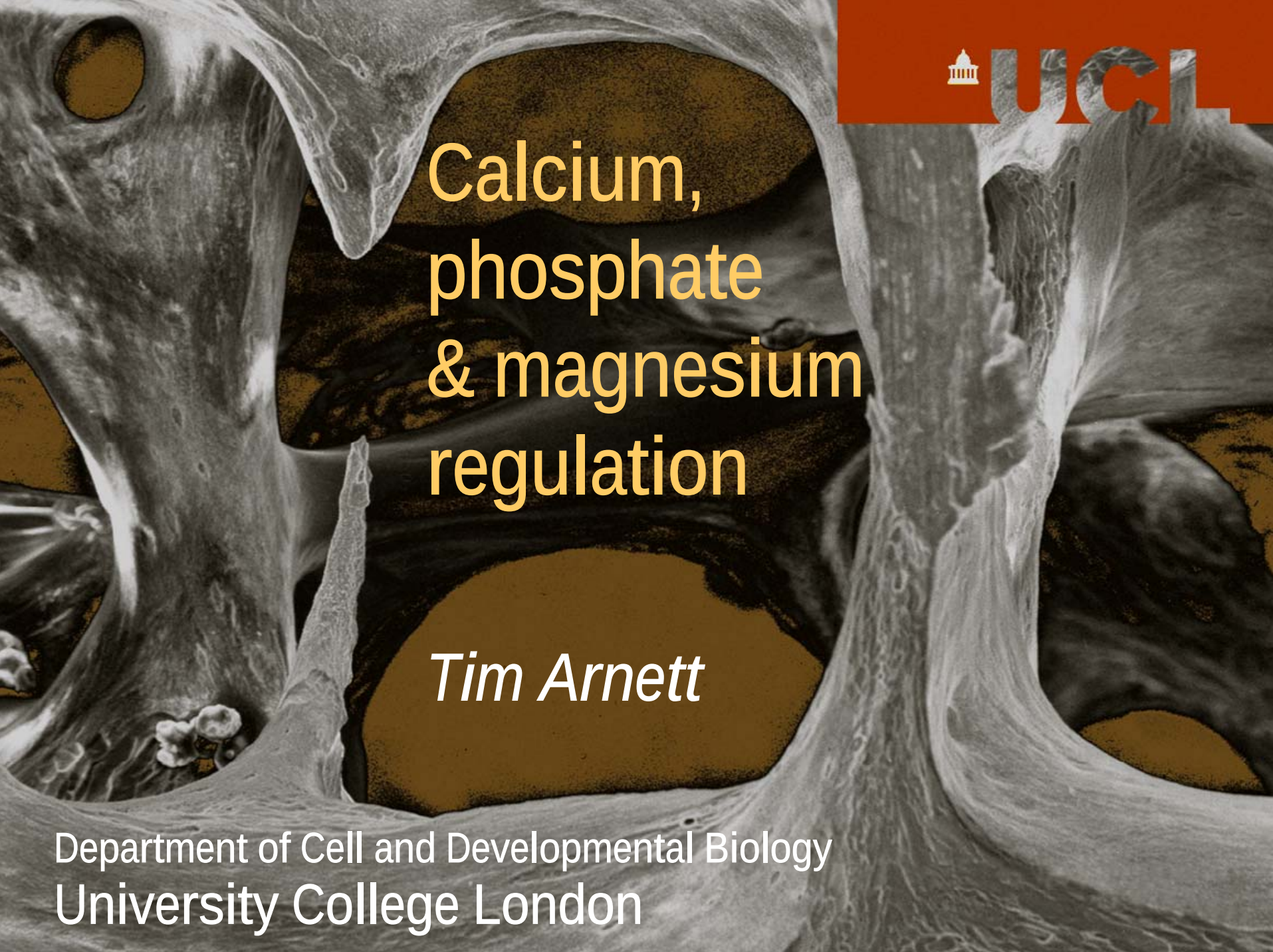
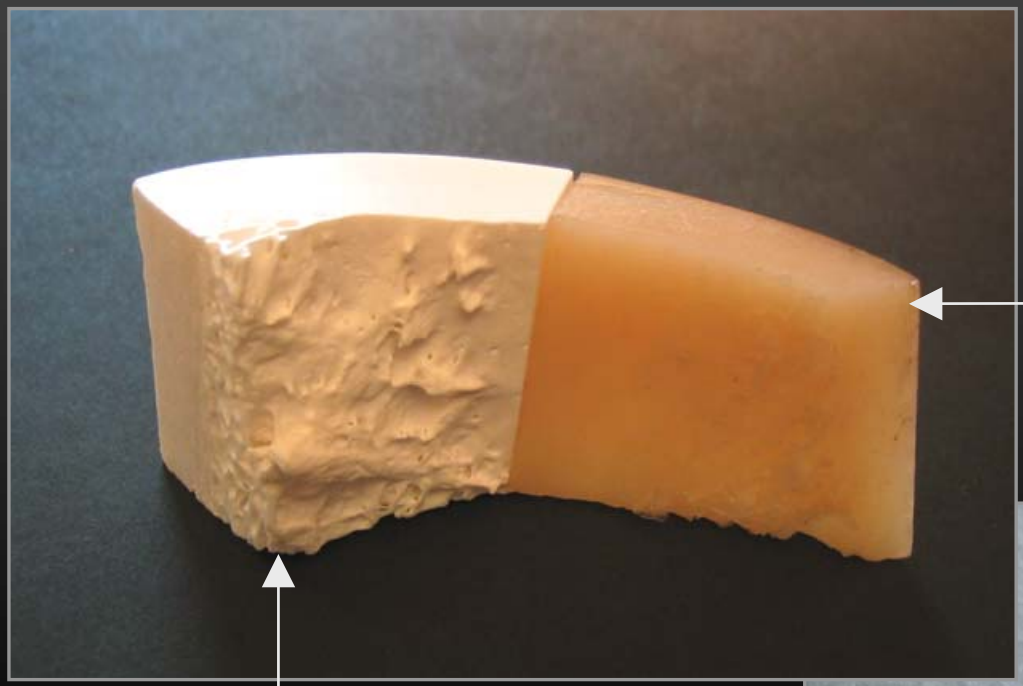


# Calcium, phosphate & magnesium regulation

*Tim Arnett*

Department of Cell and Developmental Biology
University College London

# Bone composition



Treated with hydrochloric acid
to dissolve mineral
*leaves organic component*
*[ mainly type 1 collagen ] intact*

Treated with bleach (hypochlorite) to
digest collagen
*leaves mineral component*
*[ hydroxyapatite – $Ca_{10}(PO_4)_6(OH)_2$ ]*
*intact*

Collagen shrinkage
on drying

# $Ca^{2+}$ regulation

- Almost all of the $Ca^{2+}$ in the body (>99%) exists as mineral deposits in the skeleton and teeth
- In man, plasma $Ca^{2+}$ is tightly maintained at ~2.5mM
- Local concentrations of $Ca^{2+}$ in extracellular fluid may vary more
- About 50% of plasma $Ca^{2+}$ is ionised and diffusible

# $Ca^{2+}$ regulation

*Why so precise ?*

- $Ca^{2+}$ is used as a vital second messenger within cells
- $Ca^{2+}$ is necessary for normal blood coagulation, muscle contraction and nerve function; hypocalcaemia results in excitation of nerve and muscle cells leading to spasms, tetany and asphyxia
- Solubility of many $Ca^{2+}$ salts (phosphate, carbonate, sulphate, oxalate) is low…

  …increases in $Ca^{2+}$ could lead to inappropriate precipitation (tissue mineralisation)

# $Ca^{2+}$ regulation

## Major $Ca^{2+}$ regulating hormones

- Parathyroid hormone (PTH)
- 1,25-dihydroxyvitamin $D_3$
- Calcitonin

# Parathyroid hormone

- Raises plasma $Ca^{2+}$ - *key minute-to-minute regulator*
- 84 amino acid polypeptide secreted by chief cells of parathyroid glands (amphibia upwards) into bloodstream in response to small falls in local $Ca^{2+}$ concentration
- Pulsatile secretion
- Normal plasma concentration ≅10-50 pg/ml;  plasma half life ≅10 min
- (*synthetic 1-34 peptide has full biological activity*)
- Actions mediated through PTH / PTHrP receptor - coupled to adenylate cyclase through Gs & to phospholipase C via Gq signalling proteins

