol / l

filtration
240 mmol / d

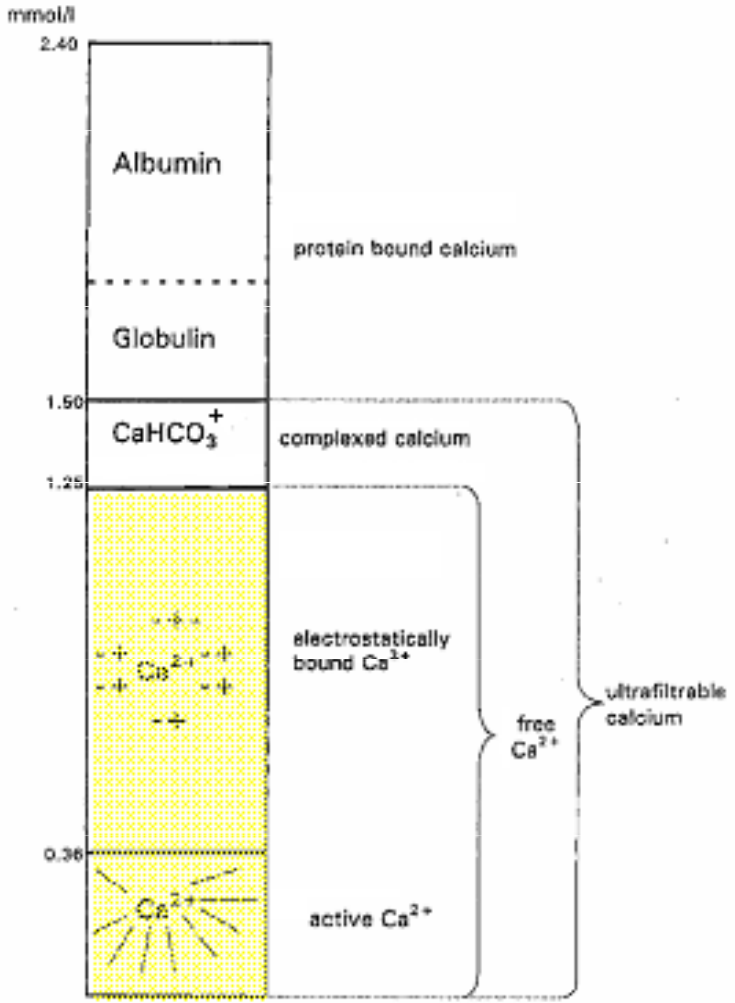
tubular resorption
230 mmol / d

Excretion fraction max. 5 %
decreased by PTH,
enlarged by calcitonin and
high supply of Na^+

5 – 6 mmol / d

Blood serum calcium

The biologically active fraction is ionized Ca^{2+} (free Ca^{2+} ions).



Chemical methods (photometry, AAS) estimate **total serum calcium**.

Approximately one half (~ 46 %) of Ca^{2+} is bound to carboxyl groups of proteins – **non-diffusible calcium**.

A small fraction (2 - 5 - 10 %) of calcium is bound in chelate complexes with carboxylate anions (citrate, lactate, oxalate, etc.), slightly also with HCO_3^- and phosphates – **non-ionized diffusible calcium** (usually not taken into account).

Free Ca^{2+} ions - ionized calcium - are decisive for an assessment of the calcium accessibility.

Total serum calcium S-Ca – mean value **2.5 mmol/l**,
reference range 2.25 – 2.75 mmol/l .

Ionized calcium S-[Ca^{2+}] – mean value **1.25 mmol/l**,
reference range 1.0 – 1.4 mmol/l .

Conclusions deduced from the values of total calcium may be erroneous, unless account is taken to protein (namely albumin) concentration and abnormal pH value.

Normal total Ca concentration doesn't exclude a change in ionized Ca^{2+}, abnormal total Ca can be accompanied by a normal value of ionized Ca^{2+}.

Protein-bound calcium increases if **plasma protein concentration**, particularly of albumin, is high.

Influence of pH can be understood as a competition for Ca^{2+} binding sites between H^+ and Ca^{2+} ions. pH value changed by 0.1 corresponds to the change in [Ca^{2+}] by approximately 0.02 mmol/l.

