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ANNIVERSARY REVIEW

The structure and mechanics of bone

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Abstract The four levels of hierarchy considered in this review are the nanoscale (the mineralised collagen fibre and the extrafibrillar mineral), the microscale (the structure as visible under the microscope), the mesoscale (particularly the relationship between cancellous and cortical bone) and the whole bone scale. The explosion of papers at the nanoscale precludes any settling on one best model. At the microscale the inadequacies of linear elastic fracture mechanics, the importance of *R*-curves for understanding what is happening to cracks in bone, and the effect of different histological types are emphasised. At the mesoscale the question of whether cancellous bone is anything but compact bone with a lot of holes in it, and the question of whether cancellous bone obeys Wolff's 'law' is discussed. The problem of not damaging bone when examining it with X-rays is mentioned (though not solved). At the whole bone level the relative roles of genetics and the external forces and the question of the way in which bones are loaded, in bending or compression, is raised, and the question of size effects, long underestimated or ignored by the bone community, is discussed. Finally, the question of why there are hierarchies at all in bone is addressed

Introduction

In this short review, I shall deal almost entirely with 'standard' bones: limb and vertebral bones of mammals, and will ignore the more exotic bones that are used as clubs, armour, springs, tough bones like deers' dry antlers, delicate bones like the turbinals, bones as ballast found in the dugongs, and so on. Many of these are described briefly in [1]. I deal eclectically with subjects that interest me. Furthermore I shall not deal with bone diseases, such as osteogenesis imperfecta, or tissue engineering. These are both very important topics that I am incompetent to deal with.

Since the Journal of Materials Science started 45 years ago there has been an enormous increase in the ability of workers in the field of bone properties to understand what is happening in this tissue. The increase has mainly come about from increases in technology rather than, primarily, wonderful insights that people have had. Or, rather, the insights have come from the enabling of technology. However, the application of materials science concepts to bone has often been very fruitful. Some of technological enhancements have come from the refining of pre-existing technologies, some from entirely new ones, to biology anyhow. Panel 1 categorises some of these. It is interesting that at the highest level, that of whole bones new insights have been much sparser than at the lower levels. This is possibly partially because, apart from FEA, little in the way of enabling technologies have been produced.

Bone's structure is hierarchical

It is a truism these days to say that bone has a hierarchical structure. I consider four levels in this review. These are the nanoscale (the mineralised collagen fibre and the extrafibrillar mineral), the microscale (the structure as visible under the microscope), the mesoscale (particularly the relationship between cancellous and cortical bone) and the whole bone scale. For a good review giving seven levels see Weiner and Wagner [2]. Of course, how many levels are present are not fixed, they are user-specific, and

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dependent on the interest of and availability of equipment to the user. For instance, before the invention and use of the electron microscope, all levels below the microscale would have been a closed book. However, the implications of the hierarchy, both structural and mechanical, are not always fully appreciated. In particular, there is still a great deal of simplification, little of it sensible, that goes on in the thinking about the transition from the tissue level to the whole bone level. Furthermore, it is also not always obvious what mechanical properties should be considered important for the real animal. For instance, mechanical properties are usually assessed quasi-statically, because it is much easier to do so, whereas in life bones are loaded traumatically at quite high strain rates or they may be loaded repetitively to strains that would not cause failure in a single loading, and so fail, if they do, by fatigue. Fortunately, there is often a good relationship between quasi-statically determined properties and properties determined at higher strain rates. However, toughness, one of the really important mechanical properties of bone, often *decreases* somewhat at high strain rates whereas stiffness, another important property, *increases* slightly. Repetitive loading usually comes about as a result of locomotion or mastication, and in humans, for instance has a frequency of about 2 Hz, resulting in a rather low strain rate. For smaller animals, the characteristic frequency is higher.

What is bone for, mechanically?

We can start by asking what are the desirable mechanical and physical properties for bone tissue, and for whole bones. We should consider what bone is designed by natural selection to do. Often, because if its clinical importance, fracture versus non-fracture is considered to be the important feature of bone mechanics. However, natural selection has, over geological time, weighed up all the features of bone (and of everything else in the body including behaviour), and resistance to fracture is but one. Probably the main adaptive feature of bone is its stiffness, with also a certain amount of toughness. Bones would be stiffer if they were thicker, but this would increase their weight, bulk, cost, time to build and so on, which would be bad for obvious reasons. Therefore, natural selection has come up with some optimisation; the bone material must be stiff, and for this it needs to have a certain density. (Differences in density of the bone *material* are insignificant in determining whole bone mass compared with differences in whole bone structure—the cortical thickness, for instance.) If the volume of the whole bone increases, then the bone will become stiffer, heavier and more massive, and there comes a point where the disadvantage of the increase in weight and mass becomes greater than the advantage of the increase in stiffness. If the bone is a certain size, and the bone material has a certain strength, then the bone will break under a certain load, or impulse of energy. How much greater this load is than those to which the bone is subjected in its day-to-day life when the animal is in extreme activity, such as sprinting, gives a 'safety factor'. Safety factors in mammalian bones are not large, usually being of the order of about 2–4 [3 page 325 et seq.], and that is why people break their bones quite frequently. Considering the variability of loading that bones must endure, it is clear that a safety factor of 3 would be negligently small in a critical engineering structure, such as nuclear reactor vessels but natural selection is concerned only with the success of organisms in continuing the genetic lineage down the generations; living dangerously may be one of the keys to success.

As well as Young's modulus, static yield strength gives quite a good idea of the general fitness of a bone for its function, but one must always be aware that except in the case of fatigue, *toughness*, rather than yield strength, is probably what is important in the real world. Strength and toughness, however, are not the only features determining whether a bone fails or not. Failure of a structure may be described as becoming unfit for its purpose, whatever that may be, and does not necessarily imply fracture. Bone may fail for not being *stiff* enough, through buckling, either Euler or local. Furthermore relatively compliant bones would increase the cost of locomotion, requiring as they would longer muscles. Such bones might not break, but nevertheless might impose unadaptively high metabolic costs on the animal, and could be said to have failed to fulfil their function properly.

Another problem is that bones in life are probably normally loaded mainly in compression [4, 5] though also to some extent in bending in particular directions [6, 7] but when they fail they can have been loaded in any direction, particularly if they fail from traumatic loading. It is much easier to test bone in bending or tension than in compression, and as a result many more properties of bone are known from tensile tests, or bending tests, than from compression tests, so much of the information about bone mechanics is from tension, which is probably not the habitual mode of loading. This is often important. For instance, George and Vashishth [8] showed that the mode of failure and loads at which it occurred were quite different in tension and compression.