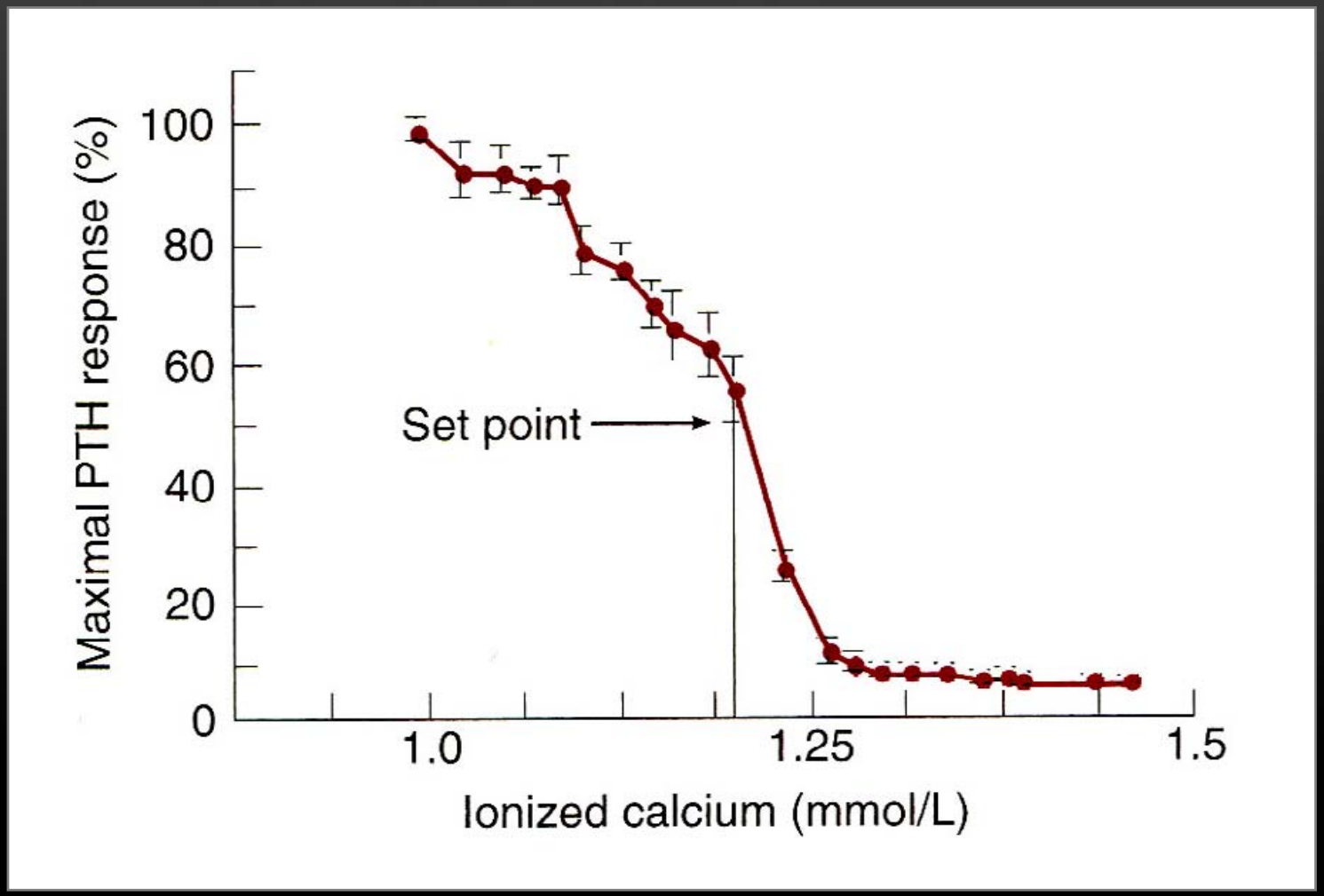
# Parathyroid hormone

*Increases plasma $Ca^{2+}$ by:*

- ↑ osteoclast formation (↑ RANKL) and resorptive activity *(direct & indirect actions)*
- ↑ $Ca^{2+}$ reabsorption in distal renal tubules
- ↑ $1,25(OH)_2D_3$ production by stimulating the activity of the critical $1\alpha$- hydroxylase enzyme in kidney

- *PTH is also anabolic for bone when administered intermittently…*
  *… thought to involve suppression of sclerostin production by osteocytes*
  *… not fully understood – PTH does not directly promote bone formation by cultured osteoblasts*

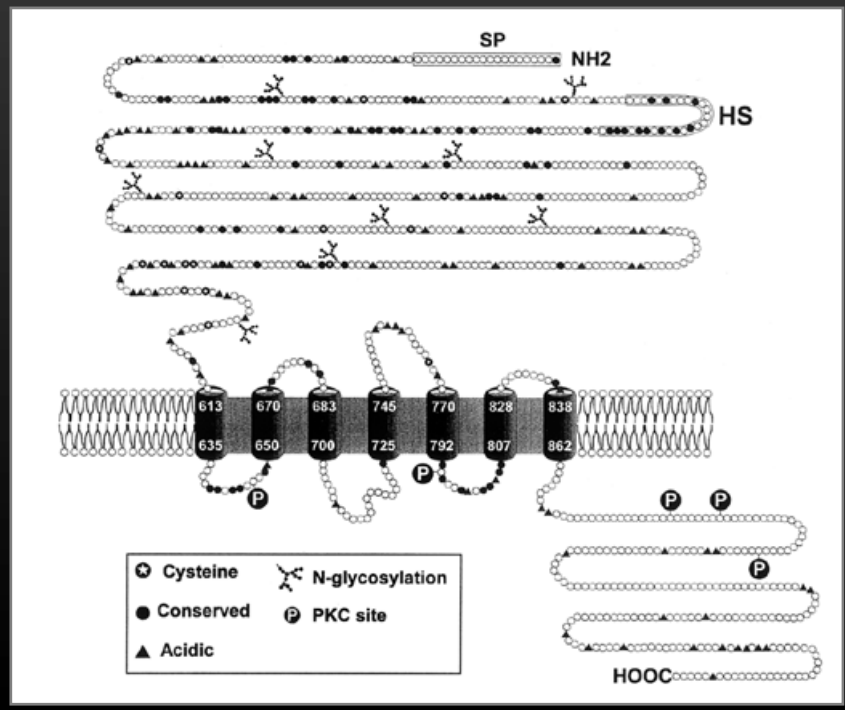
# PTH production is very sensitive to plasma [$Ca^{2+}$]



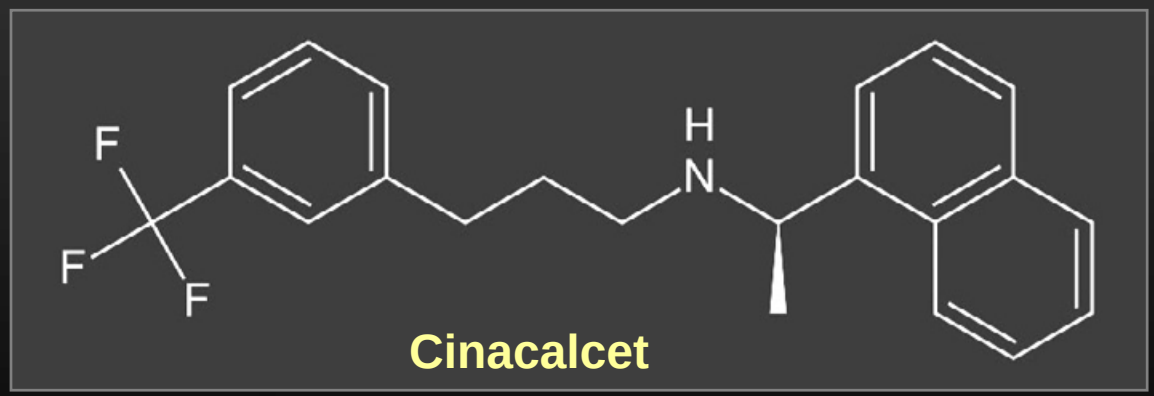

WF Ganong (2005) Review of Medical Physiology, ed 22, after Brown (1991) Physiol Rev 71: 371

# $Ca^{2+}$ sensing receptor (CaSR)

- CaSR is a G-protein coupled receptor (GPCR)
- Molecular mechanism underlying $Ca^{2+}$ sensing by parathyroid chief cells and renal tubules
- CaSR acts as the body's thermostat for $Ca^{2+}$ or 'calciostat'
- Detects perturbations in the ionized $Ca^{2+}$ of only a few percent, leading to alterations in parathyroid function & PTH secretion that are designed to normalise plasma $Ca^{2+}$



