# Evolving Concepts in the Mechanobiology of Bone

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Biological Foundations of Orthodontics and Dentofacial Orthopaedics

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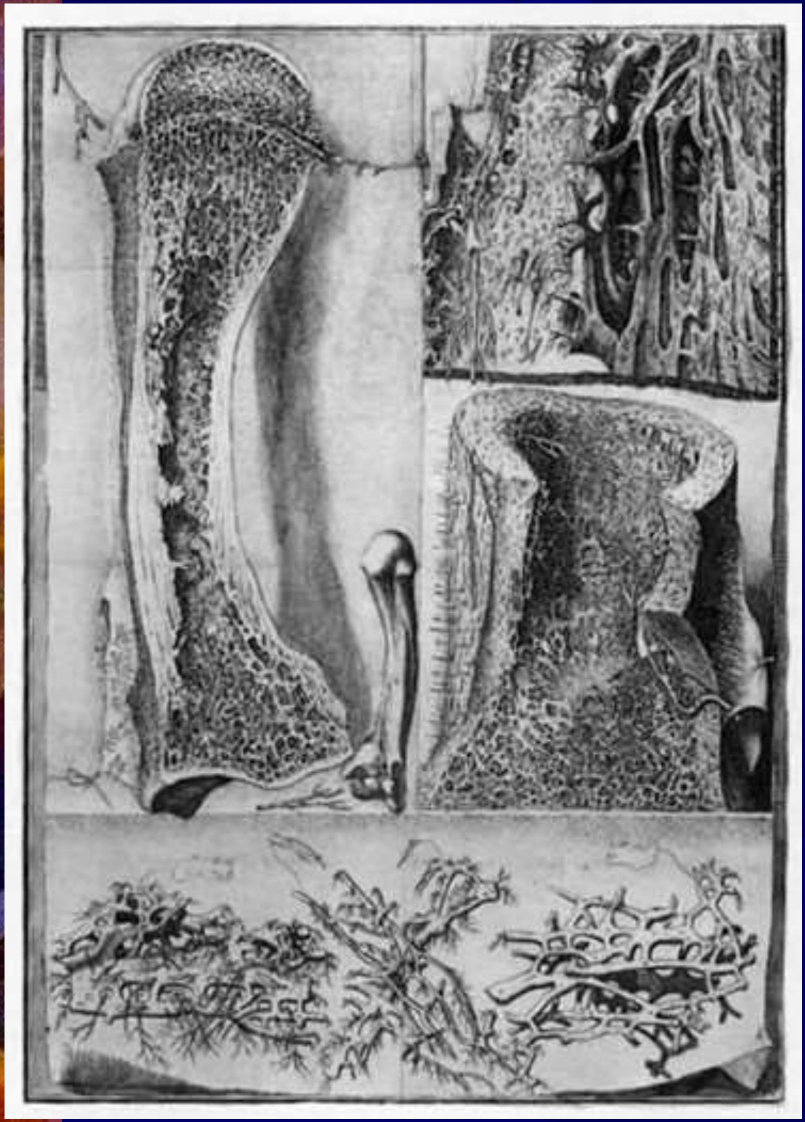
SAPERE AUDE
Te Whare Wānanga o Otāgo

# Regulation of bone mass by mechanical stimuli

- Mechanical stimuli play an important role in the differentiation, growth and maintenance of skeletal tissues. It has been estimated that environmental factors such as physical activity and nutrition account for 20–40% of individual variation in bone mass, the remaining 60–80% being determined by genetic factors.
- Understanding the mechanisms by which mechanical stimuli are converted into cellular events such as bone remodelling, is clearly important for the practice of orthodontics, orthopaedics and sports medicine based on sound biological and bioengineering principles.
- The importance of mechanical stimuli in the structure of skeletal tissues has been recognized since the 18th century. Most of the early attention was concerned with how function determined the osseous structure of the long bones of man, particularly the details of the cancellous architecture of the femoral head. The outcome was the trajectoral theory, the traditional starting point for any discussion of mechanically-induced bone remodelling.

# Relationship between form and function



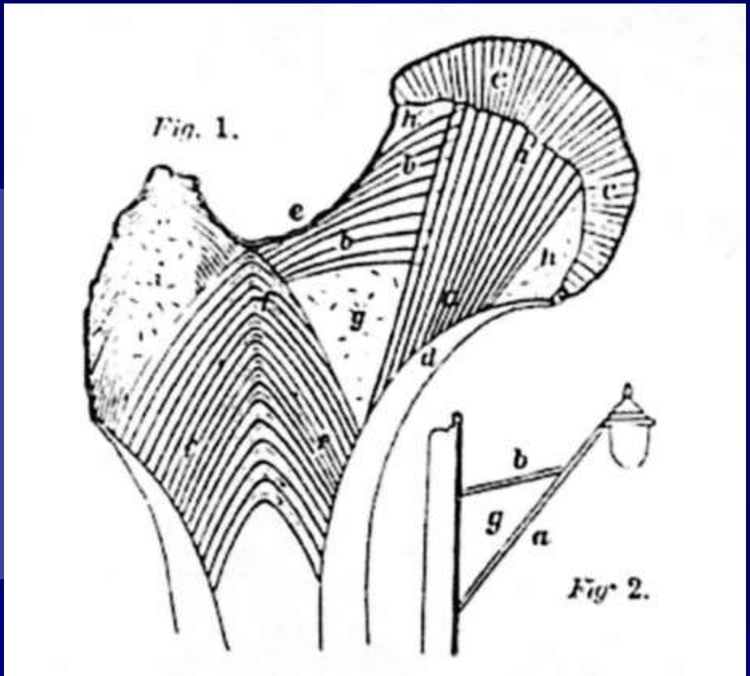
- Plate X ‘metatarsus’ (*circa* 1690) from *El Atlas Anatómico de Crisóstomo Martínez. Grabador y Microscopista del siglio XVII*. Edited by Jose Maria López Piñero, Valencia, 1964. Martínez was the discoverer of trabecular bone.
- Using a simple microscope with an optical resolution of 0.7 µm, he was able to record for the first time the internal divisions of bone into an outer cortex of compact bone, and an inner trabecular network of cancellous bone, in a series of accurate and beautiful engravings.
- Martínez died in 1694 leaving just 19 engraved plates and his *Atlas* was never published, except as a facsimile edition in 1964.

# John Hunter and the femoral head

Courtesy of the Hunterian Museum, Royal College of Surgeons of England.

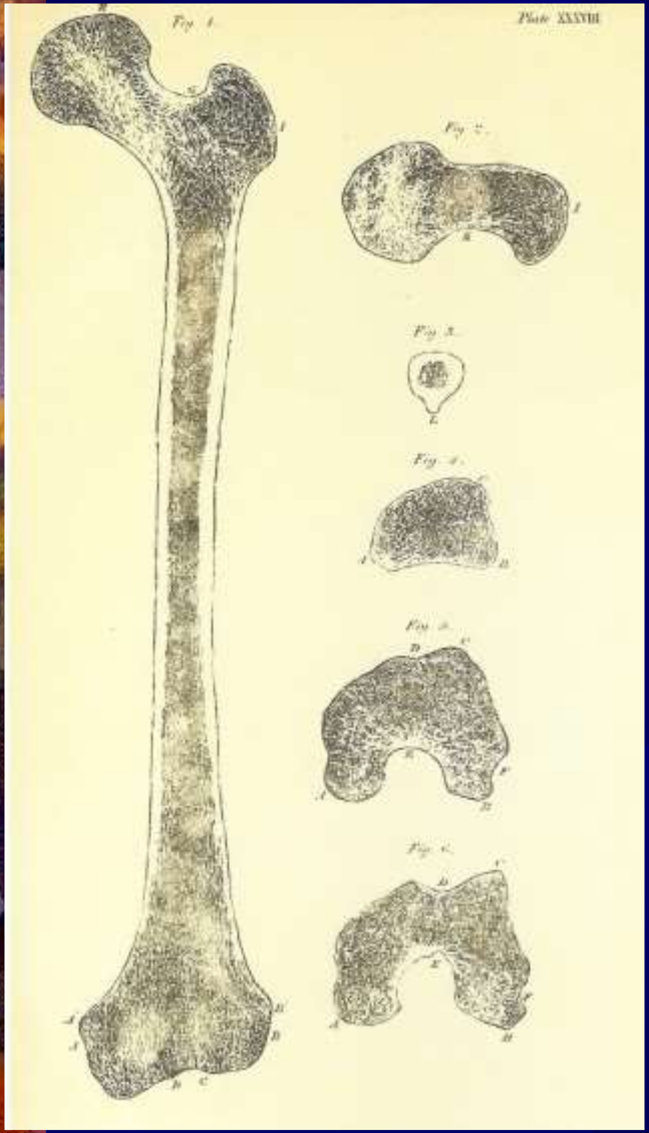
- The study of the role of mechanical stimuli in the maintenance and structure of skeletal tissues can be traced back to 1754 and the work of John Hunter (1728–1793), Scottish anatomist and surgeon, painted in 1786 by Sir Joshua Reynolds, President of the Royal Academy.
- Hunter's specimen of the head of the femur on display in the Hunterian Museum, Lincoln's Inn Fields, London, showing the trajectories followed by the trabeculae of the femoral head.

# Frederick Oldfield Ward




- In 1838 a 20-year-old medical student at King's College Hospital, London, Frederick Oldfield Ward (1818–1877), published a small pocket sized book *Outlines of Human Osteology*, in which he described not only the trabecular pattern of the proximal human femur, but also its functional significance.
- The proximal femur viewed as a bracket. From Ward (1876). *Outlines of Human Osteology* 2nd Edition.

