
Biomechanical aspects of bone microstructure in vertebrates: potential approach to palaeontological investigations

S MISHRA

*School of Engineering Systems and Institute of Health and Biomedical Innovation, Queensland University of Technology,
Brisbane 4000, Australia*

(Email, sk.mishra@qut.edu.au)

Biomechanical or biophysical principles can be applied to study biological structures in their modern or fossil form. Bone is an important tissue in paleontological studies as it is a commonly preserved element in most fossil vertebrates, and can often allow its microstructures such as lacuna and canaliculi to be studied in detail. In this context, the principles of Fluid Mechanics and Scaling Laws have been previously applied to enhance the understanding of bone microarchitecture and their implications for the evolution of hydraulic structures to transport fluid. It has been shown that the microstructure of bone has evolved to maintain efficient transport between the nutrient supply and cells, the living components of the tissue. Application of the principle of minimal expenditure of energy to this analysis shows that the path distance comprising five or six lamellar regions represents an effective limit for fluid and solute transport between the nutrient supply and cells; beyond this threshold, hydraulic resistance in the network increases and additional energy expenditure is necessary for further transportation. This suggests an optimization of the size of the bone's building blocks (such as osteon or trabecular thickness) to meet the metabolic demand concomitant to minimal expenditure of energy. This biomechanical aspect of bone microstructure is corroborated from the ratio of osteon to Haversian canal diameters and scaling constants of several mammals considered in this study. This aspect of vertebrate bone microstructure and physiology may provide a basis of understanding of the form and function relationship in both extinct and extant taxa.

[Mishra S 2009 Biomechanical aspects of bone microstructure in vertebrates: potential approach to paleontological investigations; *J. Biosci.* **34** 799–809] DOI 10.1007/s12038-009-0061-z

1. Introduction

Biomechanics can be broadly defined as an application of the principles of Mechanics in Biology. Thus it covers vast areas of our knowledge of Mechanics and Biology including several sub-areas with their own principles and domains. For example, within Mechanics one can study statics, dynamics, kinematics or fluid mechanics and these tools can be applied to understand animal or plant morphologies. Furthermore, these biomechanical (or biophysical, as reported in some palaeontological literature) principles can be applied to study biological structures in their modern or fossil form. The biomechanical analysis of complex morphologies help understand the organismal form and function relationship which has been discussed in

the last two centuries (e.g. Darwin 1859; Thomson 1936). The biomechanical investigations can be used to study the relationship between environmental demand and the physical or mechanical design of an organism; mechanical constraints under which the organism lived and functional significance of evolutionary changes. Thus, combining the knowledge gained from extinct and extant taxa, one can better understand animal and plant designs.

There are important review articles in the area of biomechanical application into palaeobiology, palaeontology and evolution e.g. Alexander (1989, 2006) provides an excellent account of vertebrate biomechanics with emphasis on dinosaurs; Rayner and Wootton (1991) discuss the evolution of mechanical specialisations in organisms and constraints on the processes of adaptations; Carter (2001) and

Keywords. Bone; canaliculi; evolution; osteocytes; osteon; vertebrate

Currey (2002) discuss the biology, mechanics and adaptation in mammalian bones. Furthermore, with the advent of Finite Element Analysis (FEA) and its application in biology, and the ease of computational power, the vast majority of biomechanical studies now tend to use FEA. However, despite its use in engineering and orthopaedics for more than 30 years, this powerful *in-silico* tool has only recently been used in palaeontological studies. An extensive summary of current research, future potential and pitfalls has been given by Rayfield (2007) where the author rightly points out that “stress strain analysis in vertebrate morphology... has much promise and is increasingly shaping our understanding of vertebrate morphology, function and evolution”. Thus, during the last decade there were some studies where biomechanical principles and tools have been used by palaeontologists to study fossilized bones. However, most of these studies analyse bone at an organ level such as femur, skull or mandible. Palaeontological studies in which bone microstructure has been investigated are still rare, with the exception of Rensberger and Watabe (2000) and Chinsamy-Turan (2005).

In the above context, this paper aims to elucidate the application of two tools of Biomechanics, Fluid Mechanics and the Laws of Scaling. Based on our previous studies (Mishra and Tate 2003, 2004, 2008), this paper shows how the application of the above tools help our understanding the bone microarchitecture of vertebrates and its implications for the evolution of transportation of fluid in biological structures. In other words, this study explores the biomechanical constraints in the evolutionary trail when nutrients were transported from very small distances in primitive organisms to larger distances in modern taxa. To address this question a case study of transportation of fluid within bone tissue has been investigated.