# Calcimimetics

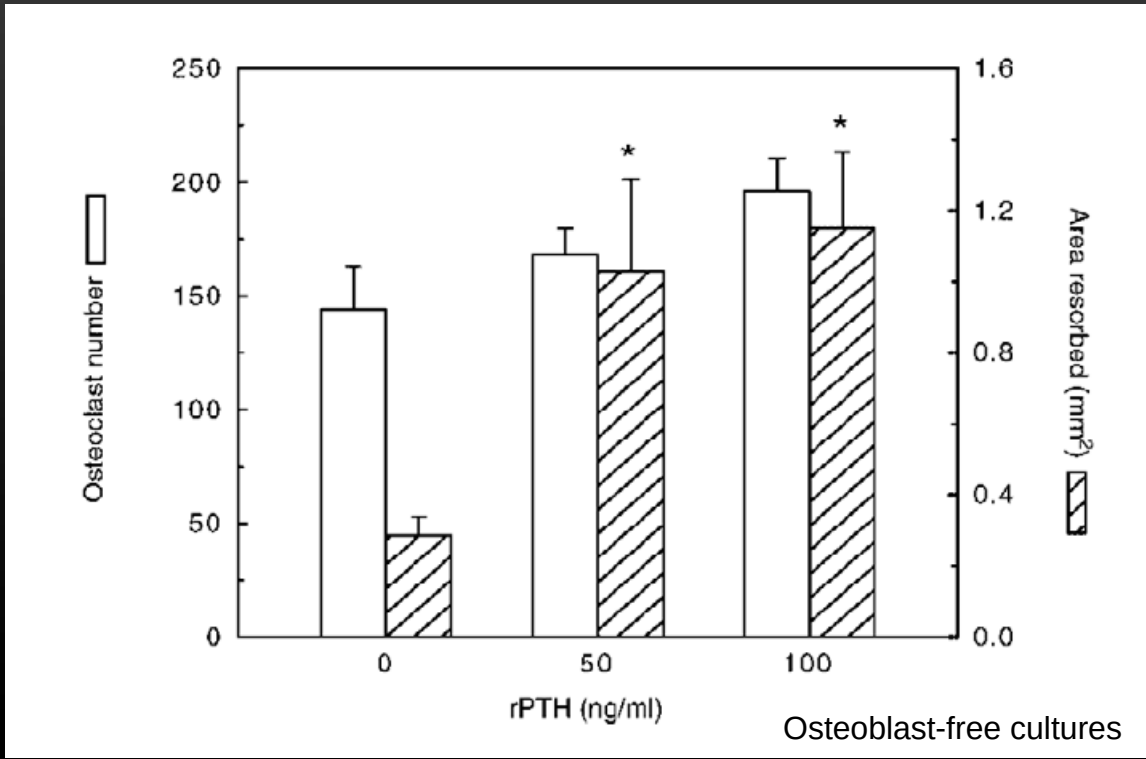



**Cinacalcet**

- Synthetic agonist which activates $Ca^{2+}$ sensing receptor
- Treatment of hyperparathyroidism / hypercalcaemia

# PTH directly stimulates resorptive function of human osteoclasts

PTH1R highly expressed on mature osteoclasts



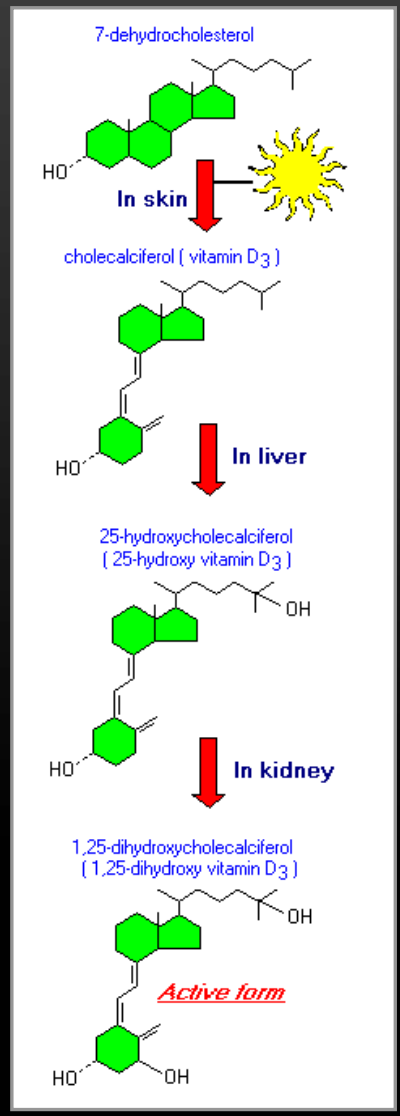

*Dempster et al (2005) J Cell Biochem 95:139–148*

# PTH-rP

- Parathyroid hormone – related protein
- Action very similar to PTH at receptor level (high homology in 1-34 region)
- Paracrine agent expressed by many normal cell types (not controlled by $Ca^{2+}$ & CaSR)
- Also expressed by tumour cells – contributes to hypercalcaemia of malignancy
- Important developmental actions: required for cartilage differentiation, tooth eruption, normal development of mammary glands… etc

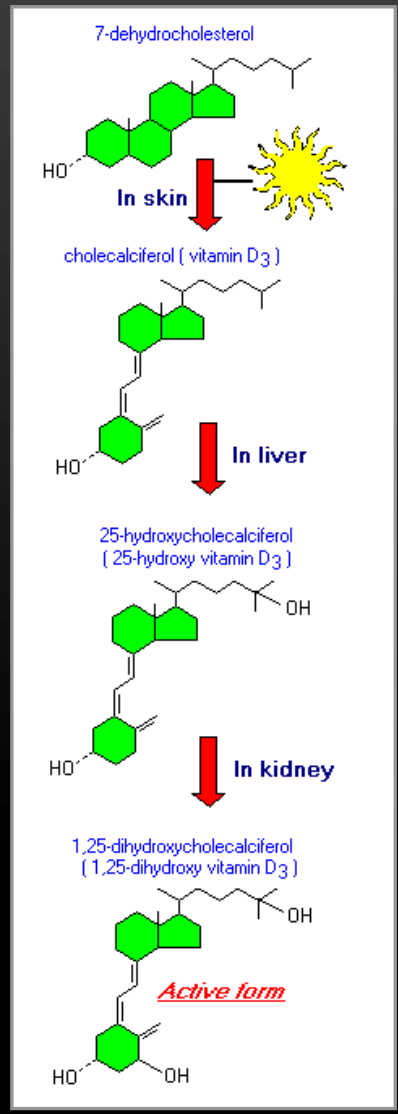
# Vitamin D

- Vitamin D (cholecalciferol), a ‘seco-steroid’ has no biological activity
- 25-hydroxy vitamin D is main circulating metabolite (nanomolar range) - very low biological activity
- 1,25-dihydroxy vitamin D is active metabolite – circulating concentration ≈ 10-50 pM
- 1,25-dihydroxy vitamin D is classed as a steroid hormone – acts via nuclear receptor (VDR)
- Vitamin D metabolites are fat soluble – slower action than peptide hormones – not involved in minute-to-minute regulation of plasma $Ca^{2+}$



# $1,25(OH)_2D$ - *actions*

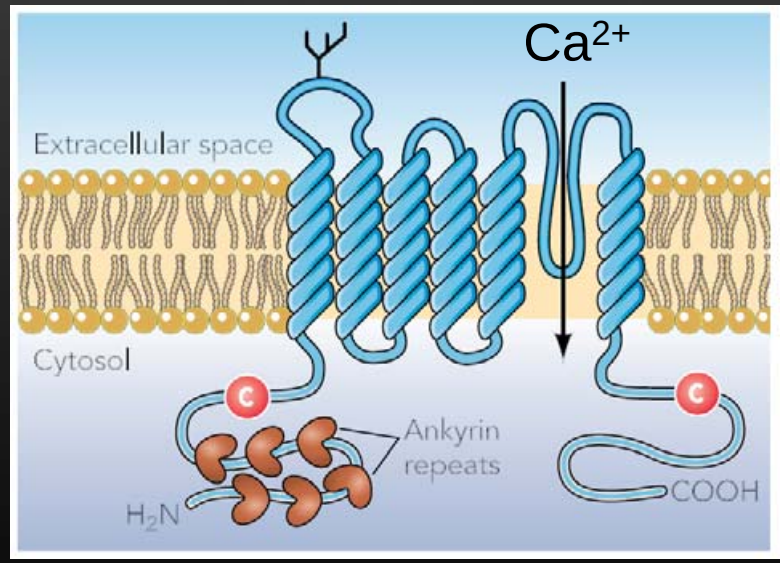
- ↑ Gut $Ca^{2+}$ uptake
- ↑ Plasma $Ca^{2+}$
- ↑ OC recruitment, activity (↑ RANKL)
- ↓ OB proliferation; ↑ OB (and skin cell) differentiation
- Required for normal matrix mineralisation. Acts mainly via promoting $Ca^{2+}$ uptake - and thus ensuring adequate local $Ca^{2+}$ supply; not much evidence for a direct effect on mineral deposition by normal osteoblasts
- deficiency → osteomalacia, rickets…



# $Ca^{2+}$ uptake / transport

- $Ca^{2+}$ uptake from the intestine now thought to occur primarily via the epithelial TRPV6 $Ca^{2+}$ transport channel (which is strongly upregulated by $1,25(OH)_2D$)
- $Ca^{2+}$ reabsorption in the kidney occurs primarily via the epithelial TRPV5 $Ca^{2+}$ transport channel (upregulated by $1,25(OH)_2D$ and PTH)
- TRPV5 also expressed in osteoclast ruffled border

(ref: Hoenderop & Bindels (2008) Physiology 23: 32-40)