Unfortunately, one cannot say that a bone must be as stiff as possible, as tough as possible, or as resistant to fatigue as possible. As we shall see, these properties to some extent run against each other, and there are also the factors of bone mass and concomitant soft tissue mass to consider. Bones, like everything else in biology, are a compromise. Nevertheless, there are probably four major mechanical properties that need to be considered: elastic

modulus, yield stress, toughness at high strain rates and fatigue strength.

Bone structure and mechanics at the nanoscale

Here one is talking about the single mineralised collagen fibril and one would hope, though this is often not attempted, the extrafibrillar mineral, which must certainly be important. This extrafibrillar mineral is difficult to measure directly, and estimates based on models of mature bone produce very different answers, from about 30 [9] to 75% [10, 11]. Personally, I think that the higher values are probably nearer the truth.

There has recently been an explosion of interest in modelling mechanical properties of bone at the nanoscale, e.g. [11–20]. Also, the use of gene-based methods is beginning to come on stream, e.g. [21] and there has been a great increase in the number of papers, and even understanding, coming from such work. I shall mention, as an example, only one recent study, which is particularly relevant. Chang et al. [22] report on one of the interesting and great unsolved and under-researched problems about bone, that is, how is the particular mineral content of the bone regulated? The content is variable between bones, between parts of bones, between bones of different ages, and has profound effects on the bone's mechanical properties. Chang et al. examined the cochlear bone of the mouse, which is normally heavily mineralised (as are all mammalian cochleas, for auditory reasons). They produce evidence that the transcription factor Runx2 regulates the properties of the collagenous bone matrix. (Transcription factors are proteins produced by genes that have their effect in regulating the extent to which other genes produce their products. Their importance has only recently become obvious.) Less Runx2 production, for instance in heterozygotes with one gene inactive, results in a lower Young's modulus of the cochlear bone, which has a bad effect on hearing. It is to be hoped that now we are beginning to see from studies like these what gene products are important for the matrix/mineralisation properties, and that we shall be able to see what it is about the collagenous matrix that is different in highly mineralised bones.

The number of models at the nanoscale level at present vying for supremacy is daunting. Luo et al. [18] write (I leave out references) 'A number of models have been proposed recently to explain mineral–collagen interaction in bone…the organic phase interacts with the mineral phase through either ions or hydrogen bonds…ionic interactions…sliding of layered water films…sacrificial bonds and hidden lengths…virtual internal bonds.' With such a plethora of models available (and many are not mentioned here) it is unsurprising that it is difficult to see the wood for the trees, and I do not attempt to be in favour of any one model.

Elastic behaviour

The elastic behaviour of bone is probably easier to analyse than the fracture behaviour. The consensus is that the plate shape and very small size of the carbonate apatite crystals in bone are very important. In the elastic region, it is supposed that the collagen takes loads from the mineral crystals and transfers them to 'downstream' crystals via shear forces. Immediately one of the questions that arise is what is it that keeps the mineral crystals small and plate-like? Viswanath and Ravishankar [23] for example suppose that it is the physical conditions in which the crystals are deposited that determines this, whereas Hu et al. [24] claim that citrate ions, bound to the surface of the crystals, inhibits them from expanding much.

Hellmich and coworkers, in two massive papers [14, 15], deal with the elastic and the fracture behaviour of bone respectively. They apply homogenisation techniques to models of the material, thereby bypassing detailed consideration of differences between different histological types. They regard the extrafibrillar mineral as a multiply connected foam. For elastic properties, their modelling produces answers that are really quite well in accord with the ultrasonically determined elastic properties observed in bone specimens.

There is, on the other hand, a general consensus, when overall strain is partitioned between its various components, that tissue strain, fibril strain and mineral particle strain are quite different. For instance, Gupta et al. [17] find that if elastic tissue strain, measured on very small specimens, has a value of 12, then fibril strain and mineral strain, measured by X-ray diffraction in the same specimens as were used to measure tissue strain, have values of 5 and 2, respectively. However, what it is that keeps the collagen and mineral together is unclear, and much debated.

Fracture behaviour

It is useful to distinguish in all this modelling and experimentation between pre- and post-yield behaviour. This is attempted in [15] (not for the faint-hearted, for instance there are two complete pages of definitions of abbreviations!). These authors suggest that water is a key 'gluing' agent between the collagen and the mineral and that fracture is initiated by 'ductile sliding of hydroxyapatite mineral crystals along layered water films followed by rupture of collagen crosslinks.'

One of the limiting factors in working out what happens in the fracture of very small volumes of bone, down to the single-crystal level, is that the game changes at the nano-level. Fratzl and co-workers suggest [25] that when crystals are of the size of, or even smaller than, dangerous Griffith

flaws, then bone becomes insensitive to the cracks' presence. However, there are counterarguments that assert that very small structures are affected by flaws. For instance, nanotubes will show a considerable reduction in strength if even one atom is missing from an otherwise perfect structure [26], and some argue that cracks always weaken brittle solids [27].

Stiffness and toughness run contrary to each other

One of the gloomy facts that people who try to get the best out of bone must face is that stiffness and toughness go against each other, so that it is not possible to have bone material that is both very stiff and very tough [28]. As the mineral content of mechanically tested specimens increases (which nearly always produces a concomitant increase in material stiffness) so the toughness, or at least the area under the stress–strain curve, which is a fair measure of toughness, decreases (Figs. 1 and 2). This is not peculiar to bone, in materials in general stiffness and toughness run against each other.