Bone is a special tissue for zoological and palaeontological studies for several reasons. Firstly, bones of all living vertebrates have the same basic components and they provide the most direct palaeontological evidence for cellular physiology because bone cells and tissues are the direct records of metabolic activity (Padian 1997). Secondly, during the process of fossilization, it is the mineralized skeletal components such as bones and teeth which are usually preserved, not the other tissues. Furthermore, the microstructure of cortical bone can be used for assessing the life histories of animals. For example, important conclusions about the ambient environmental conditions, life style adaptations, locomotion and feeding can be drawn from the bone microstructure of dinosaurs by comparing them with extant relatives i.e. birds and crocodiles (Rensberger and Watabe 2000; Chinsamy-Turan 2005). Finally, a brief discussion of the some of the vertebrate fossils with considerable biomechanical importance, such as the skeleton of fossil whales and their close terrestrial relatives found recently in India (e.g. Bajpai and Thewissen 2000; Thewissen

et al. 2007) and the late Cretaceous Indian dinosaur eggshells (Srivastava *et al.* 2005), has been discussed to suggest the possible course of future work in this area in India.

2. Bone microarchitecture

Bone contains approximately 25% fluid and 75% organic and inorganic “solids” (Guo, 2001). The fluid space of bone includes large vascular spaces (on the order of 50 to 120 μm in diameter), the so-called lacunocanicular network comprising the pericellular space (dimensions: 100 nm, canaliculus wall to process; outer diameter: 500 nm) and the matrix microporosity (dimensions: 5–12 nm) (Knothe Tate 2003). Of these spaces, the lacunocanicular network represents the greatest interconnected volume of fluid within bone and this pericellular fluid “medium” constitutes the local environment of bone cells that are the living component of the tissue.

Osteocytes are the most prevalent cells found in bone tissue. In mature bone, the osteocyte bodies and their processes are contained within spaces called lacunae and channels called canaliculi. The lacunocanicular system (LCS) is derived from the stellate shape of the osteocytes (Aarden *et al.* 1994) and their interconnectivity; it is a conduit for metabolic traffic and exchange (Copenhaver 1964; Cooper *et al.* 1966). The extended osteocytic network, comprising cells interconnected by multiple cell processes that are joined at gap junctions (Doty 1981), forms a “functional syncytium” (Knapp *et al.* 2002).

Bones of all vertebrates have the similar basic components (Chinsamy-Turan 2005) and share a common microarchitecture comprising osteocytes within lacunae that are interconnected by canaliculi (Remaggi *et al.* 1998; Rensberger and Watabe 2000). Although dinosaurs and humans share this common hydraulic architecture, human bone architecture provides a more sophisticated hydraulic network than that of dinosaurs (Rensberger and Watabe 2000). This is due to the fact that in humans and some large vertebrates such as dogs and sheep, cortical bone is comprised of basic units called osteons (Polig and Jee 1990) arranged to form a composite structure; similarly, cancellous bone consists of trabeculae that augment mechanical stability along lines of principal stress. An osteon consists of several concentric layers of bone, called lamellae, surrounding a central Haversian canal which houses one or more capillaries. In contrast, trabecular lamellae are surrounded by the vascularized marrow bed. Given the striking uniformity of osteon diameter and trabecular thickness across species (Kanis 1996), it appears that osteonal and trabecular structure are optimized for survival of the tissue. Hydraulic conductance in bone tissue is a function of tissue architecture and porosity, matrix biochemistry, and pericellular fluid properties. It is expected to play a

critical role in establishing a baseline for endogenous fluid and solute transport in bone, which is augmented by fluid flow induced through mechanical, chemical and/or electrical effects (Knothe Tate 2003). This aspect of biotransport in bone tissue has been relatively ignored in the literature.