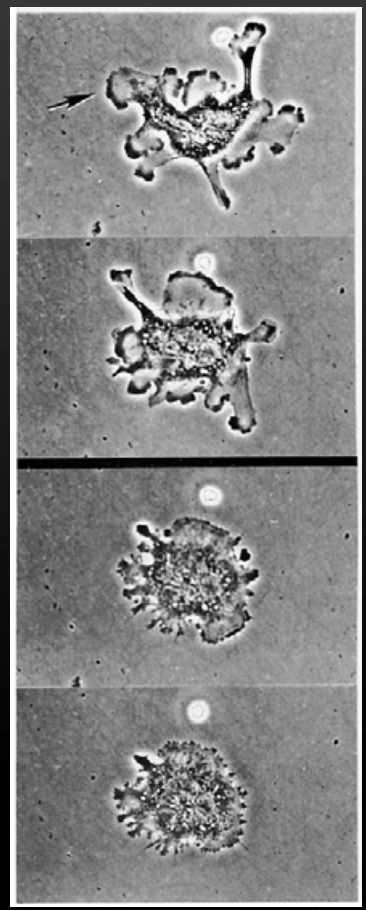



TRPV5/6 calciotropic channel

- $1,25(OH)_2D$ also increases expression of intracellular $Ca^{2+}$ binding proteins, esp. calbindin D 9K *(old mechanism!)*

# Calcitonin

- 32 amino acid peptide hormone secreted by parafollicular ‘C’ cells in thyroid / ultimobranchial gland (elasmobranchs upwards)
- ↓ plasma $Ca^{2+}$ in young / hypercalcaemic animals
- ↓↓ osteoclast resorptive function & recruitment
- Inhibits $Ca^{2+}$ and $PO_4^{3-}$ reabsorption by the kidney tubules
- ‘emergency’ hormone; not much effect in normal adults




Time-lapse sequence showing rapid inhibition of rat osteoclast motility in response to salmon calcitonin *(Arnett & Dempster, Endocrinology, 1987)*

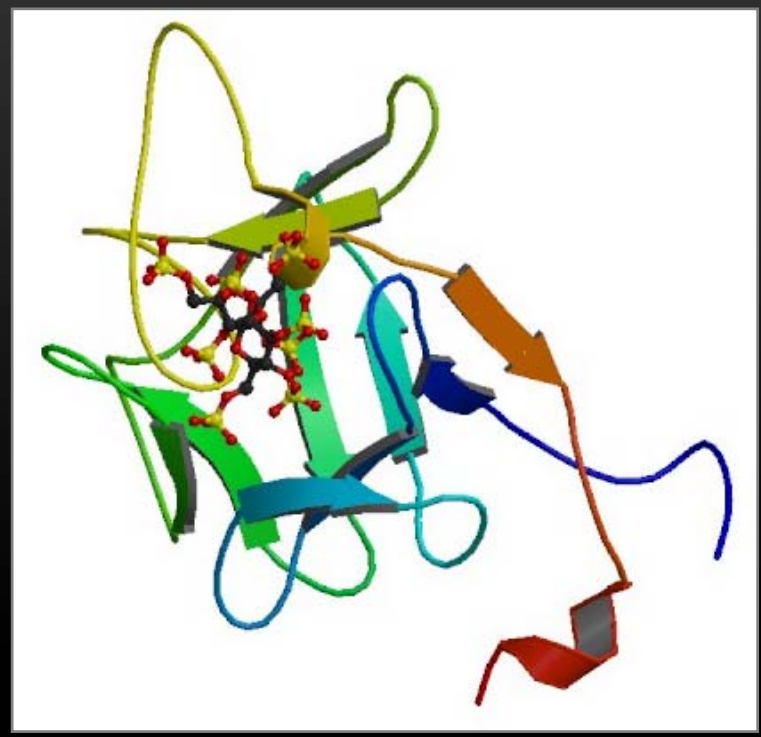
# $PO_4^{3-}$ regulation

- Plasma $PO_4^{3-}$ is less tightly regulated than $Ca^{2+}$ - normal range is ~0.8-1.5mM in adult humans
- PTH decreases $PO_4^{3-}$ reabsorption from proximal tubules of kidney – but this is compensated by enhanced 1,25-$(OH)_2$ vitamin D-mediated $PO_4^{3-}$ uptake from intestine
- FGF23 is the key circulating $PO_4^{3-}$ -lowering hormone. Reduces $PO_4^{3-}$ reabsorption in proximal renal tubules and also decreases $PO_4^{3-}$ absorption from the intestine
- Calcitonin inhibits $PO_4^{3-}$ reabsorption in renal tubules
- $PO_4^{3-}$ stimulates PTH secretion from the parathyroids… mechanism not understood
- Is there a $PO_4^{3-}$ receptor (analagous to $Ca^{2+}$ -sensing receptor) ?

  *… no real evidence at present*

# FGF-23

- Phosphaturic hormone; 251 amino acid protein, produced by bone (osteocytes)
- Identified in 2000 via gain-of function mutations associated with autosomal dominant hypophosphatemic rickets
- Prior to discovery, it was hypothesised that a protein existed which performed the function of FGF23. This protein was known as phosphatonin
- Acts through several FGF receptor subtypes in concert with Klotho, a co-receptor for FGF23,



*Review: Kuro-O M 2008 Trends Endocrinol Metab19: 239-245*

# Klotho

- Klotho identified in 1997: affected / deficient mice show premature ageing and altered mineral homeostasis resulting in osteopenia
- Klotho is a type I transmembrane protein with β-glucuronidase activity in extracellular domain; expressed in kidney and parathyroids, co-localises with TRPV5
- Klotho is required for normal $PO_4^{3-}$ elimination
- Lowering blood phosphate levels by restricting dietary $PO_4^{3-}$ intake or by blocking vitamin D function in Klotho / FGF23-deficient mice rescues not only hyperphosphatemia but also many ageing-like phenotypes

  *ie*, $PO_4^{3-}$ retention is toxic – and responsible for accelerated ageing…

*Review: Kuro-O M 2008 Trends Endocrinol Metab19: 239-245*

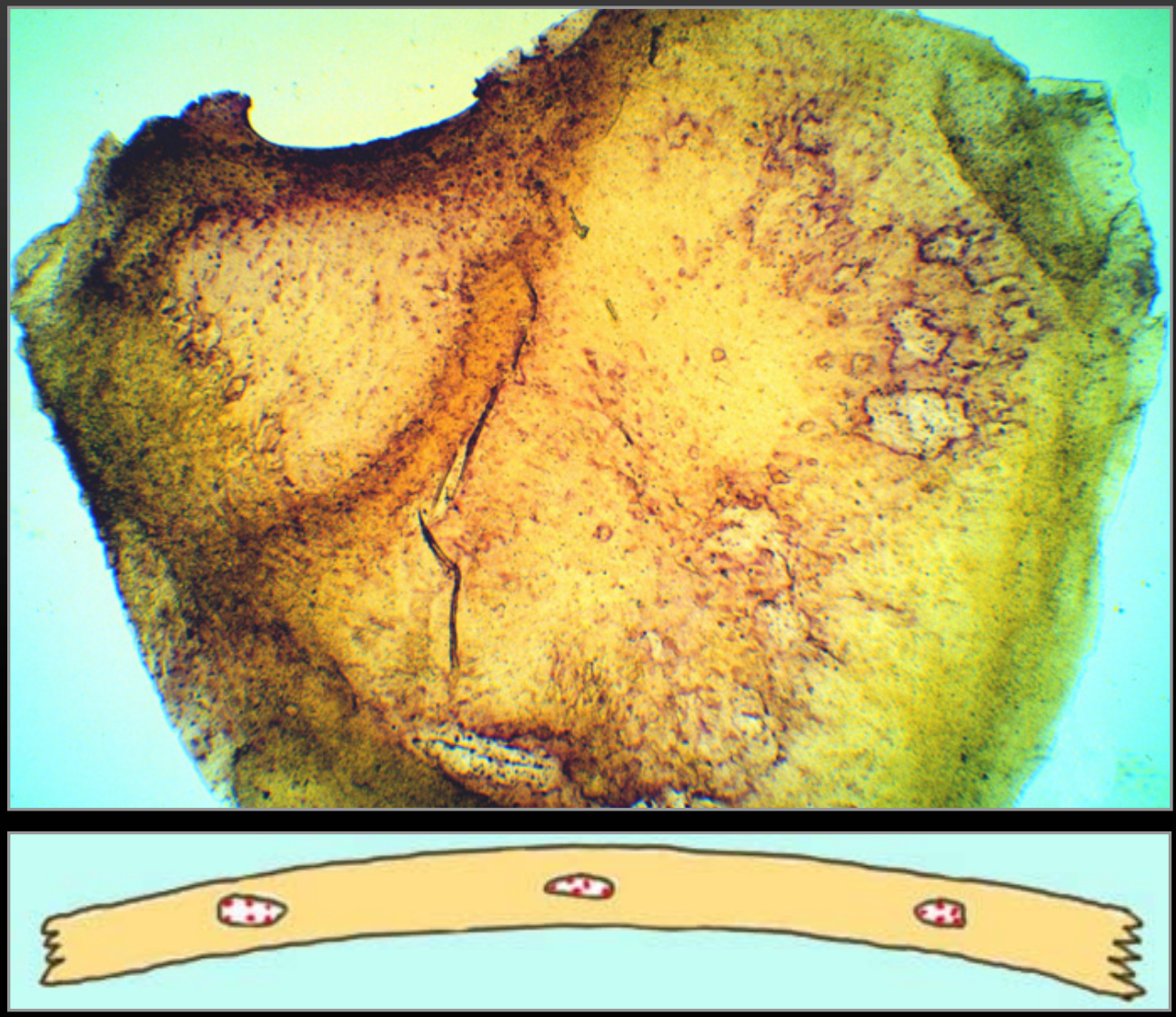
# Regulation of bone cell function by $Ca^{2+}$ & $PO_4^{3-}$