# Sir George Humphry

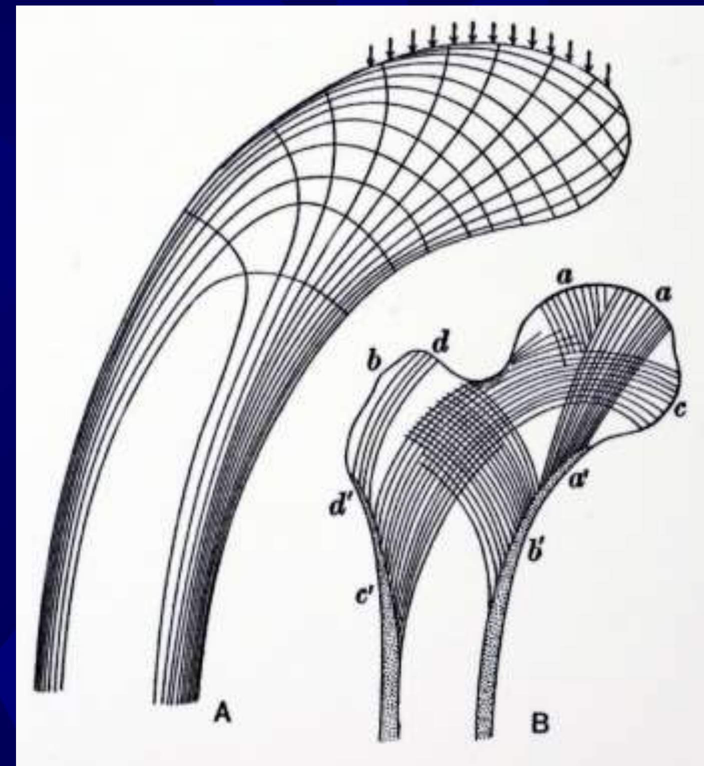


- Plate XXXVIII from a *Treatise on the Human Skeleton* (*Including the Joints*).by GM Humphry (1820–1896). This plate is a very full description of the cancellous architecture of the femur, drawn by his wife. In the year of publication (1858), Humphry was Surgeon to Addenbrooke’s Hospital, Cambridge and Lecturer in Surgery and Anatomy in the Medical School.
- His paper *On the growth of the jaws* (1866), confirmed experimentally in the pig mandible, Hunter’s observation that bone growth involved resorption, as well as deposition to maintain its shape and proportions.
- Sir George Humphry FRS, MD, FRCS, was later Professor Anatomy at Cambridge, and in 1883 the unpaid Professor of Surgery. He was knighted in 1891.

# Trajectoral theory of bone

- In July 1866 at a gathering of naturalists – the *Zürcher Naturforschende Gesellschaft* – Georg Hermann von Meyer (1815–1892), Professor of Anatomy at the University of Zurich, proposed that the trabeculae in cancellous bone were laid down along the lines of maximal compressive and tensile stress.
- This suggested that both pressure and tension could behave as morphogenetic stimuli in bone, and the stress lines became known in anatomical theory as trajectories.
- Karl Culmann (1821–1881), a structural engineer was in the audience; he noted that the cancellous architecture of the femoral head closely resembled the trajectories an engineer would draw in a crane of the same extended form as the femur (a Fairbairn crane) and of homogeneous or solid structure.

# The Fairbairn crane




- In case you were wondering what a Fairbairn steam crane looked like, it was a type of harbourside crane of an 'improved design,' with a curved jib that could reach further into the hold of a ship, patented in 1850 by Sir William Fairbairn (1789–1874). The only surviving, and still operational Fairbairn crane in existence is located in Bristol Harbour.

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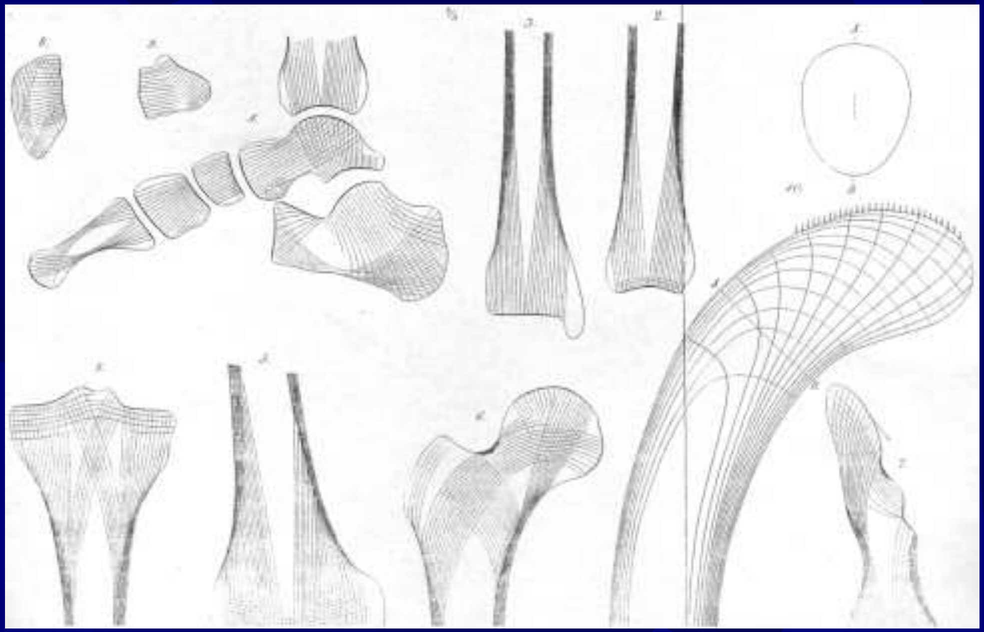
# *Die Architectur der Spongiosa* (1867)

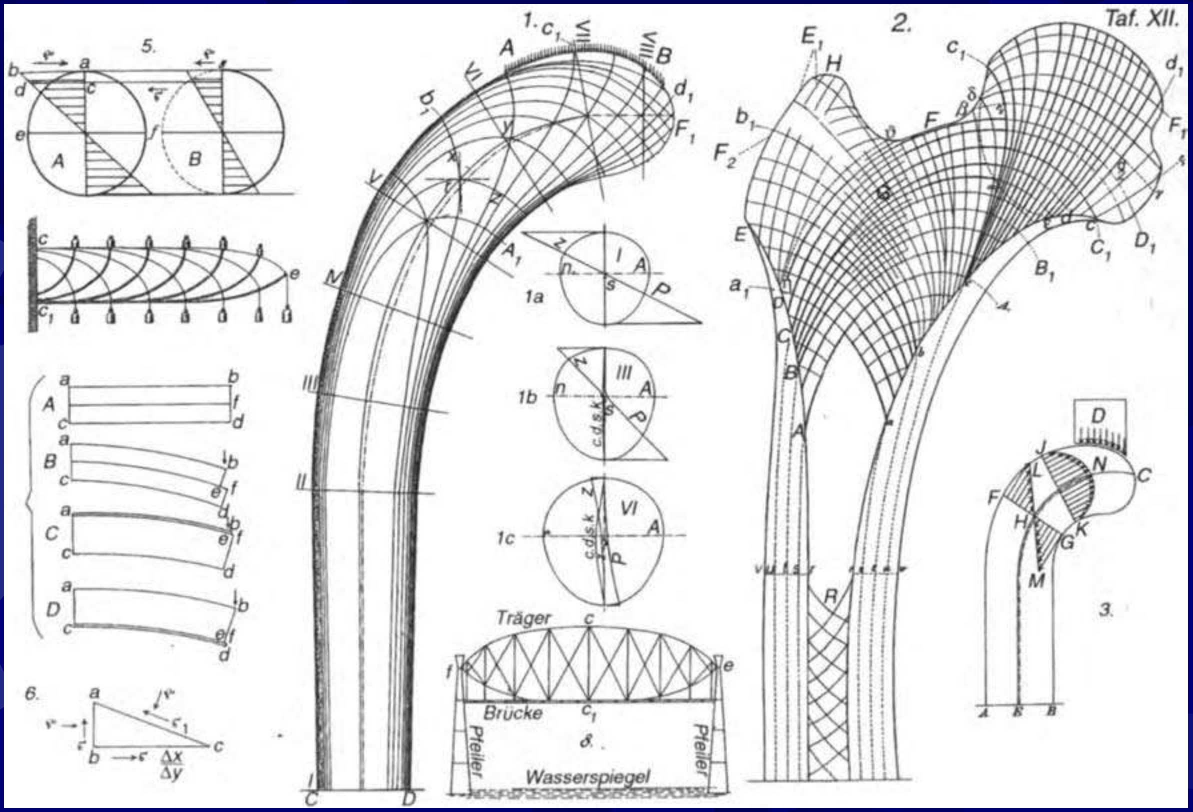


- This illustration (Tafel XVIII) showing that the trabeculae of cancellous bone were laid down in various bones according to specific stress trajectories, was included in von Meyer's 1867 article *Die Architectur der Spongiosa*. (He used the term spongiosa for trabecular bone.)
- Image 10 is a drawing of the stress trajectories calculated by Culmann's students for a Fairbairn crane.
- From von Meyer (1867). Die Architectur der Spongiosa. *Archiv für Anatomie und Physiologie* 34: 615-628.