Calcium homeostasis
– hormonal control of plasma calcium concentration

PARATHYRIN

(**PTH**, the older name parathormone is currently taken as an improper one)

PTH consists of 84 amino acid residues (prepro-PTH 115 AA, pro-PTH 90 AA). Biological activity is exhibited by the N-terminal sequence, the initial 28 residues.

Plasma intact PTH (1-84) **–** the reference range **1 - 5 pmol** / **l –** is degraded rapidly in the liver (half-life 3 – 5 min) by splitting off the inactive C-terminal segments (35-84).

PTH secretion is regulated by plasma Ca^{2+} concentration:

hypercalcaemia inhibits the release of PTH,
secretion of PTH is stimulated in hypocalcaemia.

Phosphates don't have a direct influence.

The calcium sensor is a receptor cooperating with G_q proteins. In contradistinction to other secreting cells, an increase in intracellular Ca^{2+} due to IP_3 inhibits secretion of PTH.

High level of intact PTH is usual in primary hyperparathyroidism, the secretion is not suppressed by hypercalcaemia.

Biological effects of PTH

In bone,

– PTH **stimulates bone resorption** through differentiation and activation of osteoclasts. This effect is mediated by osteoblasts that, after PTH binding, expose one of the ODF proteins. Those proteins are recognized by the receptors of osteoclasts and initiate the activation.

In the renal tubules,

– **the Ca^{2+} reabsorption increases** increasing so calcaemia and decreasing calciuria (but at a high Ca^{2+} supply, the maximal transport capacity of the tubular cells is exceeded and excretion of Ca^{2+} increases);

– **the HPO_4^{2-} reabsorption decreases** and so excretion of phosphate increases; in hyperparathyroidism, there is also the **decrease of HCO_3^- reabsorption** that may be the cause of a mild hyperchloridaemic metabolic acidosis.

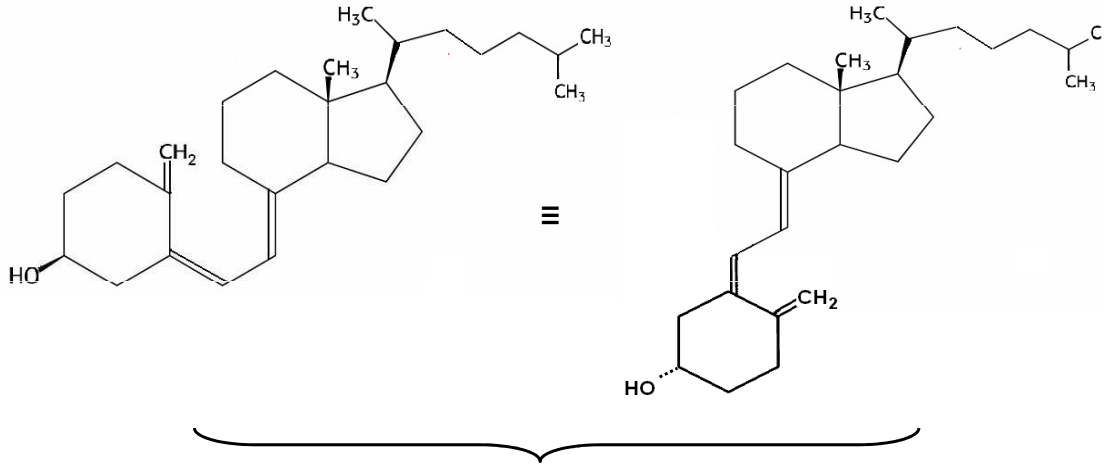
PTH exhibits an **indirect effect on the intestinal mucosa**:

– **increased Ca^{2+} absorption** results from **stimulation of the 1α-hydroxylation of calcidiol** to calcitriol in the renal tubules.

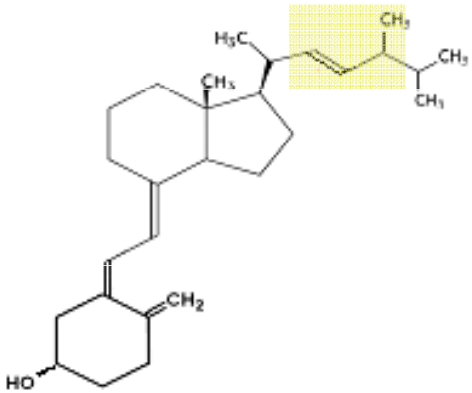
CALCIOL (calciferol, vitamin D_3)

and **ercalciol** (ergocalciferol of plant origin, D_2) are present in the diet. In addition, calciol is synthesized from 7-dehydrocholesterol in the skin due to UV irradiation.