- $Ca^{2+}$ has a minor inhibitory action on osteoclasts at concentrations >10 mM (ie, 4 x higher than physiological)
- $PO_4^{3-}$ exerts a strong, reversible inhibitory action on osteoclast function at concentrations >2 mM (ie, close to physiological) *(Yates et al, JBMR 1991)*
- $Ca^{2+}$ & $PO_4^{3-}$ both promote mineral deposition by differentiated osteoblasts

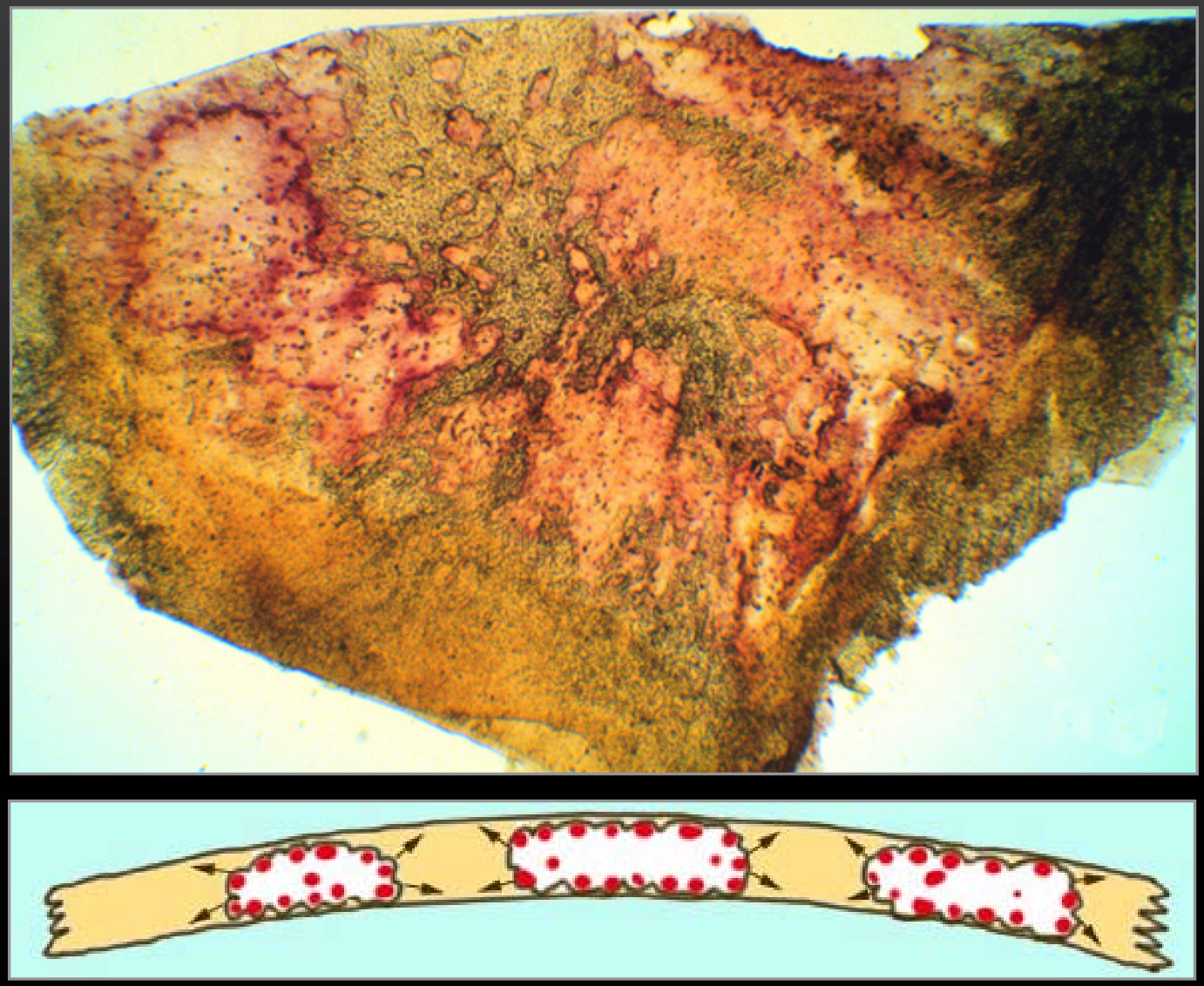
# Mineralisation

- $Ca^{2+}$ and $PO_4^{3-}$ regulation must be understood in relation to mineralisation
- Total body Ca content in adults is about 1000 g, of which 99% exists as hydroxyapatite [$Ca_{10}(PO_4)_6\ (OH)_2$] in the mineral phase of bone
- Mineralisation is a physicochemical process – but ‘managed’ by osteoblasts and osteocytes
- Release of mineral from bone largely under cellular control (osteoclasts)…

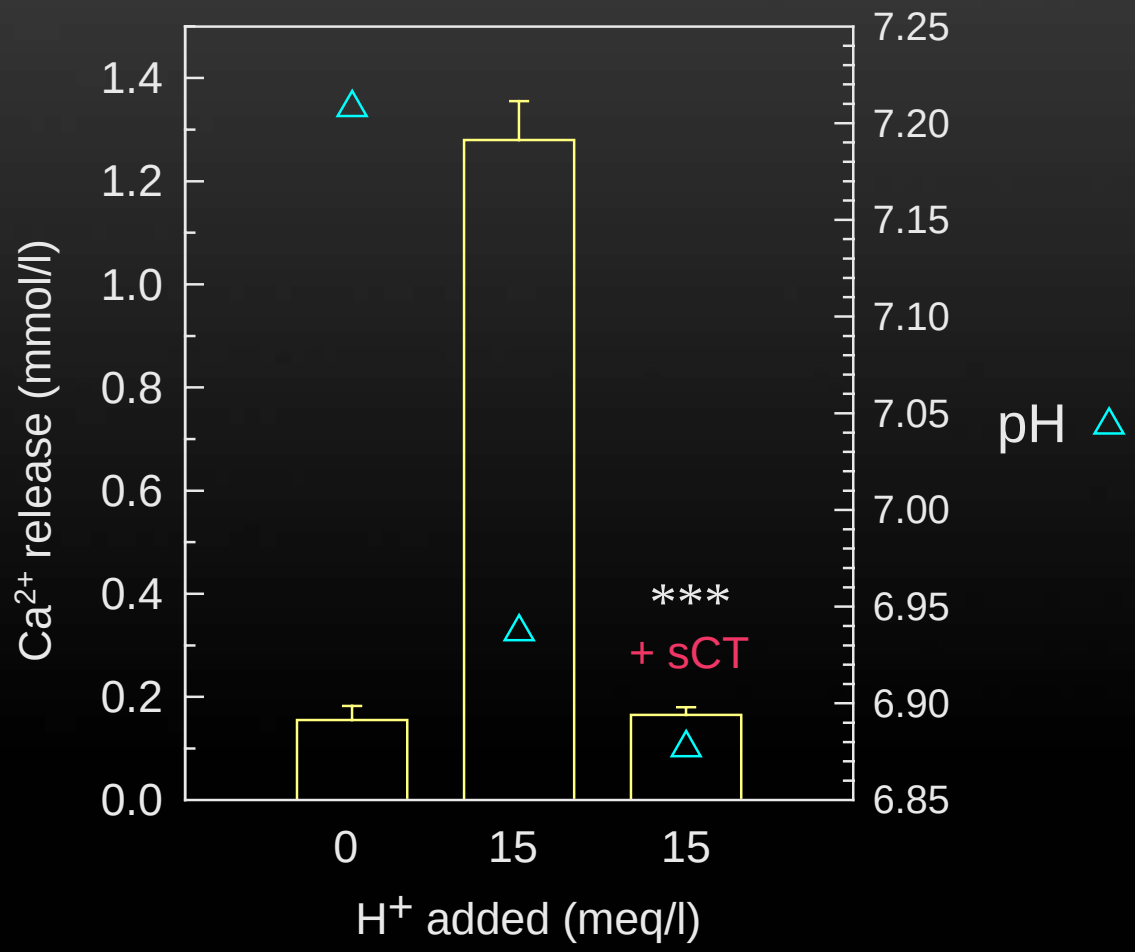
# TRAP stained calvarial bone
# 3 day culture - *control*