The increase in stiffness with increasing mineralisation is easy to understand qualitatively, because the mineral is stiffer than well-mineralised bone, and therefore whether one uses a simple rule of mixtures, or some more complex

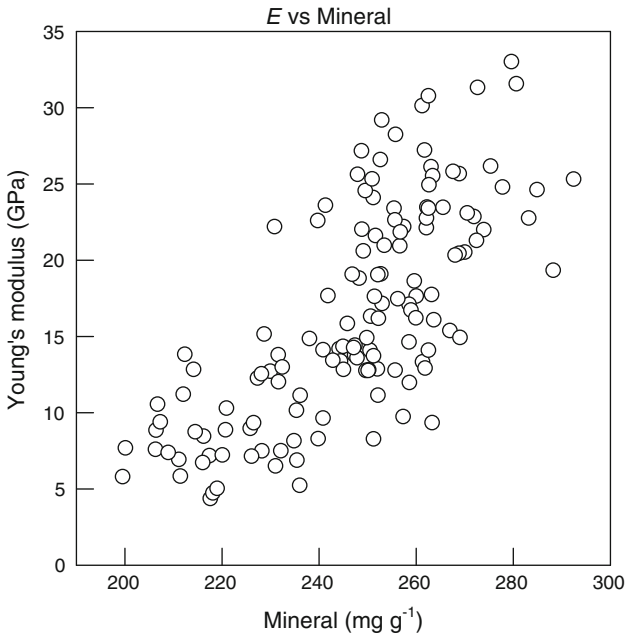

Fig. 1 Relationship between mineral content and Young's modulus (E) measured in tension (From JDC's original data). Only specimens with a porosity of less than 20% were included, which prevents obviously cancellous bone specimens from being included. Even so, inclusion of porosity as well as mineral content as another variable reduces the spread of points considerably (not shown here). The specimens were of the same size and shape. *Note*: abscissa does not start at zero

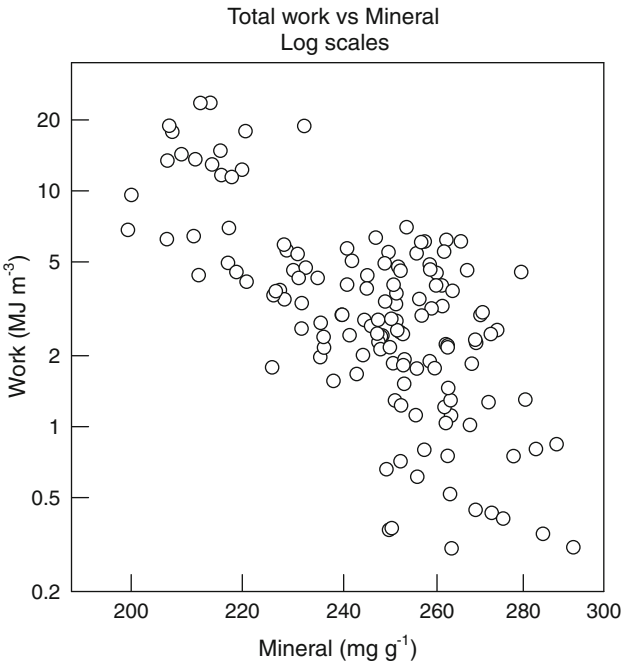

Fig. 2 Relationship between the mineral content and work under the tensile stress–strain curve. Note: log scales on both axes. The specimens are the same as those used in constructing Fig. 1 (From JDC's original data). Including porosity as another variable has little effect on improving the distribution, unlike the case for Young's modulus

model of mineral–collagen interaction, more mineral implies more high-modulus material, which will reduce the strain for any particular load. It is the reduction in toughness that is not so readily obvious. In *very* highly mineralised bone, such as found in the ear bones and in the rostrum of *Mesoplodon densirostris* presumably the mineral particles fuse together. However, it should be said that the work of Rogers and Zioupos [29] suggests that the crystals in the rostrum, though larger than those of the whale tympanic bulla, which are in turn larger than those of the human femur, and thus accord with their amounts of mineralisation, still seem to be separate (Table 1).

In less highly mineralised bones it is less clear why toughness should decrease as mineral content increases. If the material is an open foam, as Fritsch et al. [14, 15] model, then the explanation is easy, because a dangerous crack once started will travel more easily through a denser mineral foam than a less dense one. If the mineral platelets are all separate, then one has to suppose that with many crystals the collagen is inhibited in some way from deforming according to the local stress, and this is what reduces the amount of post-yield deformation, so characteristic a feature of less well-mineralised bone. Furthermore, well-mineralised bone shows much less microdamage as it fractures, which also reduces the total amount of work that has to be done on it to break it. Presumably, this production of much microdamage in less

Table 1 Properties of size of mineral crystals and physical properties of bulk specimens of three tissues

	Human femur	Tympanic bulla	Mesoplodon rostrum
Max. size (nm)	15.4	19.2	23.1
Ca content (mg Ca/g dry bone)	250	310	350
Young's modulus (GPa)	16–18	32–36	40–42
Bending strength (MPa)	200–220	45–55	50–60
Hardness (VHN)	40–60	150–170	200–220

The mechanical values were collected at a different time from the other two sets of values. Data from [29]

highly mineralised bone is because the fibrils are not inhibited from pulling apart from each other by the blocks of mineral surrounding them.

Bone structure and mechanics at the microscale

At this level one deals with structures one can see only with the microscope, the histological types of bone, for instance: woven versus lamellar bone, Haversian bone versus interstitial bone and bone with microdamage versus bone without microdamage. From earlier, relatively simple, though critically important work of people like Bonfield and Clark [30], the field has advanced enormously because of improvements and refinements of the methods available for characterisation.

Histology of bone

Bone can be rather summarily divided into woven bone, lamellar bone (variously arranged) and Haversian (secondary) bone. Woven bone is found in embryonic bone, in fracture callus, and in thin sheets in a rather special structure called 'fibrolamellar' bone. This is found in large vertebrates, such as ourselves, cows and large dinosaurs that have to increase in absolute size rather quickly [31]. 'Woven' bone is a misnomer, because weaving, with a weft going alternately one side and the other of the warp, is a very difficult trick to bring off, usually requiring a shuttle which animals and plants do not have. Rather, woven bone is a series of feltworks of fibres randomly arranged in two dimensions, but scarcely in the third dimension. Furthermore, as Weiner and Wagner [2] point out, woven bone has a large amount of non-collagenous organic material, and is really somewhat of a mess. It probably, though this is not known for certain, has poor mechanical properties. Its virtue, from the point of view of the animal, is that it can be laid down very quickly.