The daily requirement for calciols is 5 – 20 μg/d (200 – 800 IU/d).



Calciol (cholecalciferol, vitamin **D_3**)



Ercalciol (ergocalciferol, vitamin **D_2**)

The calciols are 9,10-*seko*steroids, in which the ring B is opened.

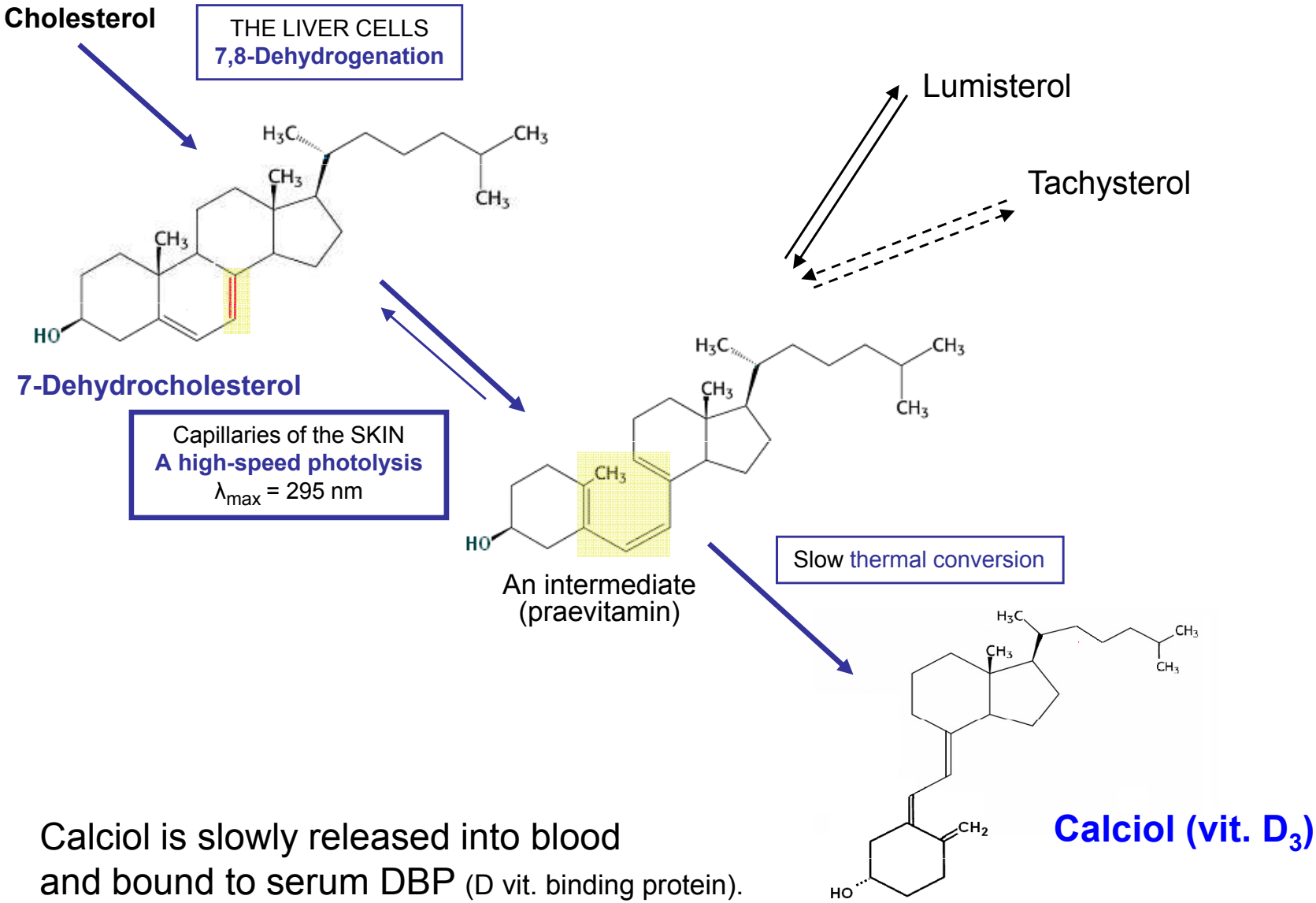
Most natural foods have a low content of **vitamin D_3**. It is present in egg yolk, butter, cow's milk, beef and pork liver, animal fat and pork skin.