# Resorption of calvarial bone stimulated by acidosis (pH 7.01)



# $Ca^{2+}$ release stimulated by acidosis is blocked by salmon calcitonin

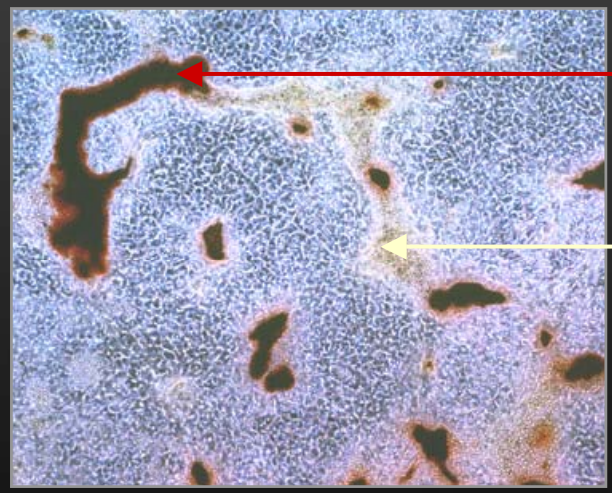


# Mineralisation

- Major function of $1,25(OH)_2D$ and PTH for the bone mineralisation process is to maintain $[Ca^{2+}] \times [PO_4^{3-}]$ solubility product in circulation / ECF in a supersaturated state (<4.5), resulting in passive mineralisation of the collagen matrix (osteoid) laid down by osteoblasts
- *So why don't all tissues mineralise ?*
- 'Vascular calcification - a passive process in need of inhibitors' (Schinke & Karsenty, 2000)
- Matrix Gla protein: MGP $^{-/-}$ mice exhibit lethal arterial calcification and inappropriate calcification of cartilage (in mice); MGP expressed by vascular smooth muscle cells but not bone cells
- ASARM peptides (Clemens Löwik)
- Pyrophosphate
- ATP & other nucleotide triphosphates…

# ATP and UTP selectively inhibit bone mineralisation

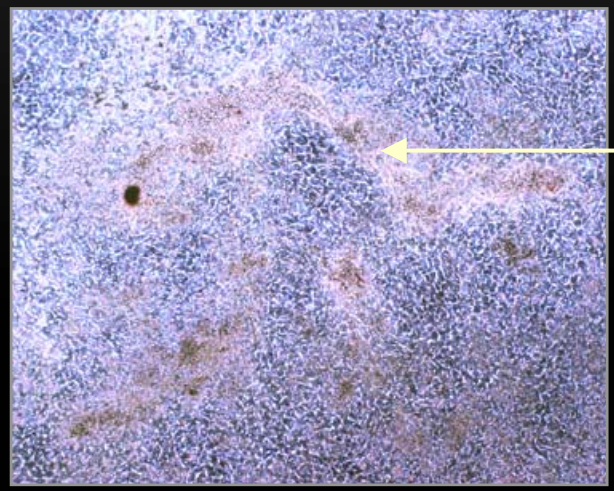
**control**



mineral stained with Alizarin red

un-mineralised collagenous matrix

**10 μM ATP / UTP**



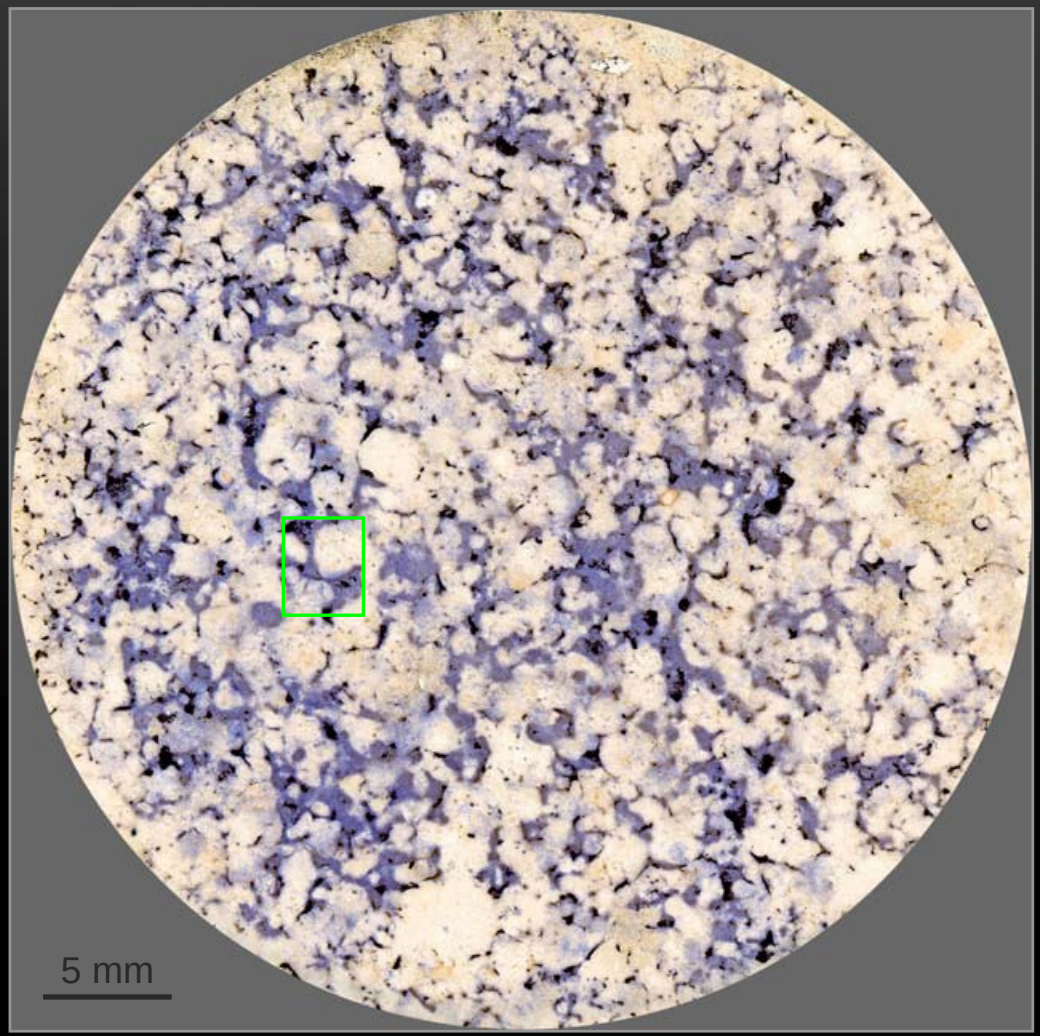
un-mineralised collagenous matrix only

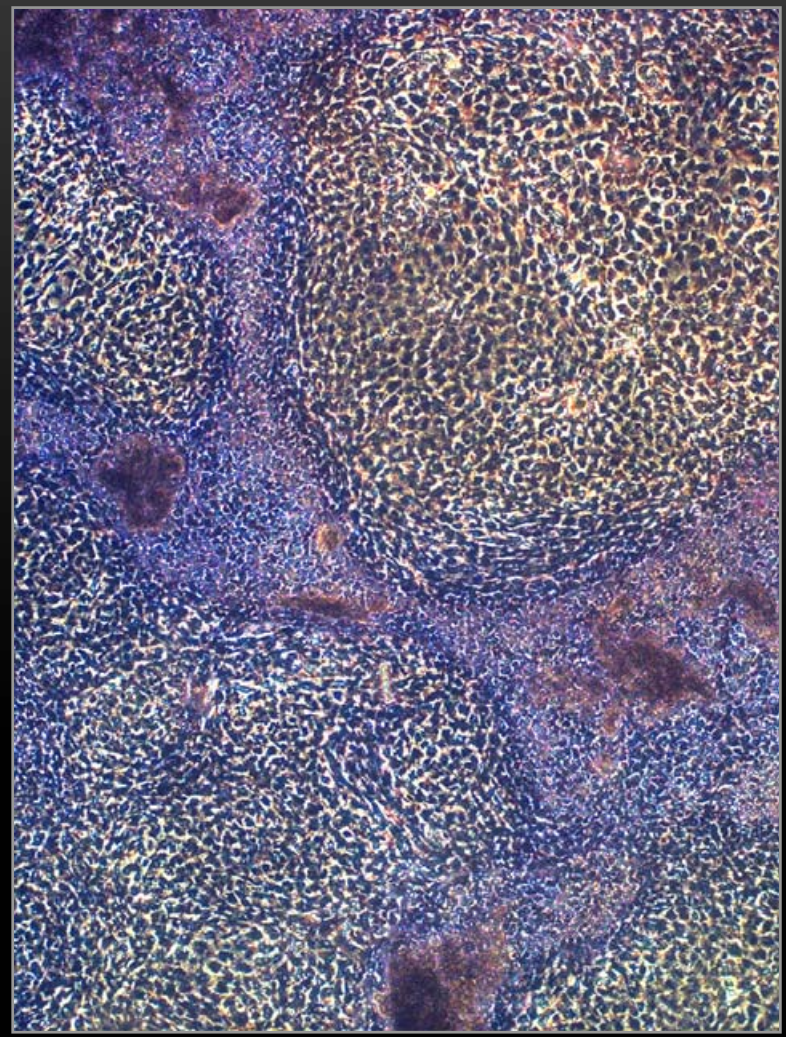
Organic matrix deposition unaffected

# Alkaline phosphatase

Differentiated osteoblasts express high levels of alkaline phosphatase (ALP)