Mishra and Knothe Tate (2003) hypothesize that the lacunocanalicular hydraulic network defined by the pericellular fluid space common to all bone tissue is optimized to transport fluid and solutes between the blood supply and bone cells. Furthermore, this network is limited to a critical radial distance beyond which an increase in pressure head losses within the hydraulic network renders further transport of fluid and solutes unviable. This has been tested in a hydraulic model of a single osteon. By varying the size of the Haversian canal, osteonal diameter, and canalicular length in an analytical model, it is possible to characterize the influence of the transport path distance on the efficiency of fluid and solute transport between the blood supply and osteocytes within the hydraulic network. Furthermore, the development of hydraulic networks in biological structures was approached from an evolutionary perspective to understand the role of hydraulic architecture in maintaining transport to cells within increasingly complex organisms.

3. Initial idealized osteon model

An idealized model of an osteon was developed with dimensions approximated based on published data (figure 1, table 1). The initial osteon configuration comprised five concentric annular regions, i.e. lamellae, housing radially extending canaliculi. In the course of this analysis, the size of the Haversian canal, osteonal diameter, and canalicular length, respectively, were varied to study the influence of the transport path distance on the efficiency of solute transport between the blood supply and osteocytes within the hydraulic network. Within the cylindrical geometry of a given osteon, the cross sectional area of each concentric annular region, i.e. lamella, increases with increasing distance from the blood supply of the Haversian canal (figure 1b,c) and is proportional to the radius. First, the annular area within each lamella was calculated (table 1). Then, the number of canaliculi on the inner and outer edge of each lamella was calculated, assuming a uniform canalicular density of $0.05/\mu\text{m}^2$ (Marotti *et al.* 1995). Finally, a nondimensional ratio was calculated for the total number of canaliculi on the inner edge (or inner surface, in three dimensions) of a given lamella to that on its outer edge (C_i/C_o), for each lamella