Lamellar bone consists of lamellae about 3-μm thick which have fibres in various angles mainly in the plane of the lamella. Sometimes the degree of co-alignment in a layer can be considerable (for a full description see [2]). This bone can be either round the inside and outside borders of bones, or wrapped up in Haversian systems, also called secondary osteons. These are cylindrical objects, of about 200-μm diameter, made of lamellar bone. They are formed when osteoclasts (bone-destroying cells) eat through previously existing bone, leaving a cylindrical cavity that is then filled in by osteoblasts (bone-forming cells). These bone-forming cells produce lamellae that are arranged round a central cavity, the Haversian canal, which contains blood vessels and possibly nerves. This replacement of bone by new bone may replace damaged bone, or old bone, or even bone that has merely undergone large strains. An important point about Haversian systems is that they are always younger than the bone they replace. Because mineralisation in large animals takes many months or even years to complete Haversian systems are for some time less highly mineralised and therefore their material has a lower Young's modulus than the surrounding bone, which is usually called 'interstitial' bone and is often the remains of previous Haversian systems. (In a few specialised tissues like some ear bones the situation is reversed, and the primary osteons, which look superficially like Haversian systems, but which have not replaced previously existing bone, are *more* highly mineralised than the interstitial bone [32].) In ordinary bone, each Haversian system is surrounded by a 'cement line (or more properly 'sheath') of rather obscure composition, although it seems clear that it is low in collagen. Since the Haversian system has a lower modulus than the surrounding interstitial lamellae, at the border between the two (the cement sheath) there is a stress discontinuity and cracks are likely to initiate there. However, cracks may also be prevented from entering the Haversian system by this mismatch and cracks often go round the cement sheath, rather than penetrating the Haversian system itself.

Anisotropy

Most studies have loaded bone specimens, usually in bending or tension, in a way that results in the main force being along the direction of the long axis of the bone. Of course in reality bone is loaded, particularly in trauma, in all sorts of directions and the question of its *anisotropy* may be important. For instance, the fibrolamellar bone found in many larger vertebrates, consisting of sheets successively of lamellar bone, woven bone, lamellar bone, two-dimensional nets of blood vessels, and lamellar bone again, is extremely anisotropic, being quite brittle when loaded (in tension) normal to the plane of the sheets, but quite tough with a much higher ultimate strain and with a higher modulus when loaded along the length of the sheets (Table 2). Haversian bone is also quite anisotropic.

Table 2 Mechanical values for bovine bone loaded in tension, all done on specimens machined in the same way

	Haversian longitudinal	Haversian radial	Fibrolamellar longitudinal	Fibrolamellar radial
Young's modulus (GPa)	23.1 (×2.2)	10.4	26.5 (×2.4)	11.0
Tensile strength (MPa)	144 (×3.7)	39	167 (×5.6)	30
Ultimate tensile strain	0.016 (×2.3)	0.007	0.033 (×16.5)	0.002

Raw data from [68]. The figures in brackets show the value of the longitudinal values divided by the radial values. Ultimate tensile strain is a reasonable indicator of toughness. It was assumed by the authors that bone was transversely isotropic for modulus

Fracture mechanics, microcracking and R-curves

The fracture mechanics of bone (that is, the mechanical tests that determine danger of cracks, strain concentrations, and the real strength of structures that are not smooth-sided, which is true of virtually all bones) is difficult. Certainly, the entry point for fracture mechanics, linear elastic fracture mechanics (LEFM), is barely applicable to bone, because LEFM assumes that the material behaves elastically, and that the region in which the elastic criterion breaks down is tiny compared with the size of the structure. Bone nearly always shows microdamage occurring well away from any flaw that may eventually spread and cause the bone to fail. Fracture mechanics in bone is also difficult because bone is full of interfaces, of varying strength, be they cement sheaths, or lamellar interfaces, or where lamellar bone butts against woven bone in fibrolamellar bone, and these are likely to be places where cracks are particularly likely to initiate, but they are also places where cracks, if they are not too long, will be brought to a halt, because the stress field surrounding the crack tip will cause the interfaces to separate, and make it more difficult for the crack to continue.

This is one of the reasons for the rising *R*-curve often seen in bone. This is not the only reason, another is the development of uncracked bridges in the wake of the crack tip [33, 34]. An *R*-curve shows how the value of the critical stress intensity factor K_C increases as a function of the length of the crack. The *R*-curve is used to examine the processes of slow stable crack growth and unstable fracture. Nalla et al. [35] showed that older people's bone had a lower initial value of K_C than that of younger people. This meant that it was possible to initiate a crack at a lower stress in older people's bone. However, perhaps more importantly, as the crack extended, in older people the value of K_C hardly increased at all, meaning that once the crack had started there was little that would stop it. On the other hand in younger people's bone the *R*-curve, the plot of K_C against crack length, rose steeply, implying that the increase in length of the crack which, other things being equal, allows the crack to travel more easily, was insufficient to continue to drive the crack forward. As a result, older people's bone was quite brittle, whereas younger people's showed considerably more post-yield deformation. Akkus and Rimnac [36] have shown that the rate of crack travel often slows down, often effectively to zero, when the crack runs up against some barrier, and then increases again if the barrier is passed. If the crack lengthens too much, its travel eventually becomes inexorable [37].

Liu et al. [38], by taking bones from baboons who had been dosed with alendronate, which inhibits remodelling and therefore the production of Haversian systems, were able to compare, from the same place in the bone, though from different animals of course, various properties of specimens from secondary bone, which has many Haversian systems, with specimens from primary lamellar bone found in the unusually large amounts of almost uninterrupted circumferential lamellae. They loaded at different angles to the long axis, and found marked anisotropy, particularly in toughness (work to fracture) but, interestingly, not huge amounts of difference in the properties of the primary circumferential lamellar bone and the Haversian bone (Table 3), except in the way the bone broke up after fracture. Liu et al. noted that the Haversian specimens tended not to break completely in two, and suggested that Haversian bone might be able heal after fracture in a way that bones made from circumferential lamellae would not.

Despite the relationship between mineralisation and toughness not being completely clear, Figs. 3 and 4 show the fracture surface of a wet specimen of deer's antler, which was very tough (as measured by the area under the load–deformation curve) though not well mineralised, and a wet specimen of the tympanic bulla of a fin whale *Balaenoptera physalus,* which was very brittle and highly mineralised. The extremely rough surface of the antler and the relatively smooth and stone-like surface of the bulla are what one would expect of tough and brittle materials.

Bone structure and mechanics at the mesoscale or tissue level

Here is what one can see with the naked eye. Particularly important at this level is the distinction between compact bone and cancellous bone.