The most important source of **vitamin D_2** is fish oil, primarily liver oil.

The **D-provitamins**, 7-dehydrocholesterol and ergosterol, are widely distributed in the animals and plants.

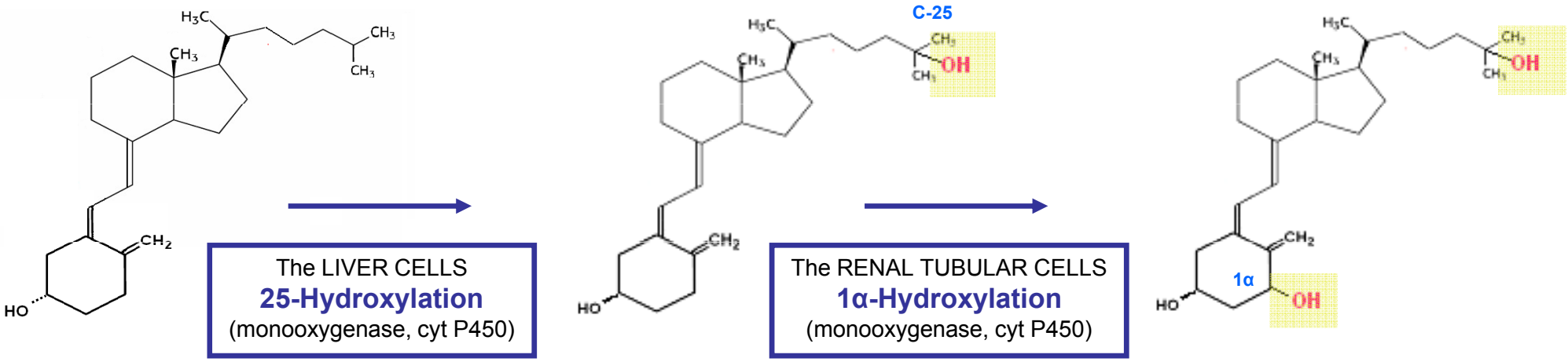
Calciols are inactive **precursors of calcitriol**, a calciotropic steroid hormone.

Calciol (vit. D_3) is synthesized from **7-dehydrocholesterol** by photolysis that is a cause of opening of the ring B:



Calciol is slowly released into blood and bound to serum DBP (D vit. binding protein).

The hydroxylation of calciols



Calciol
(Cholecalciferol)

Calcidiol
(25-Hydroxycholecalciferol)

Calcitriol
(1α,25-Dihydroxycholecalciferol)
A CALCIOTROPIC STEROID HORMONE

In the **liver**, calciol is hydroxylated to **calcidiol** (25-hydroxycalciol).

25-hydroxylation of calcidiol is inhibited by the high concentrations of calcidiol and calcitriol (a negative feed-back control), calcitonin, and a high intake of calcium in the diet.

In the **proximal renal tubules**, calcidiol is transformed by 1α-hydroxylation into **calcitriol** (1α,25-dihydroxycalciol).

1α-hydroxylation is stimulated by PTH (in hypocalcaemia), inhibited by high concentration of calcitriol, calcitonin, and high dietary calcium.

Calciol - the blood serum concentration in the range **2.6 - 13 nmol / l** .

Calciol is transported bound to the specific protein DBP (vit, D-binding protein, the concentration of which is less than 10 μmol/l and saturation by calciol not higher than 1 – 2 %).

Calcidiol is the major circulating metabolite of calciol. Its biological half-life is rather long, approx. 20 - 30 days.

The concentration of calcidiol in serum informs of the body calciol accessibility. Seasonal variations are observed: The average concentration

during summer about **75 nmol / l** (30 μg / l),

In winter about **37 nmol / l** (15 μg / l, i.e. a mild deficit).