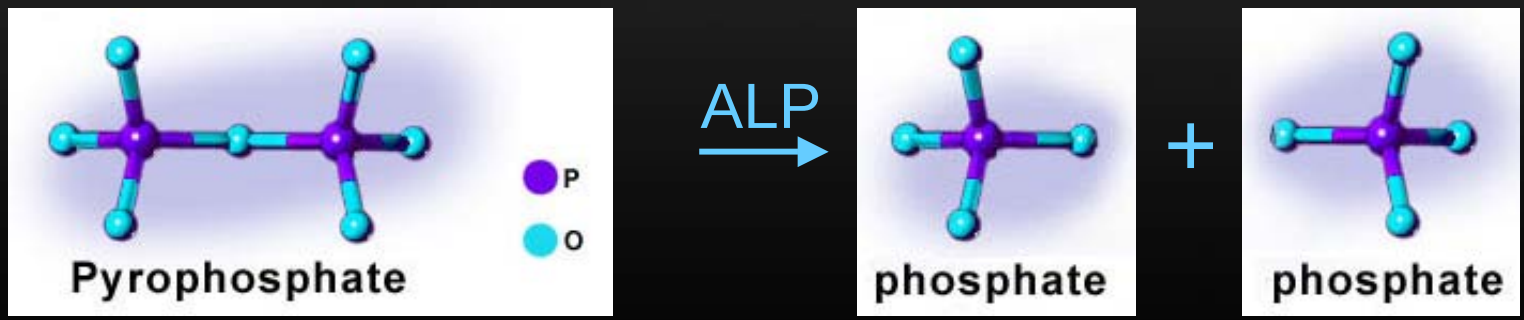
*(which is selectively inhibited by ATP / UTP…  evidence for $P2Y_2$ receptor involvement)*



# Pyrophosphate and alkaline phosphatase

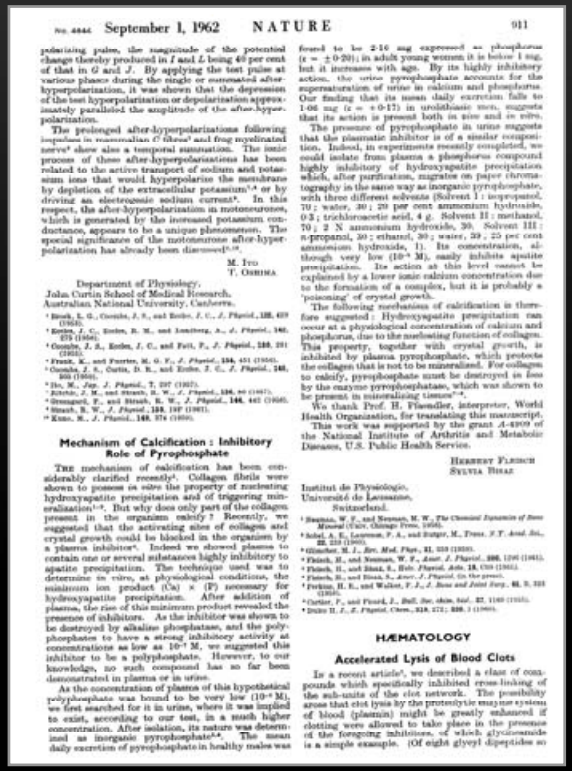
*Major function of ALP in bone is to hydrolyse pyrophosphate (PPi), a key inhibitor of mineralisation*




→ *enables mineralisation*

# Pyrophosphate: a potent inhibitor of mineralisation

- Inorganic pyrophosphate (PPi) in the low micromolar range inhibits mineralisation (Fleisch 1962 & 1966)



No. 4844 September 1, 1962 NATURE 911

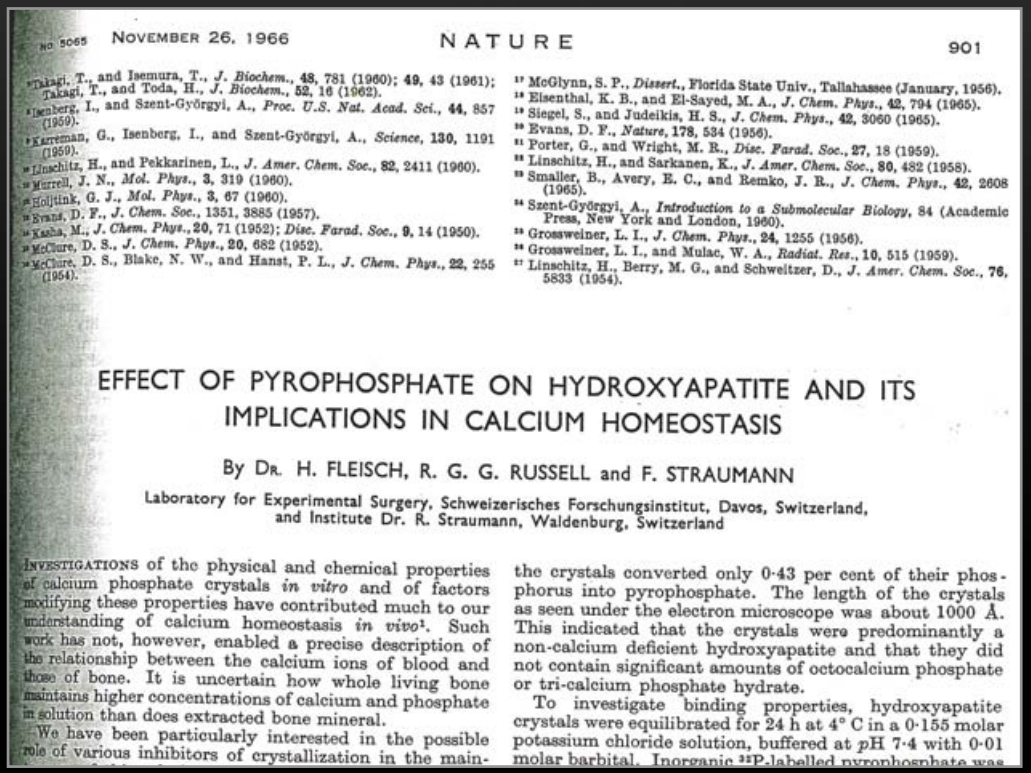
Mechanism of Calcification : Inhibitory Role of Pyrophosphate

M. Ito
T. Oshima

Department of Physiology,
John Curtin School of Medical Research,
Australian National University, Canberra.

HÆMATOLOGY

Accelerated Lysis of Blood Clots



No. 5065 NOVEMBER 26, 1966 NATURE 901

EFFECT OF PYROPHOSPHATE ON HYDROXYAPATITE AND ITS IMPLICATIONS IN CALCIUM HOMEOSTASIS

By Dr. H. FLEISCH, R. G. G. RUSSELL and F. STRAUMANN

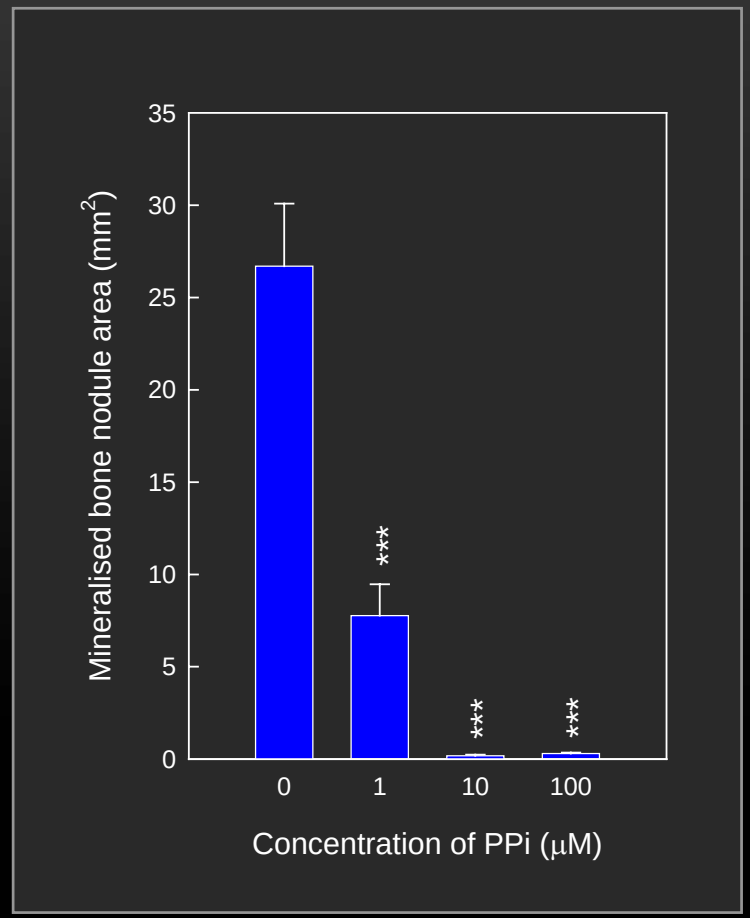
Laboratory for Experimental Surgery, Schweizerisches Forschungsinstitut, Davos, Switzerland, and Institute Dr. R. Straumann, Waldenburg, Switzerland