Table 3 Mechanical values for baboon tibias loaded in bending

	Circumferential lamellae				Haversian bone			
Angle from longitudinal (°)	0	30	60	90	0	30	60	90
Young's modulus (GPa)	15.5 (×1.8)	14.3	10.0	8.6	14.4 (×1.9)	10.7	8.1	7.6
Bending strength (MPa)	350 (×3.4)	194	120	104	289 (×4.1)	130	71	71
Work to fracture (kJ m^{-2})	7.8 (×26.0)	2.4	0.5	0.3	7.1 (×17.8)	0.9	0.4	0.4

All tests done on specimens machined in the same way. Raw data from [38]. Work to fracture is a reasonable indication of the toughness. The numbers in brackets show the value of the longitudinal value divided by the most transverse value, a measure of mechanical anisotropy

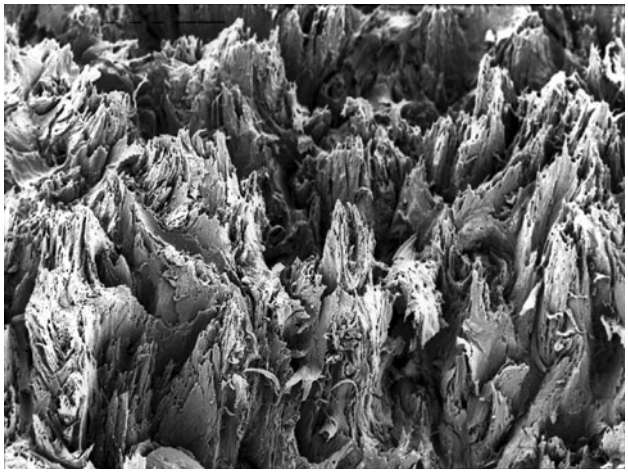

Fig. 3 The fracture surface of a wet specimen of red deer (*Cervus elaphus*) antler bone loaded to fracture in tension. The width of the picture is about 500 μm. The mineral content was about 48% of the wet weight. The work of fracture was about 6,200 J m^{-2}. (JDC's original picture)

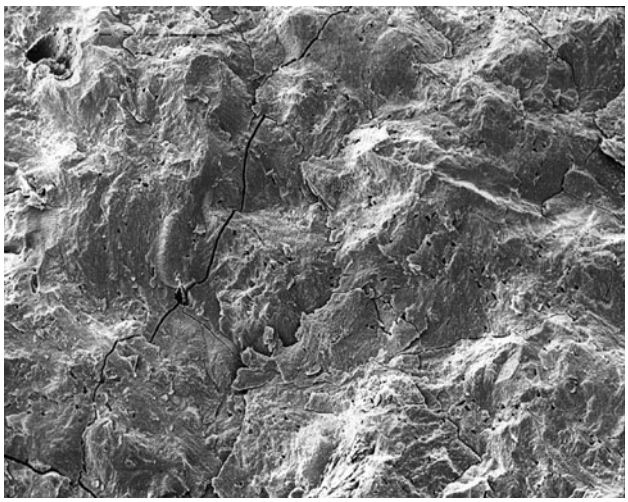

Fig. 4 The fracture surface of a wet specimen of the tympanic bulla bone of a fin whale (*Balaenoptera physalus*) loaded to fracture in tension. The horizontal dashed line (top left) is an artefact. The specimen size and shape was the same as for Fig. 3. The width of the picture is about 500 μm. The mineral content was about 85% of the wet weight. The work of fracture was about 20 J m^{-2}. (JDC's original picture)

Compact versus cancellous bone

Cancellous bone is rarely found on the surface of bones; it nearly always has at least a thin covering of compact bone outside it. It occurs predominantly in four places: at the ends of long bones, right through the length of short bones, such as the wrist (carpal) bones, in large flat bones such as the scapula, ischium and ilium, and in protuberances where muscle attach to bones. In a fifth, in the lumen of large long bones, it probably has a 'non-mechanical' function, holding the sloppy marrow in place.

The mechanical properties of cancellous bone are difficult to analyse: to some extent because cancellous bone is partway from being a material to being a structure and is always bound up with cortical bone but, more particularly in relation to '1' below, because individual trabeculae are difficult to test mechanically. Three questions one may ask about cancellous bone are:

(1) is its composition such that its *material* mechanical properties are the same as those of compact bone and;
(2) how does the bone volume fraction: bone volume/total volume (BV/TV) affect the *tissue* mechanical properties and;
(3) how does the arrangement of the trabeculae (the struts and sheets making up cancellous bone) in space affect the mechanical properties of the cancellous *tissue*?

Mechanical properties of cancellous material

The cancellous bone in any place seems to have a somewhat lower mineral content than the nearby compact bone. Whether the lower mineral content is because the tissue is turned over more rapidly than the compact bone, and is therefore younger and slightly less mineralised, or whether it is a property intrinsic to cancellous tissue is unclear. The former effect will certainly have some influence. The mechanical properties are not greatly different from the surrounding bone, certainly in dry bone anyhow, perhaps

Young's modulus is 10% less [39], and the post-yield deformation is rather greater.

BV/TV

The question of the amount of mineralisation is confused at the moment by the fact that the acronym 'BMD' (bone mineral density) is used in two quite different ways. One, mainly of interest to clinicians, is the attenuation of X-rays, whose wavelengths are arranged to give a good measure of the amount of mineral in a bony object, divided by some measure of the total volume of the material that is doing the attenuating, for instance the length of the bone being measured times πr^2, where r is half its apparent width in the X-radiograph. One should note that this measure unfortunately usually includes the marrow cavity as part of the BMD. Obtaining BMD is relatively non-invasive, and it is often the only means of measurement that clinicians can use. The other meaning, which is of interest more to materials scientists, is the attenuation of the X-rays *per voxel*. This, amongst other things, shows the variability of the mineralisation in small volumes of bone. The usage of bone mineralisation density distribution (BMDD), for the latter [40], is slowly becoming dominant, but it is by no means universally accepted, which is a pity, although the context usually makes it clear what is being talked about. The difference between the BMDDs of compact and cancellous bone specimens, particularly their variances can be marked, because cancellous bone is more likely to have bone of different ages in a region of interest, and so the BMMDs of different voxels are likely to have a higher variance in the cancellous bone than in the compact bone.

There is a strong positive non-linear effect in the relationship between the amount of bone material present (BV/TV) and the mechanical properties. Young's modulus seems to obey roughly a quadratic power law, e.g. [41], whilst strength in both compression and tension has a power law of about 1.5 to 2, e.g. [42].

Needless to say, there are differences in these values if wet versus dry testing is performed (estimates of the modulus are somewhat higher if the specimens are dry, this difference becoming greater as the organic component increases). In humans, where senile bone is often examined and is, fortunately, often the only bone available, older cancellous bone tissue is both in general weaker and has a lower modulus than younger bone for specimens taken from the same place in the bone. This is because in older bone the material is weaker, and there is less of it. The anisotropy of cancellous bone can be marked, sometimes the modulus in one direction can be seven times the modulus in the normal direction [41, 43].