Values < 20 nmol / l have to be taken as a sign of serious vitamin D deficiency.

In enormous exposition to sunlight – values up to 250 nmol / l,

in hypervitaminosis D up to 1250 - 2500 nmol / l.

Calcitriol – its biological half-life is short,

serum concentration in children about **160 pmol / l**,

in adults **3 - 580 pmol / l** .

Biological effects of calcitriol:

In the enterocytes, it increases resorption of calcium into ECF in all three distinct steps:

- Entrance into the cell. There are no special transporters for calcium, calcitriol induces a **change in the binding of calmodulin to brush border myosin** (it forms a complex with a special type of myosin that is bound to membrane actin). The complex may remove calcium from the brush border after it crosses the membrane. Changes in the phospholipid composition of the brush border also may explain the flux of calcium across this membrane after calciol administration.
- Calcitriol **induces the synthesis of cytosolic protein calbindin** (CaBP, calcium-binding protein) after a lag-period about 20 h. CaBP seems to prevent rapid increase in Ca^{2+} by mediating rapid Ca^{2+} transport in cytoplasm.
- Calcitriol **induces the synthesis of Ca^{2+}-ATPase** that removes Ca^{2+} from the cell into ECF.

In bone, calcitriol **controls both resorption and formation** by means of induction of the synthesis of numerous proteins in osteoblasts and odontoblasts. The control mechanism is not yet quite clear.

The effect of calcitriol depends on the degree of osteoblast differentiation:

- In immature osteoblasts, calcitriol **supports differentiation pathway to mature, fully functional osteoblasts.**
- In mature osteoblasts, calcitriol **induces the synthesis of osteocalcin** and (besides PTH and certain cytokines) **production of osteoclast differentiation factors** (ODF) so that it also take part in maintaining of physiologic levels of Ca^{2+} and phosphates in ECF.

Osteocalcin (BGP - bone Gla-protein) is a small conservative protein (49 AA residues) that has three γ-carboxyglutamate (Gla) centres able to bind Ca^{2+}. Its function depends on the presence of vitamin K.

Bone contains 50 - 100 μg osteocalcin per gram of dry mineralized tissue (more than 20 % non-collagen bone proteins).

Osteocalcin concentration in plasma is 5 - 10 μg / l, its biological half-life only 4 – 5 min.

Osteocalcin takes part in the control of bone mineralization. It seems to **slow down mineralization** of the bone matrix by binding onto collagen (preventing so Ca^{2+} and phosphate leakage from plasma into bone), on the other hand osteocalcin **retards bone resorption** in epiphysis.

Concentration of plasma osteocalcin is one of the biochemical **markers of bone remodelation** (turnover), perharps also of osteoblast activity in formation of organic bone matrix. In sole osteoresorption, increase in osteocalcin concentration is not observed.

CALCITONIN

is a 32 amino acid peptide secreted by the C-cells of the thyroid gland.

It is classified as a neuropeptide, because it played a role of neurotransmitter during the evolution.
In humans, calcitonin is synthesized only in the thyroid gland.

> Calcitonin structure differs in animal species. Rat calcitonin resembles human one (only 2 different amino acids), the most effective calcitonin is that of salmon (50 % homology with the human calcitonin). In the therapy of metabolic osteopathies, the salmon, human, or the dicarba analogue of eel calcitonin are used.

Plasma calcitonin about **13 - 27 pmol / l** (50 - 100 ng / l),
in women over 50 years of age the values are lower (high risk of osteoporosis).

Secretion of calcitonin is **controlled by calcaemia**. The calcium sensor is of the same kind as in parathyroid glands, but in the thyroid C-cells the increase in intracellular Ca^{2+} is the signal for secretion.

> **Procalcitonin** (116 AK, calcitonin sequence in position 60-91) is split to calcitonin and catacalcin in the C-cells and not secreted normally.
> In sepsis (not in localized infections), procalcitonin is secreted in considerable amount (from neuroendocrine cells of the lung and intestine and mononuclear leukocytes obviously), without an increase in calcitonin.
> Plasma procalcitonin is a **reliable marker of sepsis.**

Biological effects of calcitonin

Calcitonin counteracts PTH in the control of Ca metabolism.
Under normal conditions, calcitonin exhibits a **limited role in calcium metabolism control**. Its effect on hypercalcaemia is not long, but acute, and it results in blockade of bone resorption.
No disturbance of calcium metabolism occurs following removal of the thyroid gland.