# Julius Wolff

- Julius Wolff (1836–1902) an aspiring Berlin surgeon impressed by von Meyer's article made a detailed examination of the trabecular patterns of various bones, focusing like his predecessors on the proximal end of the human femur (*Oberschenkel*).
- His first papers (1870, 1873) included very detailed illustrations later expanded into his *magnum opus – Das Gesetz der Transformation der Knochen* published in 1892.
- In spite of his subsequent fame, Wolff had a remarkably poor understanding of bone physiology even for the time. He believed bone could grow interstitially, and rejected the idea bone could undergo resorption, which John Hunter had shown over 100 years earlier.
- Inexplicably, given the supremacy of German biomedical sciences in the 19th century, Wolff had little understanding of cell theory, or the function of bone cells.

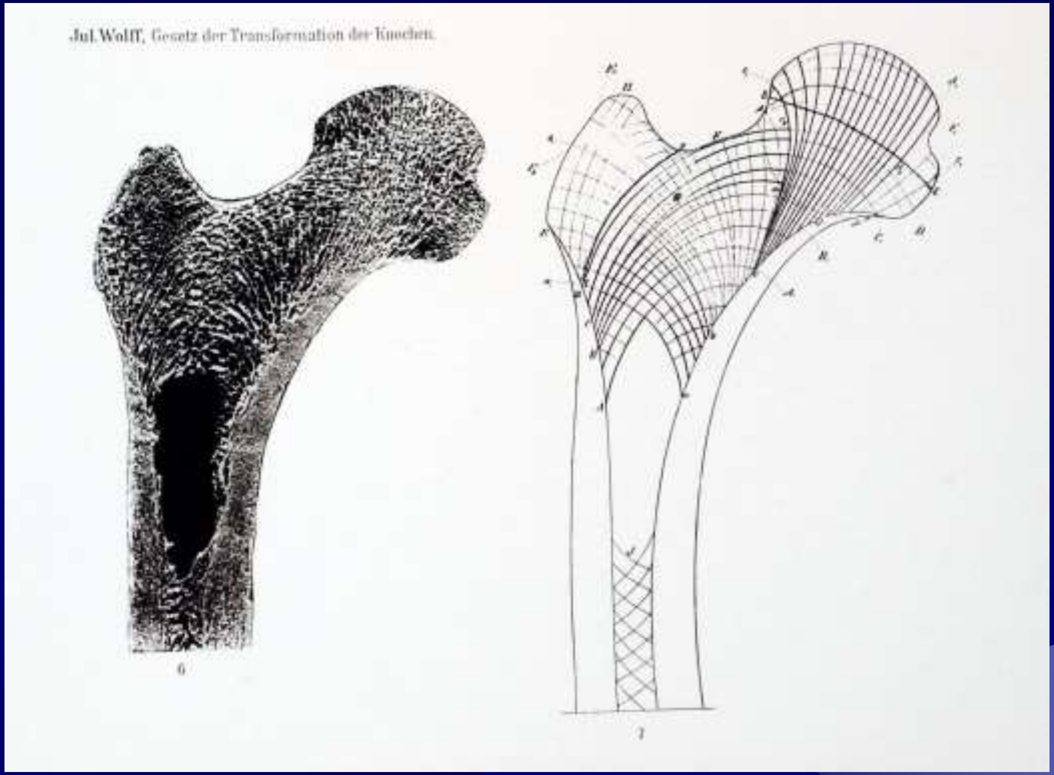
# Stress trajectories in the human femur



- Stress trajectories for (1) the curved Fairbairn crane compared with (2) the internal architecture of the cancellous bone of the femoral head. The smaller drawings provide details of the mechanics of the crane and the stress distributions in various structures.

- From Wolff (1870). *Virchow’s Archiv Pathol Anat Physiol.* **50**, 389–450.

# Wolff’s law

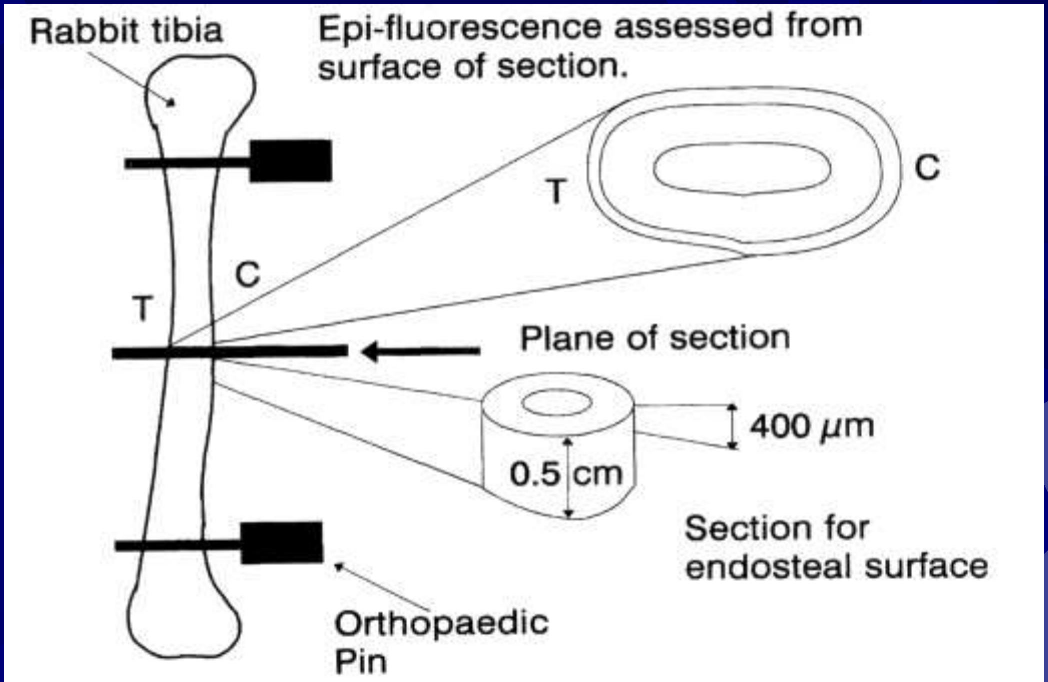



- The von Meyer/Culmann drawings were subsequently adapted by Julius Wolff in his famous book *The Law of Bone Transformation* to illustrate the bony trabeculae of the femoral head.
- From this he concluded the structure of bone is morphologically adapted to its function in accordance with mathematical principles – Wolff’s Law. This enables bone to provide maximum strength with a minimum of material.
- From Wolff (1892). *Das Gesetz der Transformation der Knochen.*

# Objections to the trajectoral theory

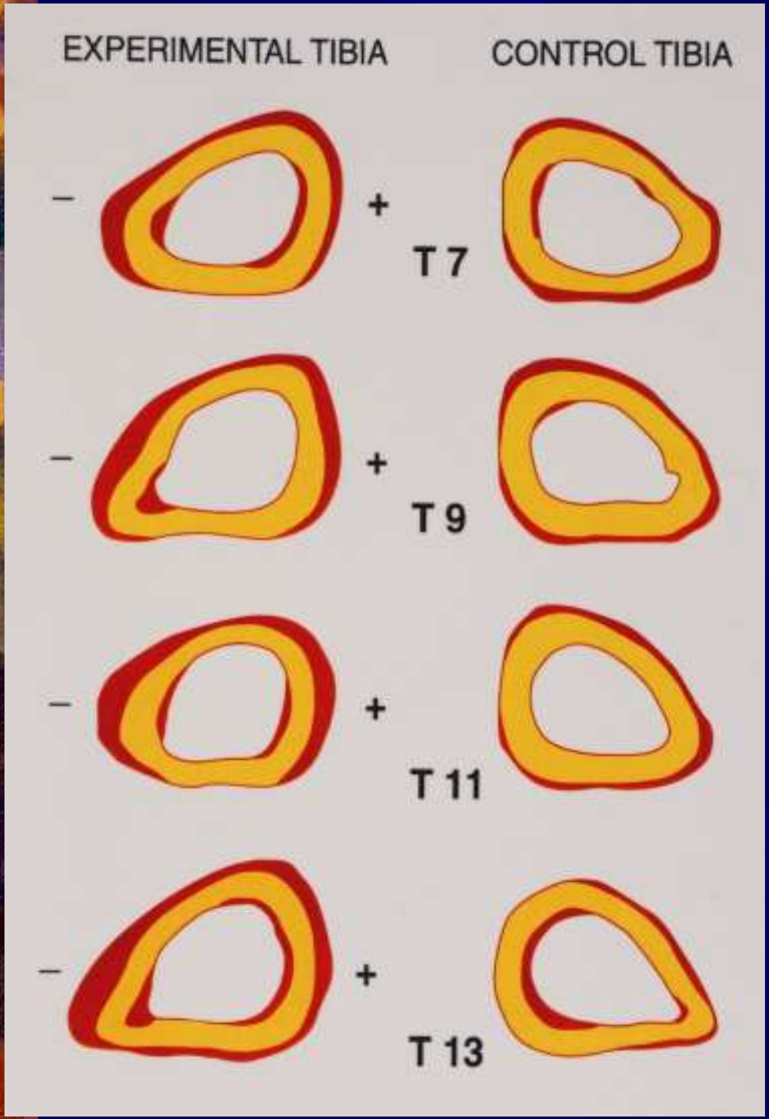
- The trajectoral theory was not without its critics, the most prominent being Triepel (1922). A difficulty of fundamental importance arose from the fact that an engineer in calculating the distribution of trajectories in a stressed body, assumes the body to be homogeneous in structure. Bone is clearly not structurally homogenous and in Triepel's opinion, the assumption that the trajectories in bone will be closely similar to an ideal homogeneous body was fundamentally flawed.
- While mechanical forces clearly play a large part in the determination of osseous structure, the trajectoral theory in its rigid form demands a degree of perfection that does not exist in Nature (unlike von Meyer, Wolff insisted the trajectories were orthogonal, conveniently ignoring the fact that trabeculae do not always cross each other at right angles).
- While such a difference of opinion can be regarded as a minor academic tiff, a more important difficulty is that Wolff's Law only takes into account the positive effects of mechanical loading, when it is evident that mechanical strain can have negative, as well as positive effects on bone mass.