The distinction between cancellous and compact bone is arbitrary. Usually one may consider bone material that has a porosity of about 25% or more to be cancellous, and if less to be compact. There are places where the cancellous bone merges seamlessly with compact bone, particularly where the arcades of cancellous bone under articulations and tendon insertions end, but in short bones and sandwich bones the distinction is clear and spatially sudden, and the porosity of the cancellous part is much greater than 25%.

Arrangement of the trabeculae

One sometimes sees the assertion that cancellous bone is 'lighter' than compact bone. Although this is true in one sense, if one considers the (Mechanical property/mass) relationship it is certainly untrue, since as mentioned above the scaling relationships are roughly (Young's modulus $\propto$ Density^2) and (Compressive strength $\propto$ Density$^{1.5}$). 'Density' is the mass of the dried, defatted specimen divided by the volume of the specimen. Given these relationships, the (Mechanical property/mass) will always be greatest when the bone is solid. And even this ignores the mass of the marrow fat in the interstices of the cancellous material, which will make the bone heavier without improving its mechanical properties. One might ask why cancellous bone is ever present in vertebrate skeletons. The reason is that in the places it is found the *spatial arrangement* of the trabeculae is such that it is better than solid compact bone.

The compact bone/cancellous bone complexes in the four characteristic places have different functions. At the end of long bones, the trabeculae serve to lead large, rather distributed, loads away from the joint and into the compact bone which has a much smaller cross-sectional area than the joint cartilage surfaces. Compact bone under the synovial cartilage would be unduly massive if it were solid, and being very stiff would expose the rather delicate cartilage to larger impact stresses than in fact occur ([3] pp. 225–231). In short bones, cancellous bone takes the loads between the two ends of the bone, where they articulate with neighbouring bones. In these short bones, there is no adaptive advantage in the trabeculae taking the loads running between the end plates via the compact sidewalls, because the angle at which the trabeculae would optimally travel to maximise the Stiffness/mass is not to the side, but straight along the short length of the bone ([3] pp. 217–218). Again, solid bone occupying all the short bone's volume would be unduly massive and cartilage-destroying. In large flat bones, which are bent about their shortest dimension, the cancellous bone forms the middle of a sandwich, with the compact shell bearing the major loads, and the cancellous bone keeping the walls of the shell apart, and dealing with such shearing loads as may arise. Calculations show that even if the marrow fat's mass

is included, there is a property/mass advantage, albeit modest, in having cancellous bone in the middle, rather than having a solid, though overall thinner bone ([3] pp. 212–217). Finally, the protuberances to which tendons attach have to accommodate loading at various angles, and the cancellous struts are present, as in the ends of the bones, to lead the loads into the compact bone, though perhaps the trabeculae are more isotropic than in the cancellous bone under joints arranged along tensile and compressive 'lines of force'.

The arrangement of the 'tensile' and 'compressive' trabeculae produced the observation of Culmann ('That's my crane') leading Wolff to propose his supposed law, based initially on the arrangement of the trabeculae in the head and neck of the human femur. However, this view has been cogently questioned (e.g. [44, 45] Panel 3). The trouble is that right from the time it was formulated, people realised it was not a scientific 'law' in the usual sense, and nearly always, when people do use the phrase, it could be replaced, as Cowin points out, by 'functional adaptation' with a considerable increase of clarity. Not only is it not a law, many of its implied postulates are wrong. For instance, the compressive and tensile arcades of trabeculae should cross at right angles, but as for example Skedros and Baucom point out [46], they very often do not. Furthermore, in the neck of the femur there seem to be many more compressive than tensile trabeculae. Not actually relating to the subject of the law's lawfulness, but revealing, was the fact that Wolff, all his life and unlike most of his contemporaries, did not accept bone resorption as a fact.

Despite the low opinion in which Wolff's law is held as regards its 'lawfulness' it must be said that the arrangement of the trabeculae, for instance in the ischium of the horse, often has a certain specious appeal. The trabeculae, which very often form networks in which trabeculae do cross each other at nearly 90°, are in some sense idealisations of the lines of force in the structure. This is certainly true of the calcanei of sheep and deer [46]. Personally I think that although 'Wolff's law' is in no sense a law, the cancellous structures, both those arising de novo or resulting from remodelling, are often arranged in a manner that fits the concept of following lines of principal stress.

X-ray damage

Many of the experiments involved in analysing bone at all levels involve X-ray analysis. It is unfortunate that often experimenters do not take enough care with the fact that wet specimens (which are of course the type that it is most informative to study) have their soft tissue structure and mechanical properties very rapidly degraded by the X-ray beam [47].

Structure and mechanics at the level of the whole bone

How is bone loaded in life?

For whole bones, which are of extremely complex shape, there is little possibility of determining the properties of the bone *material* from the bone's behaviour under load nor, usually, of producing analytical solutions for the stresses and strains produced by loading. Indeed, there is a further difficulty: the difficulty of determining how the bone is actually loaded in life. This difficulty, often not a problem at all for the engineer designing a structure, is often great for the biologist. For instance, in order to determine the stresses and strains in the human mandible caused by biting one has to consider the whole pathway of the forces, which are probably being shared unequally in some way by several teeth, and in each tooth the force must travel from the enamel, through the crown dentine of each tooth to the root dentine, from there through cementum (a bone-like material), through the thin soft tissue periodontal ligament into the cancellous alveolar bone and thence to the ramus of the mandible. This is a formidable task even for modern finite element programs. Fortunately finite element modelling, with the increasing processing power of modern computers, is becoming more and more precise (and often more accurate!). Even so, for instance, there are many models still appearing in the literature that assume, necessarily, because of the program's limitations, that the bone is mechanically homogenous and isotropic. On one hand bone is not isotropic and rarely homogeneous and such simplification may render the results inaccurate. On the other hand, if one has a program that allows one to assume anisotropy, one has to determine the degree and direction of anisotropy in various parts of the real bone. This is not a trivial problem.

There are three major ways in which the loads acting on bone in life can be estimated. None is entirely satisfactory, and all have different strengths and weaknesses [7]. Probably the most indirect is by back calculation from force plate data, and combining this with the angles that the bones make with each other, the ground and the centre of mass of the animal [48]. Of course this method can only estimate strains as determined from one or two viewpoints, and one has no idea of the muscle forces, that may alter the apparent bending moments considerably. On the other hand, it is non-invasive and if the animal can stand the bright lights it can move more or less as it would in the wild. Such studies have suggested that many long bones are loaded considerably in bending during locomotion.