In bone, calcitonin **inhibits bone resorption** (if increased) by suppressing the activity of osteoclasts and **supports both synthesis of organic matrix** and **mineralization of osteoid** by a rapid activation of osteoblasts

Calcitonin has a special role in pregnancy.

Plasma concentration increases till the end of the 2nd trimester up to the double normal values and is maintained till the end of lactation.

Calcitonin primarily supports formation of calcium deposits in the bones of mother (formation of stores for the foetus), later on the effect is diminished due to increased secretion of PTH (increasing the calcium accessibility to the foetus).

In the renal tubules, calcitonin **inhibits reabsorption of Ca^{2+} and phosphates** increasing their excretion in this way.

In the GIT, it inhibits the secretion of HCl, pepsin, and pancreatic enzymes.

Calcitonin has an **analgetic effect**, it alleviates bone pain in the course of metabolic osteopathies or bone metastases.

Hypercalcaemia

Mild hypercalcaemia (< 3 mmol/l) usually don´t have clinical symptoms.
Defects of renal tubular functions, particularly polyuria, may occur.

Hypercalcaemia > **3.5 - 4 mmol/l is a serious menace**.
If hypercalcaemia is chronic, **renal functions are impaired**,
soft tissue calcifications and renal tract stones develop.

In turn, **chronic renal failure** in chronic hypercalcaemia
disturbs calcium homeostasis:
Decrease in GFR to about one third is the cause of phosphate retention and hyperphosphataemia, FE(Ca) increases, calcium loss follows, calcidiol is hydroxylated insufficiently, and intestinal calcium resorption diminished
The consequence is hypocalcaemia that induces **secondary hyperparathyroidism**.

The most usual cause of hypercalcaemia (in about 90 %) is
primary hyperparathyroidism, the second most frequent being **malignant disease**.

Primary hyperparathyroidism is usually caused by a solitary adenoma.
It is detected mostly (up to 50 %) as calcium urolithiasis or nephrocalcinosis, polyuria, osteopathy (osteodystrophy, pathological bone fractures), etc.
The diagnosis is supported by hypophosphataemia resulting from phosphate losses, and mild metabolic acidosis from losses of HCO_3^- . **Increased PTH is conclusive**.

Malignancy-related hypercalcaemia
– **osteolytic metastases** of solid tumours (lung, breast, kidney) resorb bone either directly, or (breast carcinoma) activate osteoclasts by means of local release of humoral factors.
– multiple **myeloma** (and some lymphomas)
– **tumours producing PTHrP** (namely squamous cell carcinomas of the bronchus).

Other causes of hypercalcaemia:

Calciol intoxication in excessive intake of calciol (e.g. long-lasting treatment of hypoparathyroidism, calciol overdose is released from the adipose tissue slowly, in the course of several weeks) or when calcitriol is used (immediate effect, a narrow therapeutic range).

Long-lasting immobilization intensifies osteoresorption, namely in teenagers due to high bone turnover. A complete immobilization can cause about 30 % loss of bone minerals.

Hypocalcaemia

Clinical symptoms appear when total calcium is lower than approx. **1.9 mmol / l** (ionized Ca lower than **0.9 – 1.0 mmol / l**).

Hypocalcaemia is accompanied by typical neuromuscular manifestations – paresthesiae, increased neuromuscular excitability and **tetany** (carpopedal spasms up to a state of spontaneous tonic muscular contraction).

Spurious hypocalcaemia due to hypoalbuminaemia or haemodilution should be excluded primarily.

The most common causes of hypocalcaemia:

Alkalosis – hyperventilation (artificial, hysteric reaction), too rapid infusion of HCO_3^-.

Acute complexation – multiple blood transfusions (citrate or EDTA form complexes with Ca^{2+});

Reduced parathyroid hormone action – rare primary hypoparathyroidism,

– **secondary hypoparathyroidism** – surgery on the neck is the most common form (destruction of the glands or transient ischemia).