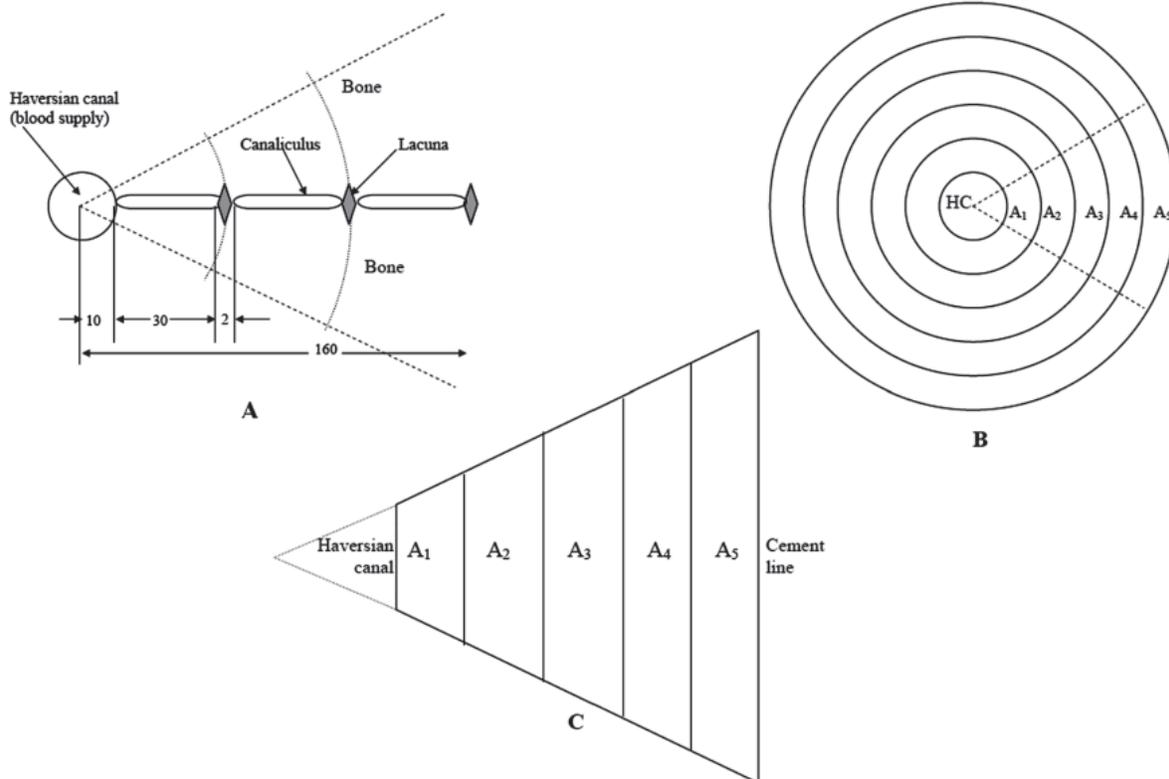


Figure 1. (A) Schematic drawing of an osteonal section (approximately 1/5 of the osteon); all dimensions in micrometer (after Piekarski and Munro 1977). (B) The lamellar regions can be idealized as concentric circular areas A_1, A_2, \dots, A_5 which, for the purpose of this analysis, are further idealized as adjacent polygons, as shown in (C). (Adopted from Mishra and Knothe Tate 2003.)

extending from the lamella adjacent to the blood supply of the Haversian canal, radially outwards toward the cement line (table 1).

For each consecutive lamella of the initial model, the ratio of canaliculi on the inner to the outer surface (C_i/C_o , referred to as the nondimensional canalicular ratio) increased with increasing distance from the Haversian canal (figure 2), approaching a threshold value of a radius of 169 μm . The rate of increase was highest (2.3 \times) in the lamella closest to the Haversian canal (figure 2) and decreased to less than 5% and C_i/C_o approaches 0.9 (figure 2).

4. Application of mechanics of fluid in bone microstructure

Assuming that canaliculus acts as a hollow pipe carrying fluid with pressure P , velocity v and specific gravity ρ , hydraulic

loss through the pipe (also referred to as “pipe losses” in civil engineering) is denoted by H_L and is calculated by applying Bernoulli’s equation (Fox and McDonald 1985) as follows, whereby g refers to gravitational force and z is the height of the pipe:

$$H_L = [(P_1/\rho g) + (v_1)^2/2g + z_1] - [(P_2/\rho g) + (v_2)^2/2g + z_2] \tag{1}$$

For fully developed flow through constant area pipe, and assuming $v_1 = v_2$, pipe losses amount to

$$H_L = [(P_1/\rho g) + z_1] - [(P_2/\rho g) + z_2] \tag{2}$$

Accounting for the flow in a horizontal pipe, $z_1 = z_2$, and

$$H_L = (P_1/\rho g) - (P_2/\rho g) \Rightarrow H_L = (P_1 - P_2)/\rho g \tag{3}$$

For laminar flow in pipes, the Darcy-Weisbach formula describes hydraulic losses as

Table 1. Dimensions, number of canaliculi and the calculated nondimensional canalicular ratio for each annular region of the osteon [osteon radius data adopted from Piekarski and Munro 1977 and canalicular density from Marotti *et al.* 1995]

Lamella	HC	1	2	3	4	5	6	7	8	9
Radius (μm)	10	41	73	105	137	169	201	233	265	297
Circular area (μm^2)	314	5278.34	16733.06	34618.5	58934.66	89681.54	126859.1	170467.5	220506.5	276976.3
Annular ring area (μm^2)		4964.34	11454.72	17885.44	24316.16	30746.88	37177.6	43608.32	50039.04	56469.76
Canalicular density ($\#/\mu\text{m}^2$)		0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total number of canaliculi		248.22	572.74	894.27	1215.81	1537.34	1858.88	2180.42	2501.95	2823.49
C_i/C_o		0.433	0.64	0.74	0.79	0.83	0.85	0.87	0.89	0.90

The nondimensional ratio (C_i/C_o) represents the ratio of canalicular number at a given inner lamella to that of the adjacent outer lamella, i.e. $C_1/C_2, C_2/C_3 \dots$, an indication of the increased branching of the transport network to provide cells with, e.g. nutrients, with increasing distance from the blood supply.

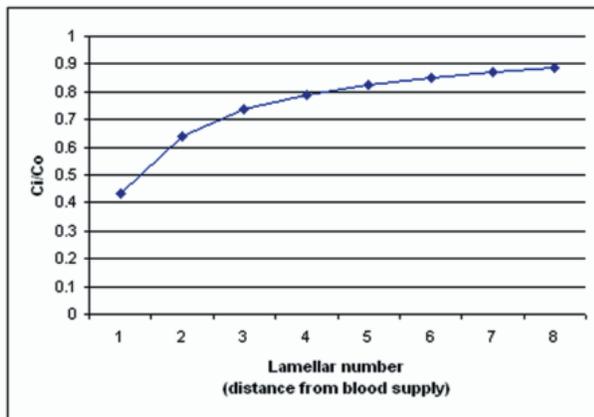


Figure 2. Nondimensional canalicular number (C_i/C_o) as a function of distance from blood supply (at origin). (Adopted from Mishra and Knothe Tate 2003).

$$H_L = f \cdot (L/D) (V^2/2), \tag{4}$$

where L = length of the pipe, D = hydraulic diameter and f is called “friction factor” of the pipe, given by

$$f = 64/ \text{Re} \quad \text{for laminar flow,} \tag{5}$$

where Re is Reynolds number of the flow.