# Development of *in vivo* experimental models




- The 1960s saw the development of experimental animal models in which carefully controlled external loads could be applied to bone.
- Hěrt and co-workers in Czechoslovakia inserted 2.0–2.5 mm Kirschner pins into the right tibiae of rabbits, which were attached to an EMG device that permitted approximation or separation of the wires which flexed the bone.
- From McDonald *et al.* (1994). *Medical and Engineering Physics* **16**, 384–397.

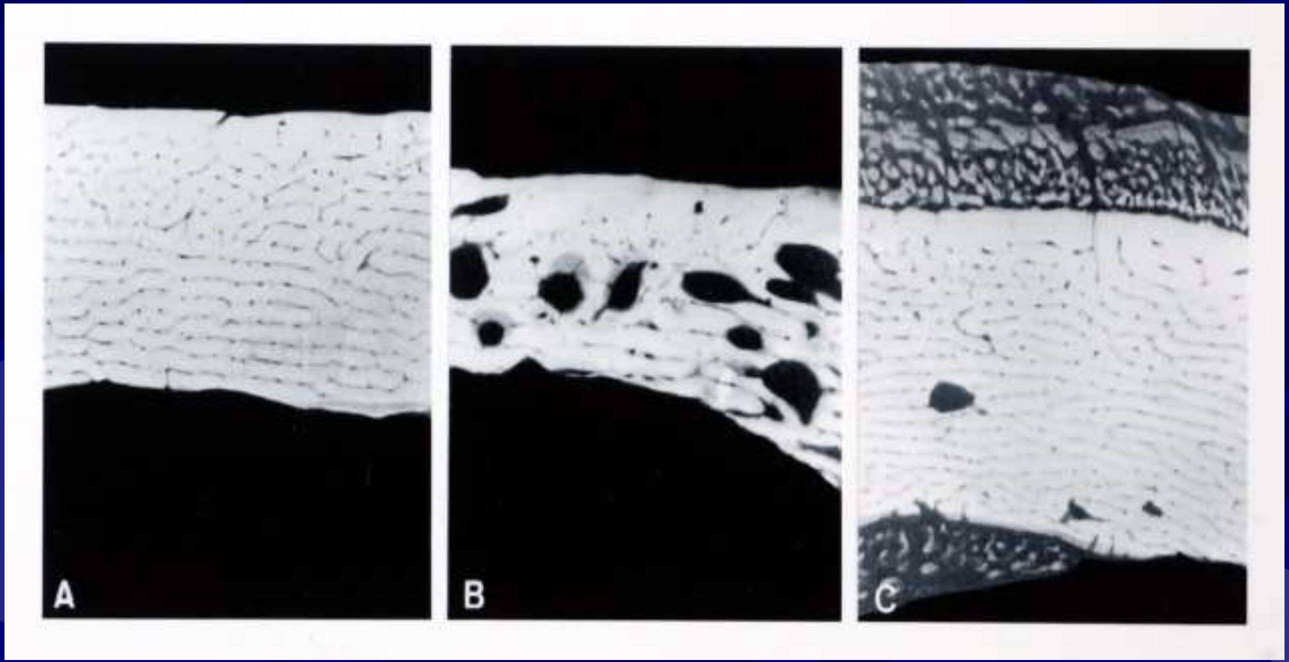
# Reaction of bone to mechanical stimuli




- In a classical series of experiments Hěrt *et al*. used the model to apply continuous or intermittent deformation to the tibiae of rabbits.
- A continuous stress applied to the surface of the tibia failed to stimulate bone formation; intermittent forces, however, increased osteogenesis on both the endosteal and periosteal surfaces of the cortical bone.
- These are sections through the tibial diaphysis after intermittent loading. Tension (-), compression (+) side. The +/- signs do not indicate the surface charge at the bone surfaces.

Redrawn from Hěrt *et al*. (1971), *Folia Morphologica (Praha)* **19**, 290–300.

# Functional loading of the turkey ulna




- Lanyon and co-workers confirmed these findings by means of a functionally isolated turkey ulna preparation. Daily loading of 100 consecutive 1 Hertz cycles were sufficient to maintain osseous architecture (A). In (B) the ulna has been isolated from load bearing for 8 weeks. The cortex is thinned and contains numerous resorption cavities leading to osteopenia. (C) Intermittent loading has produced new bone on both the periosteal and endosteal surfaces.

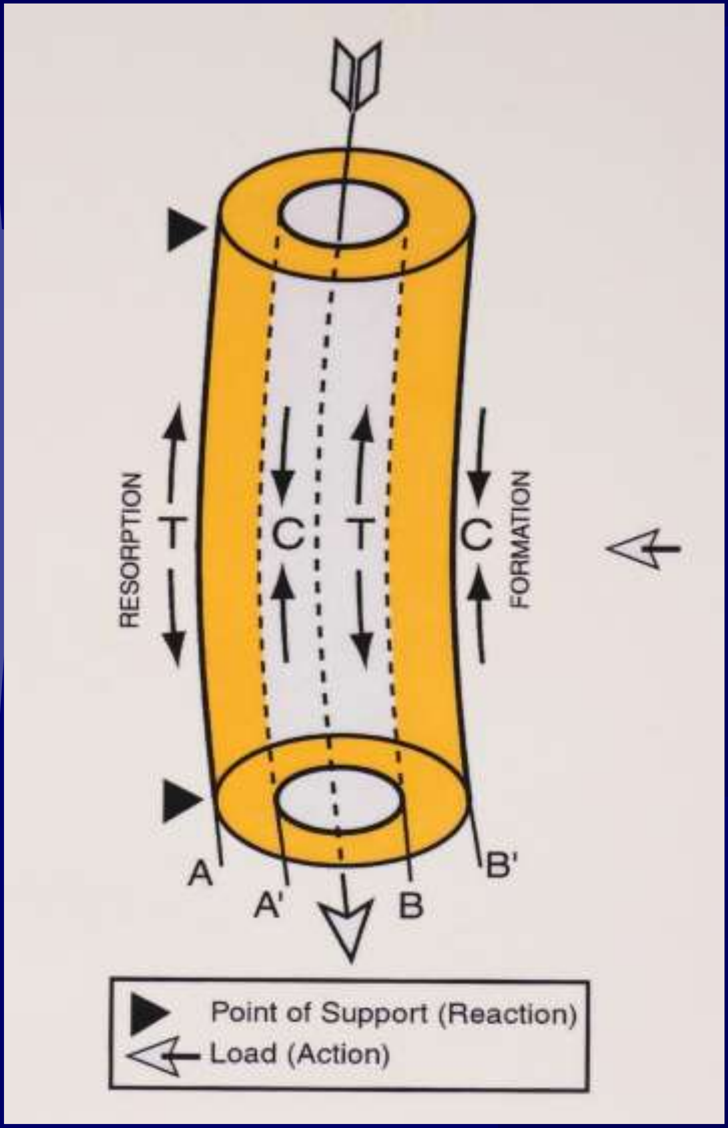
- From Lanyon (1984). *Calcified Tissue International* **36**, S56–S61.

# Changes in gene expression

- Studies of gene expression in the tibial periosteum of rats after application of an external load *in vivo* have shown a rapid increase in c-fos mRNA levels within 2 hours, but a decline in mRNAs encoding the bone matrix proteins osteocalcin, osteopontin/SPARC and ALP (Raab-Cullen *et al*. 1994). This suggests that the initial tissue response was cell proliferation. Another early response was a rapid induction in growth factor synthesis in which TGF-β and IGF-I mRNA levels peaked at 4 h.
- Evidence from a rat tail model (Chambers *et al*., 1993), indicates that following mechanical loading, the osteogenic response was not dependent upon a preceding phase of bone resorption, but was due to activation of previously quiescent bone lining cells.
- Osteocytes are also sensitive to mechanical deformation and may function as mechanosensors. Load-related increases in $^{3}H$-uridine, glucose-6-phosphate dehydrogenase and IGF-I mRNAs have been detected in osteocytes (Pead *et al*., 1988), demonstrating that intermittent loading produces rapid changes in their metabolic activity.


- Chambers *et al.* (1993). *Bone and Mineral* **20**, 167–178.
- Pead *et al.* (1988). *Journal of Bone and Mineral Research* **3**, 647–656.

# Stress-generated electrical effects in bone




- When an external load is applied to a long bone, deformation occurs producing stress-generated electrical potentials (piezoelectrical effects). The external surface of the left cortex elongates in tension, while the internal surface shortens in compression: opposite effects are produced on the right cortex.
- Increasing concavity is associated with bone formation and electronegativity. Increasing convexity is associated with bone resorption and electropositivity.
- Redrawn from Epker and Frost (1965). *Journal of Dental Research* **44**, 33-41.