Insufficient supply of calcium, **impairment of intestinal calcium absorption**, **failure to produce calcitriol**:

– **inadequate dietary intake** of calcium or calciol,
– **limited exposure to sunlight** (this sometimes occurs in elderly subjects, high sensitivity in immigrants from Asian tropical zones),
– **malabsorption** of liposoluble compounds (diseases of pancreas, insufficient bile production, intestinal by-pass),
– **chronic renal failure** (insufficient 1α-hydroxylation of calcidiol).

In adults, insufficient calcium intake results in **osteomalacia** (bands of decalcification, affecting particularly the pelvis, femur, and scapula), in children in **rickets** (**rachitis**, skeletal deformities and muscle weakness).

Urinary calcium excretion dU-Ca or fU-Ca / creatinine ratio
is not a basal test – calciuria occurs usually in all hypercalcaemias.

Calciuria increases in
– high monosaccharide intake,
– deficits of Mg and phosphates, and
– in starving.

Hypercalciuria – excretion > 7.5 mmol / d (300 mg / d) in men,
> 6.2 mmol / d (250 mg / d) in women

is the natural consequence of all hypercalcaemias.
(but it may also occur at normal calcium levels or in hypocalcaemia).

– Primary hyperparathyroidism
– "Idiopathic" hypercalciurias (at normal calcaemia, inherited?), either a hyperabsorptive type or a renal type

Phosphate distribution and turnover

Daily intake
about. **30 - 80 mmol** / d

Al^{3+} and calcium

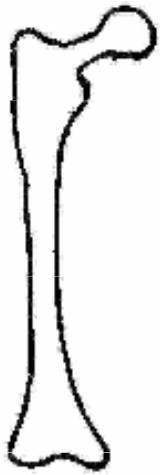
absorption
15 - 55 mmol / d

ECT (total only 55 mmol)
1.0 mmol / l inorg. phosphate
2.5 mmol / l phosphate esters

ICT (15 – 19 % of total content)
1 mmol / l inorg. phosphate
100 mmol / l organic esters

exchange
8 - 15 mmol / d

Mineral deposits
15 - 25 mol PO_4^{3-}
(80 – 85 % total P)



filtration
> 130 - 200 mmol / d

tubular reabsorption
> 110 - 150 mmol / d

Excretion fraction < 25 %
(children < 15 %)
increased by PTH or calcitonin

10 – 40 mmol / d

13 – 40 mmol / d

Phosphate homeostasis

is **maintained by urinary excretion of phosphate**
that **depends on reabsorption of phosphate in renal tubules**.

Filtration of free P_i (about 90 %) is complete, from which 50 – 70 % is reabsorbed in the proximal tubule (specific active co-transport with Na^+, inhibited by PTH), about 10 – 20 % in the distal parts of the nephron.

Of the 240 mmol of phosphate filtered daily, about 85 % (80 – 97 %) is reabsorbed.

Excretion fraction $EF_{(phosphate)}$ – in adults **less than 20 %**,
in children less than **15 %**.

Increased phosphate excretion (decrease in tubular phosphate reabsorption)
is caused by **PTH** and **calcitonin**,
– by **acidosis** (hyperphosphataemia, protons excreted as $H_2PO_4^-$),
– and indirectly (through hypocalcaemia that stimulates PTH secretion)
by **alkalosis** or **increased ECF volume**.

Retention (**decreased excretion**) **of phosphate** (increased tubular phosphate reabsorption) is caused
– by somatotropin (decrease in ECF volume) and corticosteroids,
– to a lesser degree, by calcitriol and phosphate deficiency.

Fasting serum inorganic phosphate in adults **0.8 – 1.3 mmol / l**,
in children **1.6 – 2.2 mmol / l**.

Plasma concentration is 0.06 – 0.10 mmol/l lower than that of serum due to the release of intracellular phosphate from platelets and erythrocytes during clotting:
(serum should be separated from the coagulum without delay).

In healthy adults, there is a marked **diurnal variation** (range 12 – 22 %) in phosphataemia – the lowest being in the morning and the highest during the night (this variation is abolished by fasting).
Postprandially, phosphate concentration tends to decrease presumably because of insulin release (anabolic action of insulin). Therefore, samples for serum phosphate estimation should be taken in the fasting state in the morning.

Great and rapid changes in serum phosphate concentration may have their causes in the **shifts of phosphates between ICF and ECF** that depend on the **nutritional status (the energy charge of the cells)**.