Furthermore, based on the continuity equation, the flow rate Q can be defined as

$$Q = A_1 v_1 = A_2 v_2, \tag{6}$$

and the previously described set of equations, the numerical values on the right hand side of (4) can be substituted to calculate “pipe losses” in each individual canaliculus. Then, considering whether the canaliculi are arranged in series and/or parallel, the total losses in a group of canaliculi can be calculated. In this way it is possible to calculate the net losses within each lamellar area containing osteocytes.

However, calculating the magnitude of “head loss” requires numerical values for canalicular length and diameter as well as Reynolds number and velocity of fluid flow, the latter of which are unknown. Thus, the current study deduces the losses in canaliculi qualitatively by assuming ‘ f ’, (L/D) and area (A) of the canaliculus to be constant. Hence, from (4), (5) and (6)

$$H_L \propto (Q)^2 \tag{7}$$

Therefore, under the idealized conditions mentioned above, a qualitative pattern of pressure loss can be evaluated based on projected changes in volume flow rate (Q) in the network. Further, if the flow rate (Q) in an “upstream” canaliculus is assumed to be equally divided into two branches with $(Q/2)$ in each branch (figure 3), then head loss (H_L) in each canaliculus branch reduces to 1/4 the branch immediately upstream. Similarly, if there are three branches then the pressure head loss, or hydraulic loss, is reduced to 1/9 of the feeding branch furthest upstream. In an ideal branching network where each canaliculus branches into two downstream, and keeping all other parameters constant, after six successive branching the canaliculi furthest downstream will have 1/64 the head losses of the original canaliculus furthest upstream. Hence, in an ideal case, the canaliculus situated in the outermost lamellae will have 1/64 the losses of those in the lamellae adjacent to the Haversian canal. However, in actual osteonal networks canaliculi do not always branch into two and the degree of branching decreases with increasing distance from the Haversian canal. Therefore, the actual reduction in head loss will be less than

that calculated for the ideal condition. Nonetheless, it is clear from this analysis that branching *per se* helps in reducing the head losses in the canalicular network. This may provide insight into the microarchitecture of the lacunocanalicular system in mammalian cortical bone. In mammals, cortical thickness can be up to several centimeters, necessitating a long-distance fluid transport network that is achieved through two hierarchical levels of branching for global and local distribution of fluid and solutes respectively. First, the nutrient vessels branch into Haversian (longitudinally running) and Volkmann’s (radially running) channels in order to traverse large areas of tissue. Secondly, Haversian and Volkmann’s canals branch out to the lacunocanalicular system for local distribution to the cells. In comparison to mammals, the cortex of frog bone is less than 200 μm thick and local distribution to the cells is achieved directly from the vascular bed of the endosteum and periosteum through the simple lacunocanalicular system to the cells requiring no hierarchically branching transport system.

5. Application of scaling laws in bone microstructure

Allometric scaling laws describe mathematical relationships between complex biological variables, e.g. the relationship between length (L) or diameter (D) and body volume (V) or mass (M) can be described by the equation

$$(L, D) \propto V^{SE} \tag{8}$$

whereby SE is referred to as the scaling exponent.