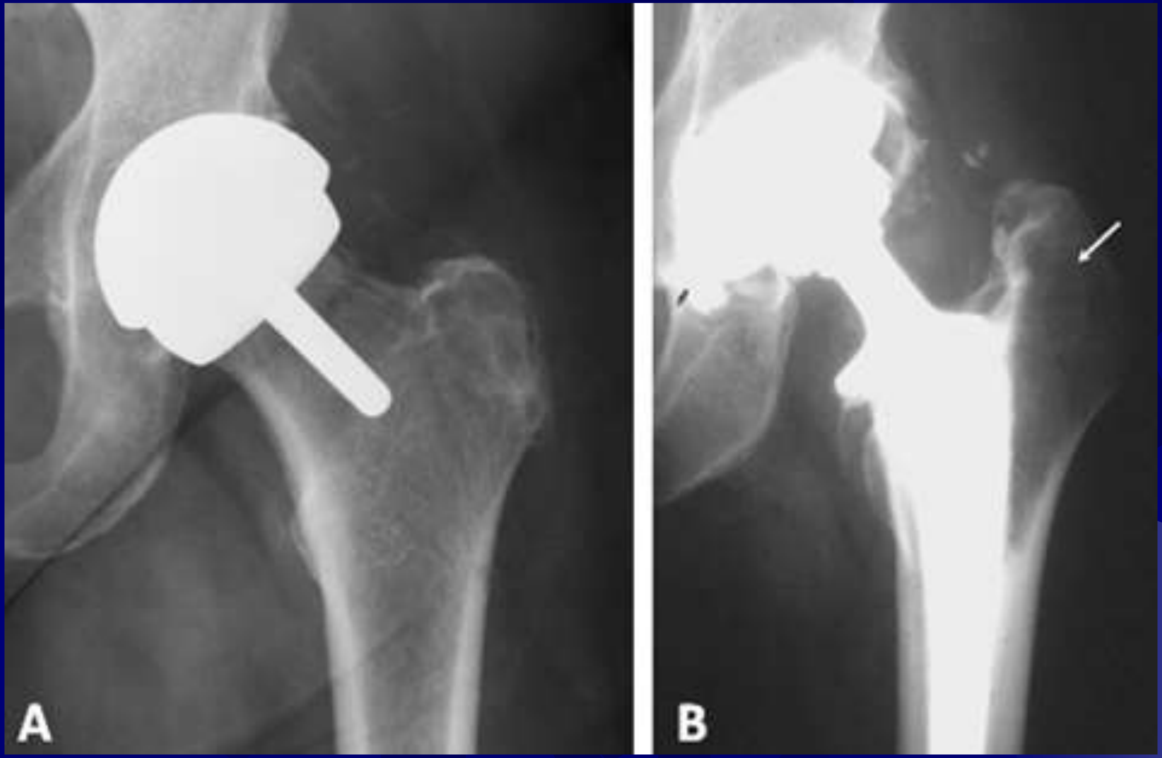
# Problems with the piezoelectrical theory of bone remodelling

- It is not sufficiently discriminatory to be able to regulate the action of cells as diverse as osteoblasts and osteoclasts which function in close proximity at bone surfaces.
- Piezoelectricity does not require the presence of living cells; dead bone displays the same effects, which appears to be generated by shearing forces acting on the collagen fibres of the bone matrix.
- It seems likely that stress-generated electrical potentials are an irrelevant by-product of bone deformation, physical phenomena that provided a plausible explanation for the regulation of bone remodelling prior to the discovery of cytokines, growth factors and other cell–cell signalling molecules in the 1980s.

# Effect of reduced bone strains

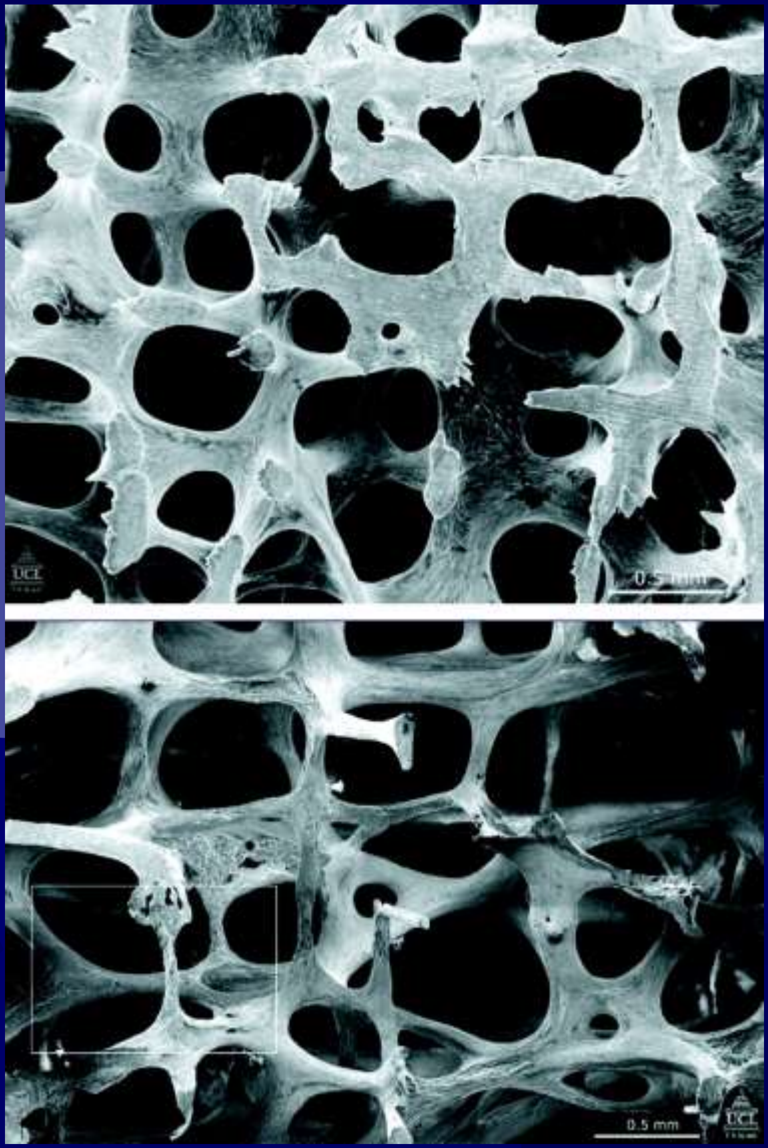
- According to Wolff's Law "The structure of bone is morphologically adapted to its function in accordance with mathematical principles." This enables bone to provide maximum strength with a minimum of material.
- The problem with Wolff's Law is that it only takes into account the positive effects of mechanical loading. But mechanical strains can have negative, as well as positive effects on bone mass.
- Reduced mechanical loading associated with prolonged bed rest or spaceflight has been shown to result in bone loss. Stress-shielding and osteopenia resulting from the implantation of rigid metallic devices into bone, is also a well-recognized complication of joint arthroplasty and fracture fixation in orthopaedic surgery.

# Stress-shielding and osteopenia




- A. Image from a patient with a femoral head arthroplasty. B. Patient with a cemented total hip arthroplasty and stress-shielding. Radiograph shows localized osteopenia (arrow) of the greater trochanter, resulting from altered mechanical loading and reduced bone strains due to the rigidity of the femoral stem.

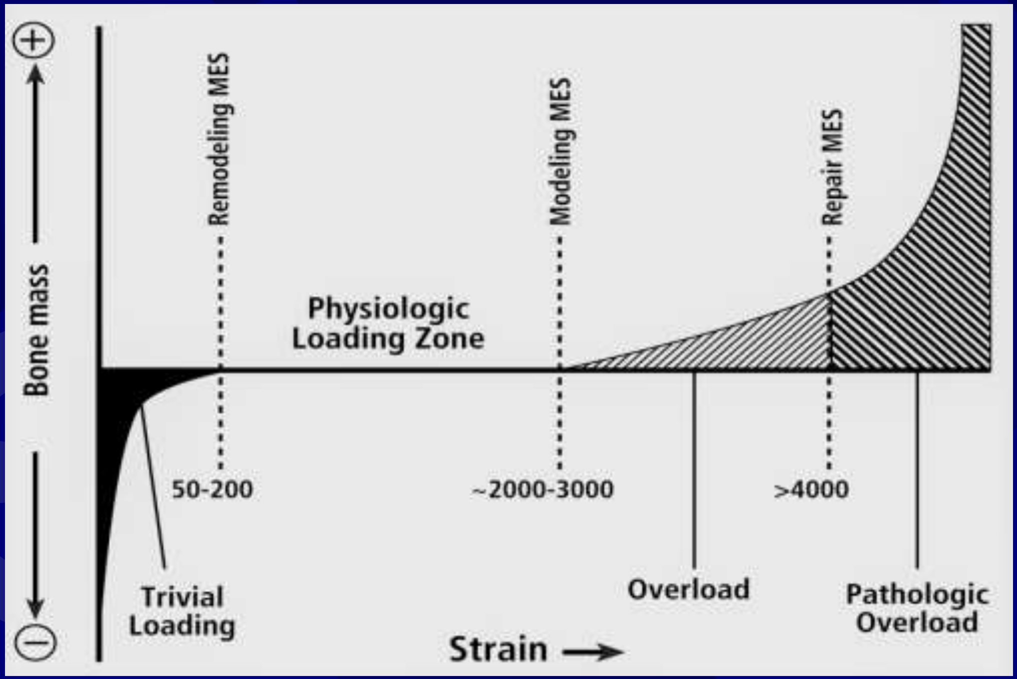
# Osteopenia and osteoporosis



- Osteopenia is a sign of localized negative skeletal balance and reversible.
- Osteoporosis is a pathological conditionmost commonly resulting from estrogen deficiency in postmenopausal women. Bone mass in elderly men is also positively related to estrogen levels.
- Vitamin D deficiency and secondary hyperparathyroidism, reduced physical activity with ageing, and decreased production of IGFs are also risk factors.