Urinary phosphate excretion

depends considerably on dietary intake of phosphates.

At the average daily intake of phosphate (about 50 mmol/d),
dU-P_i is in the range **13 - 29 mmol / d** in healthy adults.

Measurements of tubular phosphate reabsorption are important in **assessing parathyroid function** (diagnosis of primary hyperparathyroidism and other hypophosphataemias).

The **threshold phosphate concentration** is thought to be the best parameter – the theoretical phosphate concentration in glomerular filtrate (equal to plasma) below which all phosphate is reabsorbed and above which phosphate is excreted in urine: the **ratio of maximal tubular reabsorption** to **glomerular filtration rate**

TmP / GFR (normal range **0.74 – 1.2 mmol / l**).

It can be easily determined using fasting urinary and serum concentrations of phosphate and creatinine and the Walton's nomogram.

In primary hyperparathyroidism, TmP/GFR is considerably lower than 0.6 mmol/l, and higher than 1.2 mmol/l in hypoparathyroidism.

Hyperphosphataemia

Even a mild form (> 1.6 mmol/l) is a rare finding among hospital patients (1.5 %).

Consequences – Acute hyperphosphataemia can lead to hypocalcaemia.

In chronic hyperphosphataemia, the low production of calcitriol may lead to bone changes (osteomalacia or rickets) or to soft tissue calcification in myocardium, lungs, and the liver.

Artificial (spurious) hyperphosphataemia may result from haemolysis due to the release of intracellular phosphate from red blood cells and platelets.

Causes of hyperphosphataemia

Reduced urinary excretion

– **Renal failure** accounts for more than 90 % in hospital patients; retention of phosphate occurs when the GFR falls below 20–30 % of normal and may be a cause of secondary hyperparathyroidism.

– **Hypoparathyroidism** – increased tubular phosphate reabsorption.

Excess phosphate administration to patients on parenteral and enteral nutrition, also in sucklings nourished with undiluted cow's milk.

Shift of intracellular phosphate into the ECF is seen in

– **hypercatabolic states** and in **metabolic acidosis** (untreated lactic acidosis and diabetic ketoacidosis) due to increased ATP breakdown and tissue hypoxia,

– excessive **haemolysis**.

Hypophosphataemia

is much **more frequent** than hyperphosphataemia.

Mild and moderate hypophosphataemia (**0.6 - 0.8 mmol / l**)
is quite common in hospital patients (14 – 39 %, namely in acutely ill, malnourished, or with diabetic ketoacidosis), but it is rather exceptional in out-patients (incidence 0.9 %).

In moderate hypophosphataemia of long duration, **skeletal changes** (i.e., rickets or osteomalacia) are the only consistent abnormalities.

Severe hypophosphataemia (in particular < **0.3 mmol / l**) is rare, but if it is of at least four days duration, important consequences may be seen:

> **haemolysis** due to decreased 2,3-BPG and ATP in erythrocytes,
> muscular weakness that may result in **respiratory failure**,
> **impaired cardiac contractility** and **nervous system disorders.**

Hypophosphataemia does not always indicate intracellular phosphate deficiency, phosphate depletion may be present with normal or even increased phosphataemia.

The most common causes of hypophosphataemia:

Haemodilution – water retention, parenteral nutrition.

Reduced absorption

- **Low dietary intake** is an unusual cause (strict vegetarians) as phosphate occurs widely in foods,
- **oral phosphate-binding agents** (Al^{3+}-containing or other antacids in excessive quantities),
- **diarrhoea** or long-lasting **vomiting**,
- **malabsorption**.

Increased uptake of phosphate into cells

- **Administration of glucose** (both intravenous and enteral) or hyperalimentation leads to increased insulin concentration, increased formation of phosphorylated intermediates leads to the shift of phosphate into the cells. Life-threatening hypophosphataemia may occur in rapid realimentation of starved patients ("**refeeding syndrome**"), treatment of diabetic ketoacidosis, alcoholics during alcohol withdrawal and glucose refeeding.

Increased phosphate elimination

- **Decreased renal tubular reabsorption** due to high **PTH** secretion in hyperparathyroidism, rickets and osteomalacia.
- **Haemodialysis**.