The next method is to attach strain gauges to the bones of the living animals, and record the surface strains directly. Use of rosette strain gauges allows the strains to have any orientation in the plane of the strain gauge, and

still be estimated. Of course, attaching strain gauges to the bone is fraught with difficulties, and indeed there are many problems associated with this method. For instance only the strain in the bone underneath the rosettes is known, and one can only implant strain gauges in places where the surgery required in small. Nevertheless, this direct method, pioneered by Lanyon in the 1960's and 1970's [49], has been used quite extensively, and has shown, for instance that many bones are loaded in bending during locomotion, that in many cases the principal strains are not aligned along the long axis of the bone, and that in general the loading situation is very complex [50].

A third method of estimating the strains in bones is by using finite element methods. This has many advantages and some big (and often unappreciated) disadvantages. For instance often not enough attention is paid to validation in many studies using finite element analysis, and people writing (and reading) papers using it need to be wary. However, despite finite element analysis being a good exemplar of the old saying 'garbage in–garbage out' there is no doubt that already many great insights have come from its use, and with the increasing power and sophistication of computers, much more will come in the next decade or so.

A great advantage of the finite element method is that it enables one to estimate the strains wherever one likes in the bone. It is also much more capable of including muscle forces in the model. For instance, Sverdlova and Witzel [5] have shown that *if* the muscles acting on the human femur cooperate to reduce bending forces, then the loading on the femur can become almost entirely compressive in relation to the forces acting on the femur. If such modelling is correct, then the weight of the femur can be much smaller than it must be if bending forces are important and, concomitantly, the safety factors can be greater. The great problem here is the 'if'. This is because one can do more or less what one wants with a finite element model, given time and money, including modelling the relative forces that the muscles exert in the various parts of the gait cycle. However, unless the model is validated by loading with realistic forces and actually measuring the strains, usually with strain gauges, one has little idea whether the muscles cooperate in this particular way, or whether they are mainly, for instance, exerting the forces that will minimise the total weight of muscle plus bone, which is another, and quite different, possibility.

The complexity of bone shape

One of the fascinating things about bones is their extraordinary complexity of form. Anyone who is unfamiliar with this fact should look at the splendid photographs in [51, 52]. As a colleague emailed me recently: 'I think a large part of the wonderful shapes that bones have is just a simple result of their function and the muscle forces and attachments to produce that function'. However, an immediate thought aroused by such thoughts is 'How is 'function' turned into 'form'?' The bones of a cod's skull, for instance, show that the structures of the individual bones are so varied and complex that it would surely be impossible for them to differentiate in the way they have merely through the influence of loading.

An early book [53] and a later review [54] produced several examples in which the mesenchyme that would normally develop into an ordinary bone was put into an almost stress-free environment, such as into the spleen or, in the case of chick mesenchyme, into the chorio-allantoic membrane of the egg. The mesenchyme developed, before nerves arrived, into a recognisable though rough version of the bone into which it would normally have developed, often with joints. This shows that the mesenchymal cells had in them the genetic information for making a rough copy of the bone. Usually, this rough version would then be fine-tuned by the muscular and other loading on it to produce the final version. These experiments show that the form of bones must to a large extent be determined genetically.

A very interesting structure that also shows that at least some development in bone must be genetically determined is deer's antler [55]. This is true bone. It develops each year on the head of male deer, and has a more or less complicated structure. The shape is species-specific: an experienced naturalist can easily tell to which species an antler belongs and, often roughly how old the deer was [56]. The antler becomes larger each year. It is clearly designed for battles with other males, for instance having protective 'tines' over the face. An interesting thing about antlers is that they achieve their final form *before* they are loaded to any significant extent. This is because whilst they grow they are covered by a skin-like 'velvet', and the deer is very careful not to bang the delicate structure against anything. The velvet is then shed, the antler bone dies and the antler is ready for battle with other males for access to females. Any large loads the antler is subjected to are produced during the battle, well after the antler bone could adapt to them. The species-specificity and the fact that the final form is produced when no significant stresses are acting show that the genetics of the animals must be the main thing determining the shape.

Size effect

There is an important phenomenon, until recently almost ignored in the bone literature, though well known to mechanical engineers, that of the 'volume' or 'size' effect. Of course, when considering the strength of a structure one

applies normalising factors to load and deformation relating to the size and the shape of the specimen to get an estimate of the stress and strain in various parts of the structure. Thus, stress in compression is F/A, where 'F' is the compressive force and 'A' is the cross-sectional area of the specimen. However, the size effect is over and above these calculations, because even after these factors have been taken into account, larger structures are weaker than they 'ought' to be. This partially arises from the fact that larger structures have a larger volume to contain defects that reduce the strength locally and therefore globally. Of course, the larger volume will also contain more strong bits, but that is little help when it comes to fracture. This matter was examined in bones by Taylor [57] who worked on fatigue loading. The effect is not trivial. For instance, if the volume of a tensile specimen is increased by a factor of 100, the calculated fatigue strength falls by a factor of 2. This is particularly a problem in fatigue studies because fatigue specimens necessarily tend to be machined from whole bones, to uniform size, so the size effect cannot be taken directly into account. Since bones vary in size from those of mice to those of elephants and whales, which are many thousands of times greater in volume than mouse bones, this should imply a considerable difference in the strength of different bones. The size effect will have little effect on stiffness, some effect on toughness and static strength, and will have its largest effect on fatigue, where the spread of a single crack is all-important [58]. However, the safety factors found in bones during arduous exercise are rather similar, about 2–4. So animals seem not to limit their activities according to the strength of their bones; that elephants do not jump is perfectly well explained by ordinary calculations of muscle forces. Taylor [57] however suggests that the bone of small animals is intrinsically weaker than that of large animals, and that these two effects counteract each other almost exactly. This seems to me unlikely, since why should the strength of the bone material of smaller animals be limited? If it were stronger the bones could be thinner and therefore lighter. There is no doubt that the size effect is important, and Taylor's results are, as a result of the importance of the effect, puzzling.

A general introduction to the phenomenon of the size effect is given, for instance, by Le et al. [59]. They assert that the weakest link theory does *not* in the main account for the size effect. Unfortunately, the subject of size effects is extremely difficult and still the subject of intense argument amongst materials scientists [59–63] and I shall discuss it no more.

Why is bone arranged hierarchically?