SEMs of L3 vertebrae in a 31- and a 70-year-old woman. Courtesy of the *BMJ* and JE Compston

# The Mechanostat of Harold Frost



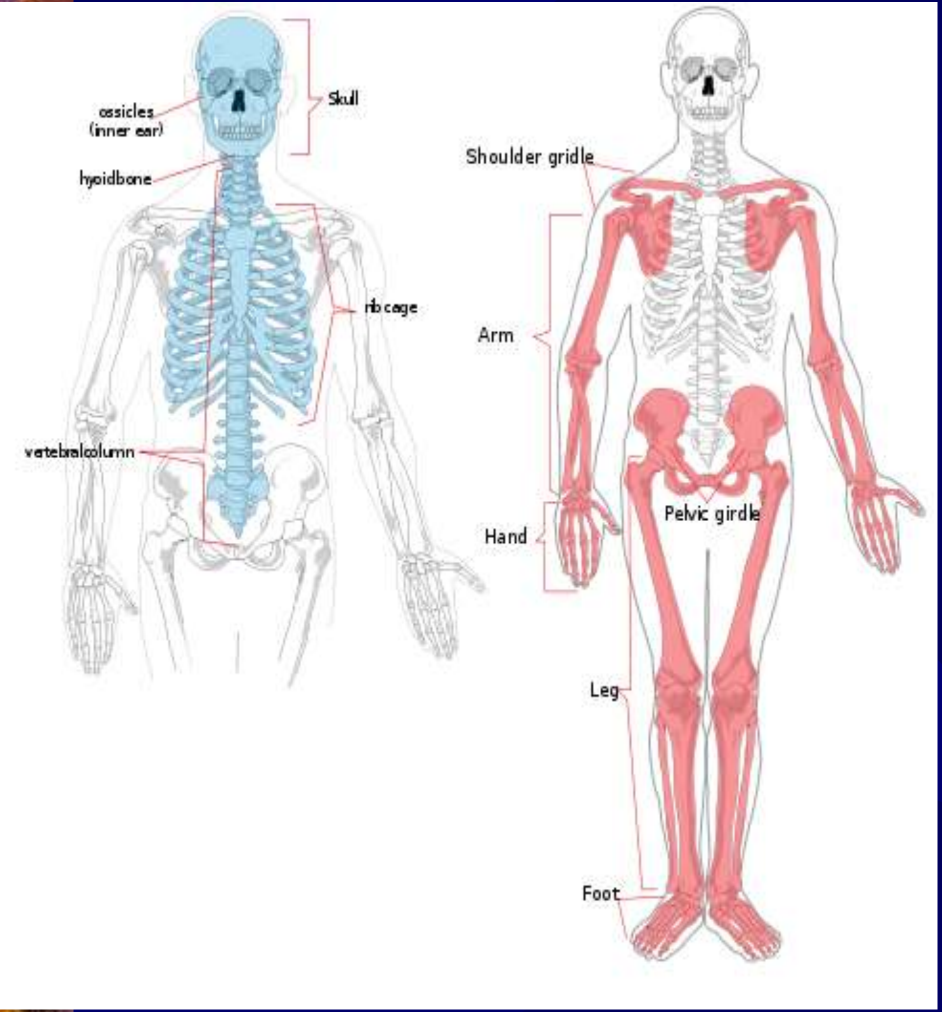

- To account for the negative effects of reduced bone strains on bone mass, in 1987 Harold Frost introduced the concept of a mechanostat for regulating bone mass (the Utah Paradigm). He proposed for each bone, there is an adapted state within which normal bone mass is maintained.
- If bone strains exceed a certain critical threshold the result is bone formation - reduction below another threshold value will lead to bone loss and osteopenia. Image from Wikipedia.

# Difficulties with the mechanostat

- The mechanostat has proved to be a useful concept, but doesn't explain everything. Phenotypic differences exist between individual bones depending on their embryonic origin and anatomical location, and calvarial and limb bones do not demonstrate the same response to dynamic mechanical strain.
- Rawlinson *et al.* (1995), recorded normal functional strains as low as 30 με on the rat parietal bone.
- In direct strain measurements on a human volunteer, the highest strains recorded (200 με) in the skull were 10-fold lower than for the tibia (Hillam *et al*., 1996), levels that in the rest of the skeleton would have led to profound bone loss.


- Rawlinson *et al*. (1995). *Journal of Bone and Mineral Research* **10**, 1225–1232.
- Hillam et al. (1996). *Bone* **19**, 686 (Abstract).

# Not all bones are created equal




- In Seminar 1 we saw that the bones of the skull are derived from the neural crest (apart from the basi-occiput formed by the first five somites). Not mesoderm as in this diagram.
- Apart from the skull, (which this illustration has got wrong) the rest of the skeleton is derived from mesoderm. In the case of the axial skeleton (Blue) from the somitic or paraxial mesoderm, and for the bones of the appendicular skeleton (Red), the lateral plate mesoderm.

# Differences in bone metabolism

- Concentrations of growth factors are significantly higher in calvarial bone, than iliac crest or vertebral bone, differences that may account for the superiority of neural crest bone in grafting procedures.
- The most striking difference, however, is in their osteoclasts. Long bones depend on cysteine proteinases (cathepsins B and K) for resorption, whereas calvarial osteoclasts produce MMPs (collagenase) as well as CPs.
- This is dramatically illustrated by pycnodysostosis, an autosomal recessive lysosomal disease caused by a mutation in the cathepsin K gene. French artist Henri de Toulouse-Lautrec is thought to have had the disease.

# The mechanostat does not explain everything

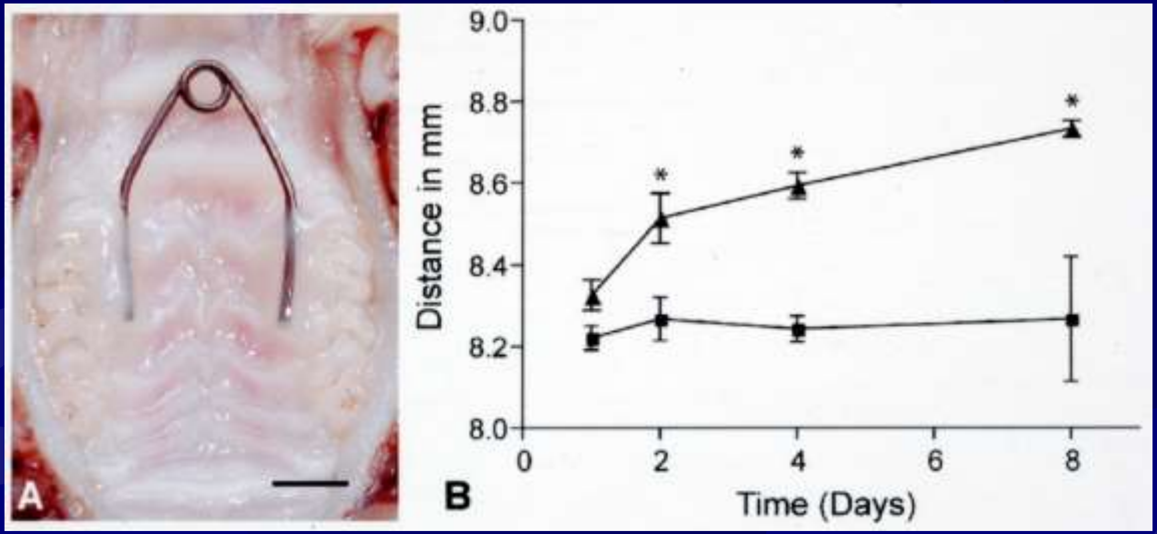
- Unfortunately, none of the above provides an answer to the original question: what is it about calvarial bone that makes it resistant to levels of mechanical strain that in the rest of the skeleton would lead to profound bone loss?

- It can't be just because calvarial bone is derived from the neural crest – the bones of the jaws are also of neural crest cell origin and do not show the same resistance to reduced mechanical loading. The mechanostat is very helpful in understanding the relationship between mechanical strain and bone mass but does not explain everything.

# The appliance effect

- Tooth movement studies to date have largely focused on histological changes in the PDL and the alveolar bone–PDL interface. However, there is another important question that has received little if any attention that also needs to be addressed.
- What is the effect of the appliance itself on the metabolism and remodelling dynamics of the tooth-supporting tissues?
- If nothing else an orthodontic fixed appliance is a metallic device of varying rigidity, and therefore might be expected to alter the loading dynamics of the tooth-supporting alveolar bone.
- The above statement is made with the benefit of hindsight, because in the course of a tooth movement study in the rat, we made the serendipitous discovery during a University of Otago research project by Bhavik Patel, that a cross-arch expansion spring resulted in a reduction in alveolar bone mass, irrespective of whether the spring was activated or not (Milne *et al*., 2009).