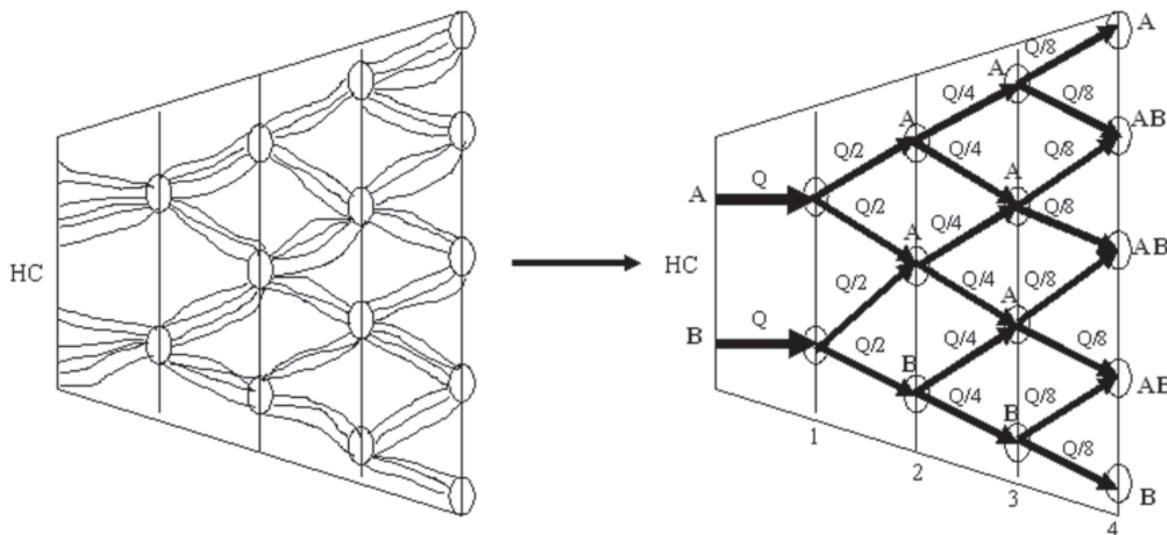


Figure 3. Schematic diagram showing alternate pathways provided by the canalicular network for fluid and solute transport between osteocytes. Osteocytes denoted by ‘AB’ may get nutrients from either A or B or both channels. There are a number of alternate pathways from the Haversian canal (HC) to each osteocytes and osteocytes further from the HC have more alternative pathways than osteocytes closer to the HC. For clarity, only a few canaliculi are shown to be originating from the HC. (Adopted from Mishra and Knothe Tate 2003.)

If one assumes that all species share a common density (Swartz and Biewener 1992), then this relationship can be described in terms of body mass or weight,

$$[(L,D) \propto M^{SE}]. \quad (9)$$

The scaling exponent is defined such that $SE = 1/3$ for interspecies *isometry*, $SE > 1/3$ for *positive allometry*, and $SE < 1/3$ for *negative allometry*.

Data including body weight, osteon and Haversian canal size were collected from published morphometric data (Jowsey 1966; Tarach and Czaja 1973) for mammalian species with different weights (10^2 to 10^6 g). Data including weight, osteon diameter and Haversian canal perimeter for rat, rabbit, cat, dog, rhesus monkey, man, cow, *Diadectes* and *Iguanodon* were available from Jowsey's (1966) study. Osteon and Haversian canal diameter data for man, monkey, horse and pig were reported by Tarach and Czaja (1973). These authors did not report weights of animals in their study, so human and monkey weights were assumed to be similar to the values reported by Jowsey (1966) and an average weight was calculated for the pig and horse. Haversian canal diameter was calculated from canal perimeter measurements (Jowsey 1966) assuming canals to be of circular cross section. The mean diameters of osteon and Haversian canal, respectively, were calculated from the corresponding maximal and minimal diameters reported by Tarach and Czaja (1973). Osteon and Haversian canal diameters were plotted against body weight for each species. A linear regression line was calculated for each resulting scatter plot (Microsoft Excel, version 2003). Finally, the ratio osteon:Haversian canal diameter was calculated for each animal species studied.

It has been reported by Mishra and Knothe Tate (2008) that, osteon and Haversian canal size increases in proportion to body weight of the animal (figures 4, 5). Based on the slope of the linear regression curve, the scaling coefficients for osteon and Haversian canal diameters are 0.12 and 0.17, respectively, indicative of negative allometry. Taking into account the positive allometric relationship between body