The question of *why* and *how* bone is hierarchically arranged is interesting. In my view the hierarchical arrangement comes naturally from the way bone is put together. Of course, if the best arrangement of material from the structural point of view had been large crystals of brittle material, natural selection would no doubt have modified the structure to produce a hierarchical one, because the mechanics would have been so bad. However, when I considered different hierarchical levels, the reader perhaps could have thought about whether things would be arranged differently if hierarchical arrangements made no difference to the mechanical properties. For instance, I have emphasised that the small size of the carbonated apatite crystals is crucial for their functioning. Larger crystals, such as those of calcium carbonate (calcite or aragonite) could have been adopted early on in the history of vertebrates and indeed otoliths, whose crystals are of calcite, are widespread in vertebrates, so it is not as if the genes for making things out of calcium carbonate are missing. Had calcium carbonate been the crystal used in bone, it would have changed, though not got rid of, the hierarchy. Could it be that fishes started off with calcium carbonate but gave it up in favour of a smaller crystal as a variant of calcium phosphate? As it happens the geological record showed that this is almost certainly not the case, but probably natural selection would have switched the mineral over to calcium phosphate had early vertebrates started with calcium carbonate. Similarly, at a higher level fibrolamellar bone and its descendant, Haversian bone, seem to have a tendency to form structures that are about 200 μm across, which is quite good for crack arrest. However, in my view the 200 μm motif is determined by the distance over which it is reasonable to have blood vessels supplying nutrients and taking away waste products from osteocytes. Indeed advanced teleosts, the most speciose of vertebrates, have an anosteocytic bone structure in which the 200 μm motif is absent. In general, across animals with 'normal' (that is human) bone, structures 20 μm across or 500 μm across would not be adaptive. Neither of these examples would get rid of the hierarchy if they were true, but would change its nature.

An interesting paper that considers the effect of differing numbers of hierarchies on composite behaviour is that of Sen and Buehler [64]. They consider materials made only from silica, but this silica exists in two forms brittle and rather tough. They find that as the number of levels of hierarchy increases so does the increase in slope of the R-curve and the size of the defects to which the material is sensitive. How much this analysis is pertinent to bone is difficult to decide, but it is clearly a promising approach.

Conclusions

There are many levels at which one can consider the structure and mechanical properties of bone. The number

of levels is not fixed, for instance Weiner and Wagner [2] use 7; I have considered only 4 levels. Perhaps the main point that comes out is that although it is possible to test material in various ways at all levels much added insight comes from at least considering the level below. This cannot apply to the lowest level of course, and there are chemists and quantum mechanists who are beginning to examine things at levels below the nanoscale considered here [65–67]. It seems to me that there are so many competing views about what is going on at the nanoscale that it would be idle to look at levels below the nanoscale at the moment.

The other thing that comes out of this survey is that the advances made have been mainly advances allowed by new, or greatly improved, technical procedures. Just for example, nanoindentation allows one to obtain information about material stiffness at a size level quite impossibly low 20 years ago. Even so, as mentioned in Panel 1, there are dangers in leaping with cries of joy on new techniques, and determining all sorts of new properties of bone, without first mastering all the intricacies of the new methods. Validation of results, if at all possible, by other techniques, is most important if good science is to be done.

Appendix

Panel 1: Enhancements in technology and new technologies that have occurred in the last 30 years or so

Improvements in already adopted methods

Computer-assisted image analysis
Light microscopes increasingly sophisticated
Mechanical testing machines driven by computers (an advance?)
Transmission and scanning electron microscopes are increasingly sophisticated
Vast improvements in the power and sophistication of computers

Methods now adopted by the bone community, or new methods

Atomic force microscopy
Computational chemistry, including quantum mechanical methods
Environmental chambers
Finite element analysis
Micromilling
Microtomography
Nanoindentation
Non-contact optical deformation mapping
Scanning confocal microscopy
Shearography
Spectroscopy (FTIR, Raman, NMR)

People should remember that new techniques bring new hazards: environmental chambers may mislead people into thinking their specimens are truly in a physiological state; images can be tweaked in Photoshop (a very bad practice); papers on Finite Element Analysis are often what Peter Medawar once called methodological chambers of horrors; not having hard copy output from computers can, indeed often does, lead to archiving problems, and so on.

Panel 2

	Adult human haversian	Bovine fibrolamellar	Whale tympanic bulla	Mesoplodon rostrum	Dry Deer antler	Wet Deer femur
Young's modulus (GPa)	13–18	11–26	35	45	17	22
Shear modulus (GPa)	3.3	5	–	–	–	–
Tensile strength (MPa)	50–130	30–170	30	Low	–	–
Tensile yield (MPa)	110	160	–	Low	–	–
Ultimate tensile strain	0.007–0.030	0.002–0.030	0.002	Low	High	So so
Compressive strength (MPa)	130–200	–	–	–	–	–
Bending strength (MPa)	–	240	33	50	350	260
Shear strength (MPa)	70	65	–	–	–	
Impact absorption	So so	So so	Poor	Poor	High	So so

Characteristic mechanical properties of cortical bone. This table merely gives some idea of the mechanical properties of cortical bone and should not be used for reference. Compare the 'Dry Deer antler' with the 'Wet Deer femur', for they were measured on exactly the same type of specimens. For more definitive information, you should refer to papers and books such as the following: [3, 28, 45, 55, 67, 68]

Panel 3

People who wish to examine the history of the so called Wolff's law, which is a concept without a legal basis, and which is still used all the time without much thought, might like to follow some of the references in this time-line.

1832 Bourgery is probably the first to publish [69] (in French) regarding the architectural structure of cancellous bone, though this had been known in a general way for centuries

1867 von Meyer publishes a paper [70] (in German) showing the supposed relationship between the directions of the trabeculae in the proximal human femur and a crane devised by Culmann

1870 Wolff 'took charge of the subject' [71] and published a paper setting out his ideas in detail [72] (in German). In the years afterwards he was very aggressive in defending his views [71]

1881 Roux published (in German) a book that was really about functional adaptation [73]. This got conflated [71] with Wolff's overspecific mathematical theory about the way in which cancellous bone was arranged, into 'Wolff's law'

1892 Wolff publishes a book [74] (in German) summarising his views ('often quoted hardly read' [71])

1922 Triepel publishes a book [75] (in German) about the architecture of human cancellous bone that lists 20 reasons for rebutting Wolff's ideas

1942 D'Arcy Thompson publishes (in English) a rather colourful account [76] of how Culmann came to see von Meyer's dissection of the end of a bone and said 'That's my crane!'

1986 [77] English language translation of Wolff's classic work is published

1987 Roesler publishes an article [71] (in English) in the course of which he essentially rebuts the concept of Wolff's law as being anything of the sort

2001 Cowin publishes (in English) a good, and one would have hoped final, rebuttal of Wolff's 'law' [44] Alas, it was not to be!

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