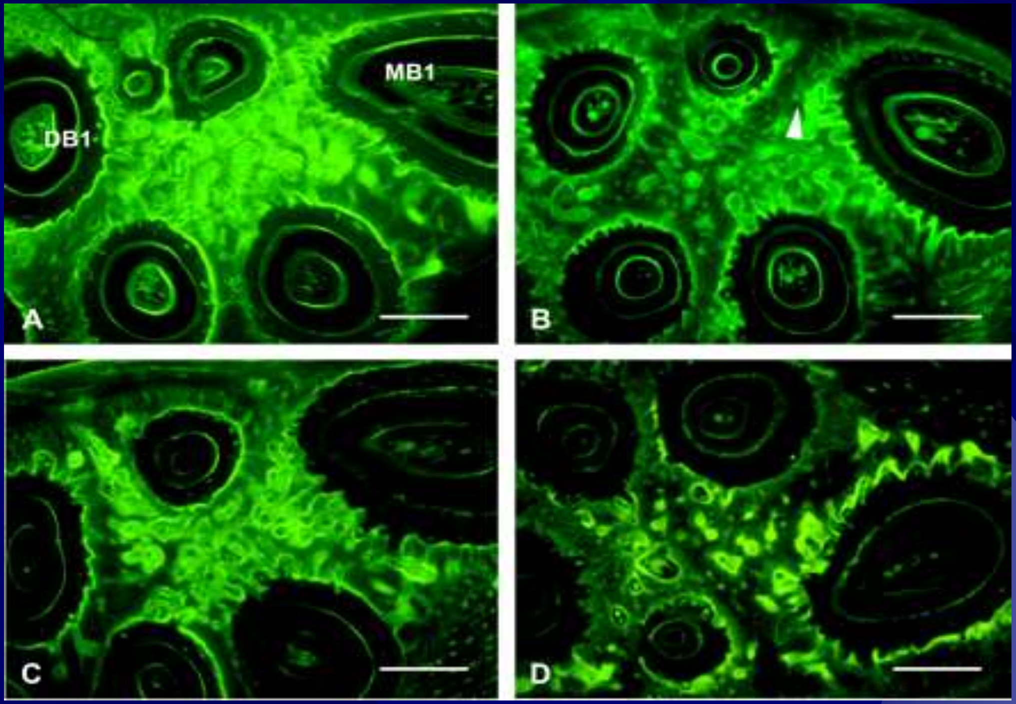
# Appliance-induced osteopenia




- The experimental model consisted of a stainless steel (0.012 inch) palatal spring bonded to M1 and M2 of 6-week-old rats with self-etching primer and light activated composite resin. When activated the spring delivered a force of 0.2N (20 gm). Negative controls (no springs), as well as positive controls (with inactive springs) were included. Movement of M1 (to 0.01 mm) was measured with a Nikon Measurescope (Sony Corp).
- The expansile force produced an increase in the intermolar width averaging 0.25 mm by day 2 and 0.5 mm by day 8.
- From Milne *et al.* (2009). *European Journal of Orthodontics.* **31**, 221–231.

# Tetracycline labelling

From Milne *et al.* (2009). *European Journal of Orthodontics.* **31**, 221–231.




- Representative horizontal sections through M1 with 3 buccal and 2 palatal roots. Each animal received a subcutaneous injection of tetracycline 24h prior to sacrifice. Tetracycline is deposited at mineralizing bone surfaces and fluoresces under UV-light.
- (A) 4-day control. (B) 4-day experimental; arrowhead shows direction of applied force. (C) 8-day control. (D) 8-day experimental. Bar = 500 μm.

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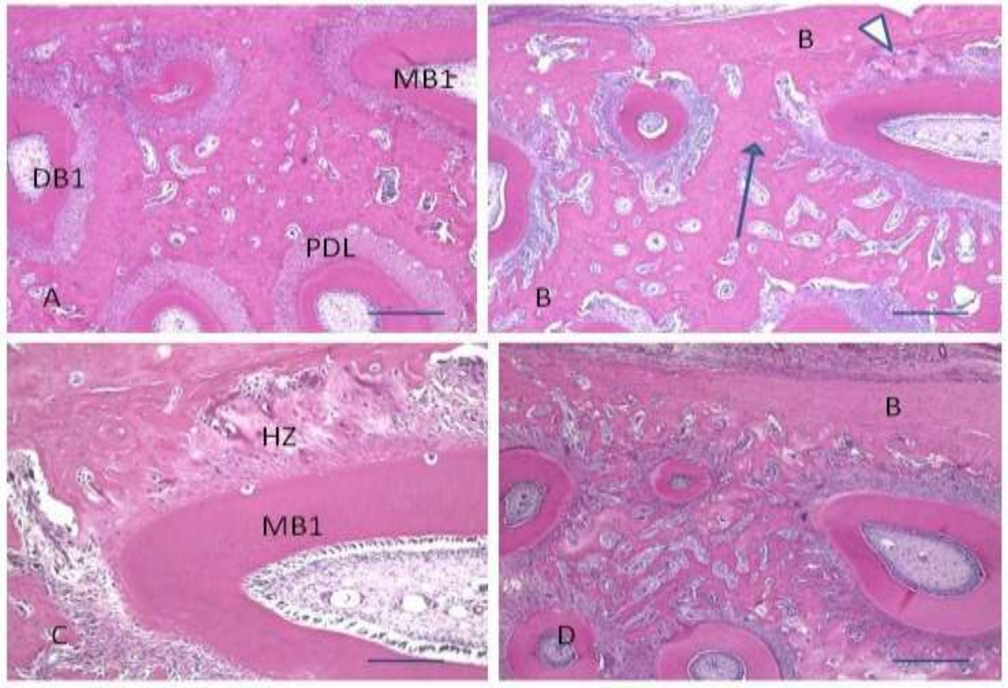
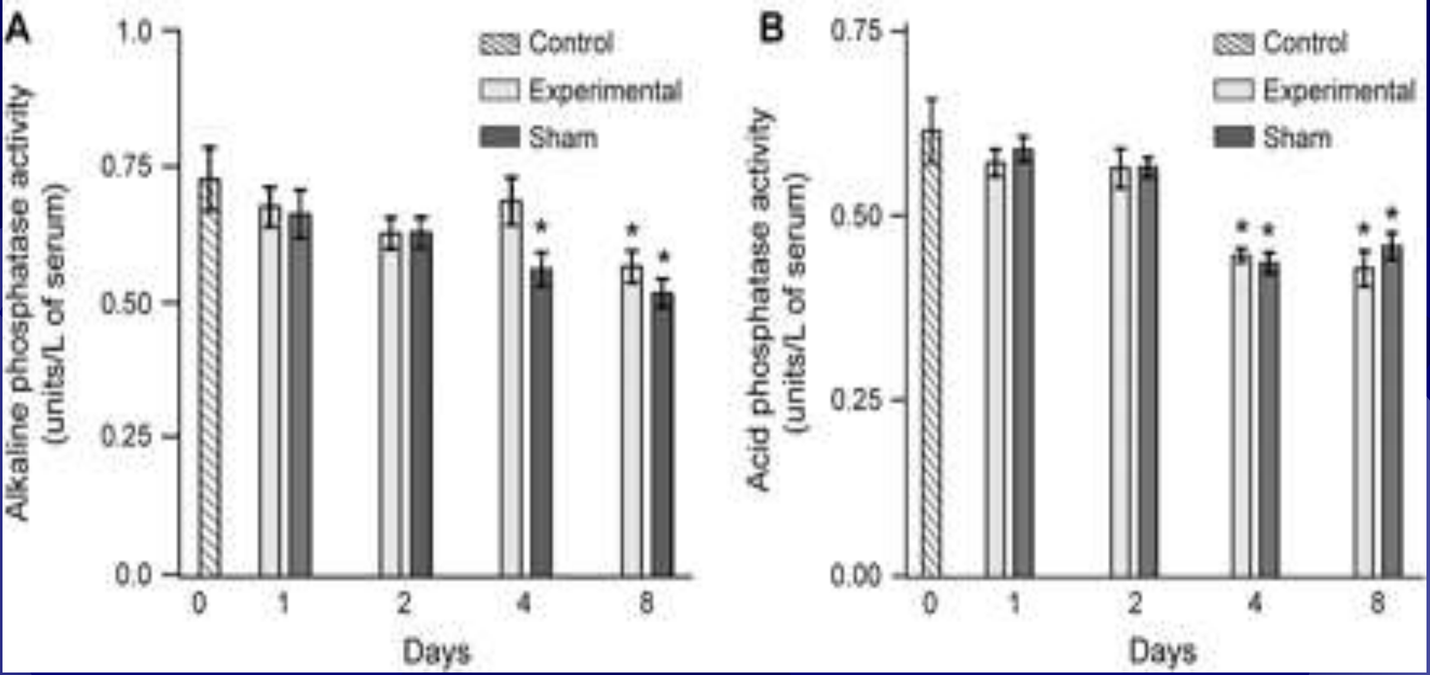
# Induction of bone loss and osteopenia

From Milne *et al.* (2009). European Journal of Orthodontics.




- Representative horizontal sections through M1 from the rat expansion spring model showing a progressive reduction in the in the density of the inter-radicular bone; the bone has become osteopenic.
- (A) zero-day control. (B) 4-day experimental. (C) Detail from B showing the hyalinized tissue. (D) 8-day experimental. Bone highly trabeculated at the bone-PDL interface.. H & E stain. Bar = 500 μm.