# Pyrophosphate: a potent inhibitor of mineralisation

- PPi is generated from many phosphate-containing molecules, including ATP
- Members of the ecto-phosphodiesterase / pyrophosphatase (ENPP) family hydrolyse:

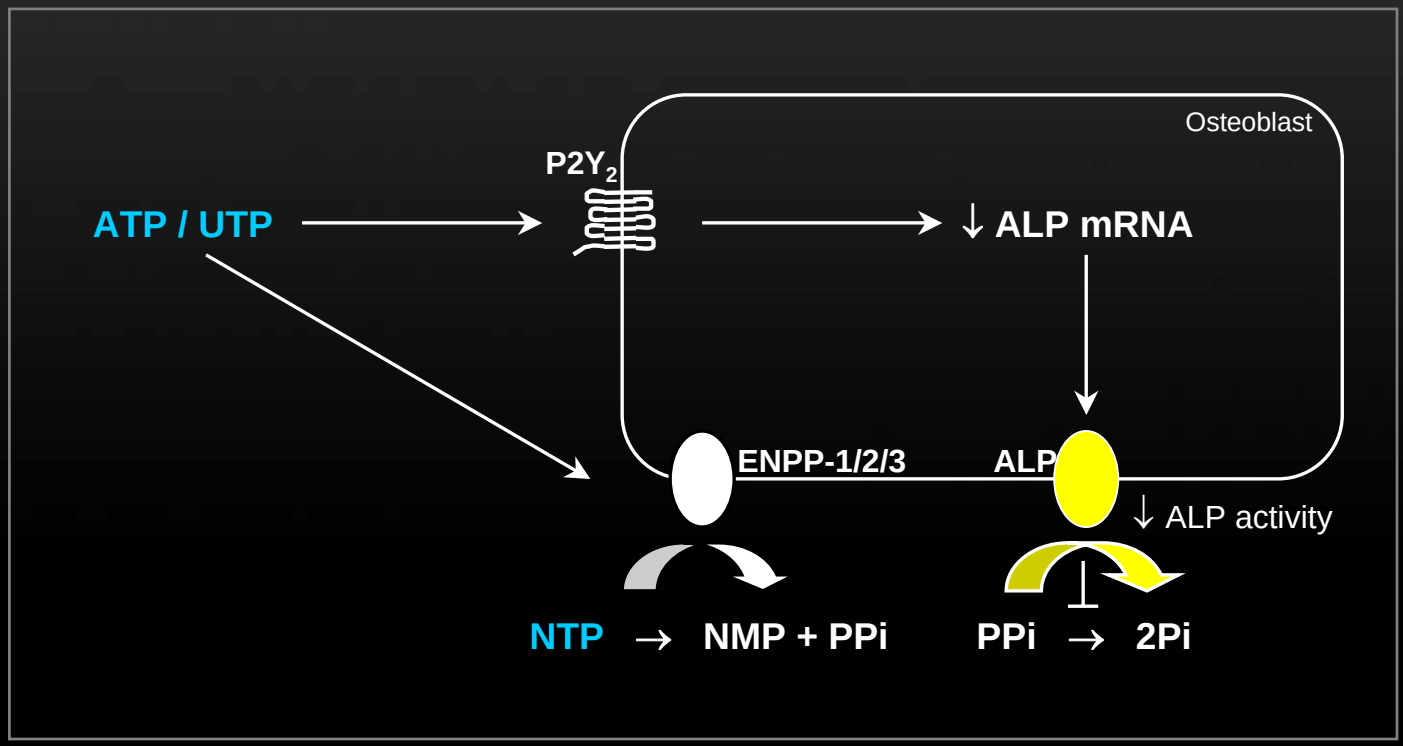
**NTP → NMP + PPi**

*Could PPi generated by hydrolysis of ATP & UTP contribute to inhibition of bone mineralisation ?*



# Dual inhibitory action of ATP / UTP on bone mineralisation

1. Via $P2Y_2$ receptor
2. Via PPi generated by ENPPs



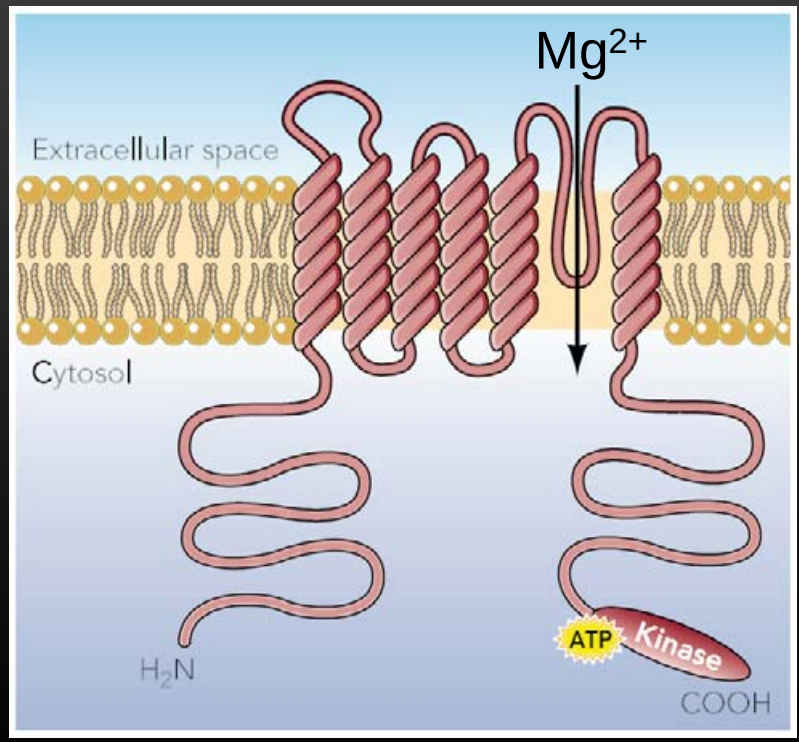

Orriss *et al* (2007) *Endocrinology* 148: 4208-4216

# $Mg^{2+}$ homeostasis

- Total adult body content of $Mg^{2+}$ is ~25g
- $Mg^{2+}$ salts have similar solubility profiles to those of $Ca^{2+}$
- ~66% of $Mg^{2+}$ located in the skeleton as mineral deposits (phosphate, carbonate)
- ~33% of $Mg^{2+}$ in body is intracellular….
  $Mg^{2+}$ required for ≥300 biochemical reactions in the body
- ≤1% of $Mg^{2+}$ in the body exists in soluble form in the extracellular fluid
- Plasma concentration of magnesium ($Mg^{2+}$) also closely regulated (to about 0.85 mM); mechanisms involved still not well understood…

# $Mg^{2+}$ homeostasis

- $Ca^{2+}$ -sensing receptor (in parathyroids) also senses $Mg^{2+}$
- $Mg^{2+}$ involved in regulation of cardiovascular function; deficiency can cause cardiac arrhythmia and impaired control of blood pressure (and also impaired PTH secretion)
- TRPM6 channels (expressed in renal tubules) thought to be important for transport of $Mg^{2+}$)
- The membrane protein GPCR6A is activated by $Mg^{2+}$ as well as other divalent cations (eg, $Ca^{2+}$, $Sr^{2+}$)
- $Mg^{2+}$ uptake from intestine not regulated by $1,25(OH)_2D$




TRPM6 magnesiotropic channel

(see: Hoenderop & Bindels, Physiology 23: 32-40; 2008

# Key reading

- ASBMR Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 6th edition (2006) *– downloadable from ASBMR website*
  - Ch 1-7: Morphogenesis, structure & cell biology of bone
  - Ch 8-12: Skeletal physiology
  - Ch 13-18: Mineral homeostasis
  - Ch 19-25: Clinical evaluation of bone & mineral disorders
  - Ch 26-41: Disorders of serum minerals
  - Ch 42-58: Osteoporosis
  - Ch 59-66: Metabolic bone diseases
  - Ch 67-70: Cancer & bone
  - Ch 71-74: Genetic & developmental disorders
  - Ch 75-76: Acquired disorders
  - Ch 77-80: Extraskeletal calcification & ossification
  - Ch 81-82: Hypercalciuria & kidney stones
  - Ch 83-87: Dental biology

- Ganong WF (2005) Review of Medical Physiology, 22nd edition. Lange, New York.


UCL
UCL