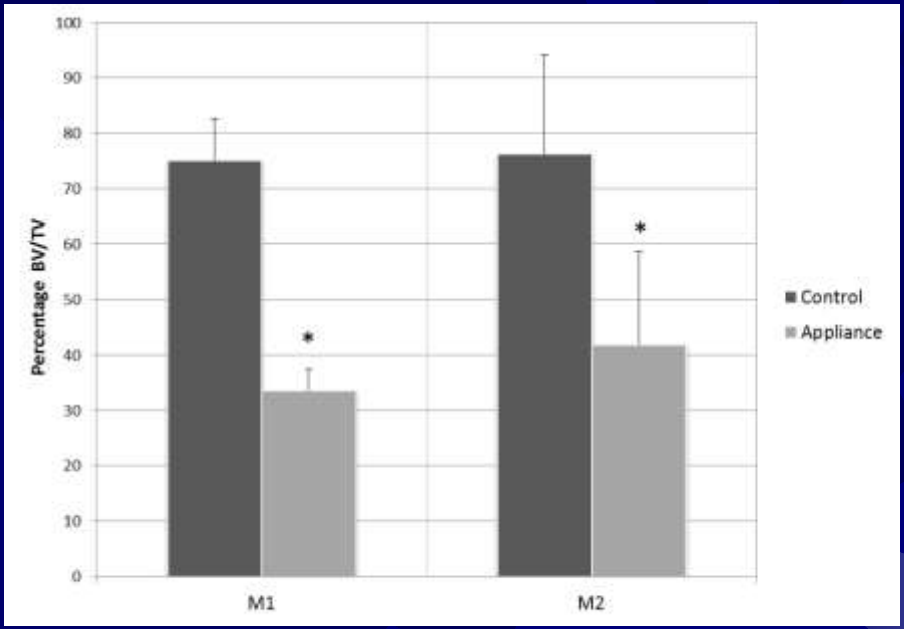
# Serum ALP and ACP activity




- Alkaline(ALP) and acid phosphatase (ACP) activity in serum (n = 5) from zero-day controls, experimental (with active springs), and sham-treated (with passive springs) groups.

- *ALP significantly less than zero-day controls. $P < 0.05$. ALP activity was also significantly reduced in sham-treated animals compared to the experimental group at day 4. ACP was also significantly less than zero-day controls < *P* 0.05. From Milne *et al.* (2009). *European Journal of Orthodontics.*

# Effect of a passive spring on bone volume



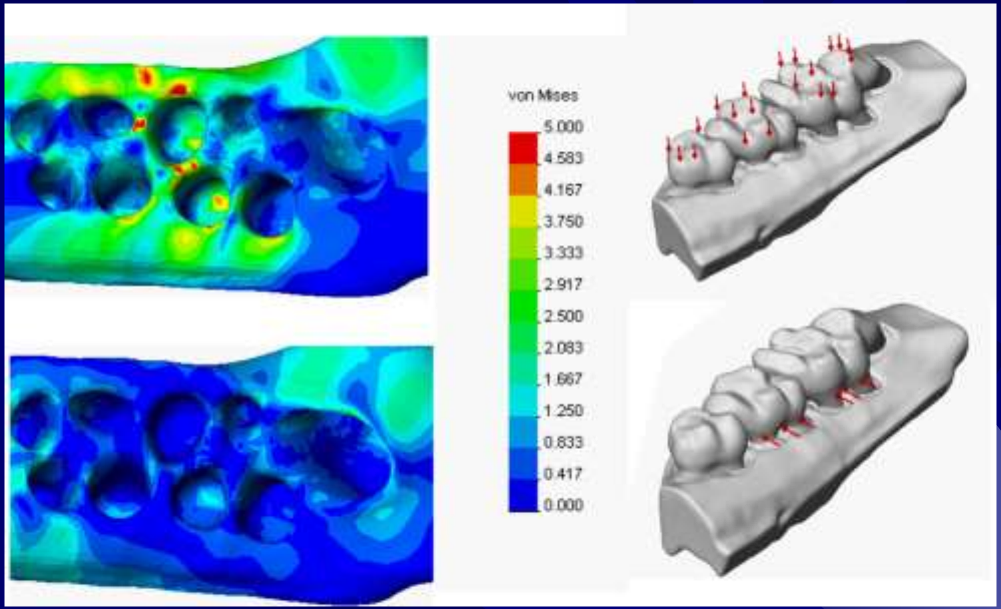

From Vinoth *et al*. (2013). European Journal of Oral Sciences **121**, 517–524.

- In a separate series of experiments, the effect of passive springs on the inter-radicular bone volume of M1 and M2 were quantified by histomorphometry. Serum bone markers from these animals were also assayed.
- Image analysis shows a significant reduction in bone volume, calculated as a percentage of total volume (mean ± SEM) in the appliance group after 8-days.

# 3-D finite element analysis

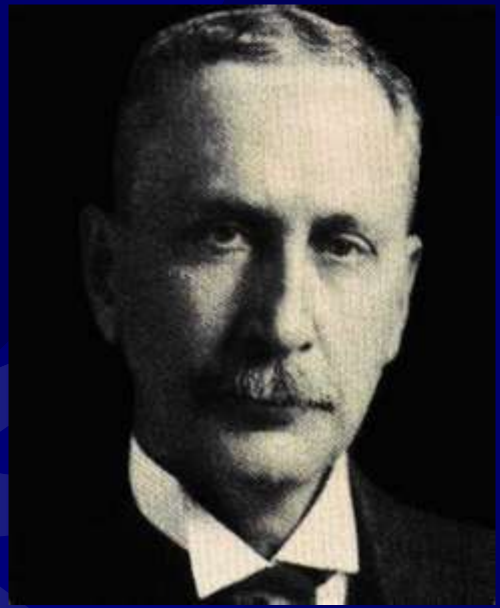
From Milne *et al.* (2009). *European Journal of Orthodontics.*

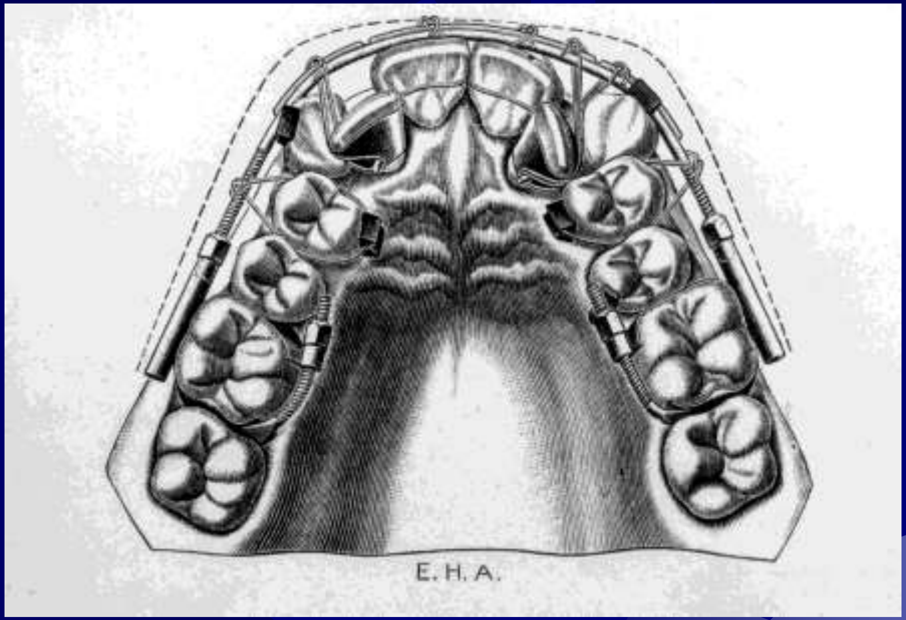
3-D finite element analysis by Dr Ionut Ichim.




- Plots showing the distribution of the distortion energy (von Mises stresses in MPa) in the alveolar bone of the first and second maxillary molars.
- (Top) Following a masticatory occlusal load of 2N, the distortion energy is spread throughout the alveolar process affecting both the buccal and lingual plates and inter-radicular bone.
- (Bottom) During an orthodontic load of 0.2N produced by an expansion spring, the distortion energy is significantly less with little or no stress in comparable regions of the bone.

# Growing bone






- Thanks to Edward Hartley Angle's interpretation of Wolff's Law, it has been an article of faith amongst orthodontists for more than 100 years that orthodontic appliances can stimulate the growth of bone.
- To quote Angle in his Seventh Edition (1907) …."If started young enough, the stimulating effect of orthodontic tooth movement and normal occlusion would enhance growth of the jaws and alveolar process." (pp 386-401).
- In other words, malocclusion could be treated without extractions by 'growing bone.'

# What does it all mean?

- In accordance with mechanostat theory, these data suggest that an orthodontic appliance produces stress shielding of the inter-radicular bone, leading to osteopenia similar to that associated with prolonged bed rest and spaceflight – or the implantation of rigid metallic devices into bone following orthopaedic surgery for joint replacement and fracture fixation.
- Three-dimensional FE analysis of the stresses generated in the bone suggest that the orthodontic appliance created a constant loading condition shielding some areas of bone from mechanically-induced strain, resulting in a reduction of occlusal loading below the critical threshold required to maintain normal osseous architecture.
- Instead of having a positive effect on bone mass, these data suggest an orthodontic appliance may exert unexpected negative side-effects on the tooth-supporting alveolar bone. Measurements of serum markers of bone metabolism indicate that the osteopenia is due to a reduction in bone formation, not an increase in resorption.

- This rather complicates how one interprets the effect of the appliance on bone metabolism in orthodontic patients – in addition to the alterations in bone remodelling associated with tooth movement mediated by the PDL and its potential buffering effect, there is the potential stress-shielding effect of the appliance itself to consider.
- These observations are also consistent with the findings of experimental masticatory hypofunction in rats, showing that reduced occlusal loading leads to a reduction in alveolar bone mass and bone mineral density.
- Finally, these findings highlight the importance of having positive as well as negative controls in experimental tooth movement studies, and recognizing that bone strains can have negative, as well as positive effects on bone mass.