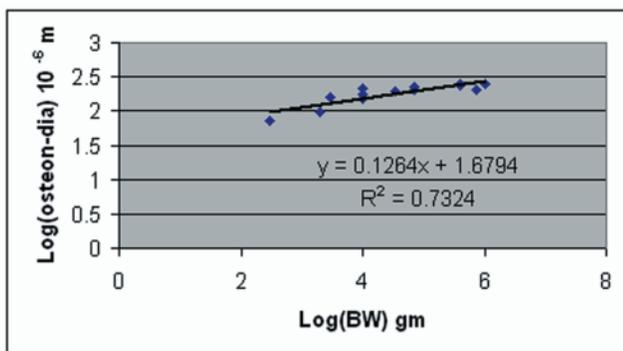


Figure 4. Plot of osteon diameter vs. body weight for increasing body mass. (Adopted from Mishra and Knothe Tate 2008.)

weight (W) and skeletal weight (W_s), as defined by Prange *et al.* (1979),

$$W_s \propto W^{1.09} \quad (10)$$

and because of the negative allometric relationship between osteon and Haversian canal diameter, larger animals tend to have relatively more skeletal mass but smaller osteon and Haversian canal size.

Normalizing for body weight, the allometric equations shown in figure 4 are divided by the body weight to get the specific osteon and Haversian canal diameter (y') i.e.

$$(y/x)_{\text{osteon}} = 47.79x^{0.12} / x \Rightarrow y'_{\text{osteon}} = 47.79x^{-0.88} \quad (11)$$

$$(y/x)_{\text{Haversian canal}} = 6.44x^{0.17} / x \Rightarrow y'_{\text{Haversian canal}} = 6.44x^{-0.83} \quad (12)$$

resulting in a scaling exponent of -0.88 and -0.83 , respectively. The negative SE for both allometric equations shows a decreasing specific osteon and Haversian canal size (i.e. per unit body weight) for increasing animal size or weight. Hence, osteons and Haversian canals in human and cow bone are relatively smaller than those in rat and cat bone with reference to their respective weights. Plotting the specific osteon and specific Haversian canal diameters against body weight on log-log axis, the resulting regression lines have negative slopes equal to the scaling exponents for each case (-0.88 and -0.83).

6. Discussion

Based on an idealized model of an osteon comprising a canal containing a central blood supply, concentric lamellae, and a lacunocanalicular network connecting the blood supply and osteocytes in the mineralized matrix, the inherent hydraulic conductance of this network is optimized for transport between the blood supply and cells up to a limiting distance of approximately five to six lamellae. Up to this critical distance, hydraulic conductivity of the network increases, and each successive annular region offers a path of reduced hydraulic losses. Nonetheless, beyond this critical distance of five to six lamellae, hydraulic conductance reaches a threshold beyond which neither the branching of the network nor redundancy in parallel networks can be overcome.

Minimal expenditure of energy provides a basis for optimization of most biological processes (Thompson 1936; West *et al.* 1997). Applying this principle to the analysis, the fifth to sixth lamellae represent an effective limit for fluid and solute transport, beyond which hydraulic resistance in the network increases and, presumably, additional energy must be expended to achieve further transportation. Furthermore, the architecture of the hydraulic network is optimized to minimize hydraulic losses during transport.

The results of the present study are also valid for fluid and solute transport in cancellous bones; thick trabeculae exhibit a lamellar structure similar to osteonal lamellae in cortical bone (Weiss 1988), except that the lamellae are oriented parallel to the vascular bed of the marrow cavity rather than being concentric to a central blood vessel contained in a Haversian canal. Moreover, given the fact that osteonal width and trabecular width are relatively constant across species (Kanis 1996), it appears that the underlying mechanism of fluid transport in bone is universal for cortical and cancellous bone and that transport path distances largely determine the dimensions of hydraulic pathways in cortical and cancellous bone.

Taken as a whole, the basic principle of minimal energy consumption in biological processes may provide the biophysical basis for the evolution of numerous parallel canaliculi networks for transportation of nutrients to cells in bone. This appears to be the end result of a natural selection process in which hydraulics and efficient transportation are likely to have played a key role.

From the perspective of evolution, primitive plants and animals had simple hydraulic architecture capable of transporting nutrients over only small distances (figure 7). Later, with the advent of large-sized plants and animals, the demand for large distance transportation resulted in the evolution of various types of hydraulic architectures (figures 7 and 8). At this stage the “factor of safety” may have played a vital role, as natural selection would have “favoured” transportation networks with high safety factor and an associated increased probability of survival. This is in agreement to the evolutionary studies of hydraulic architecture in plants (Zimmermann 1983). Water supply to leaves for photosynthesis is believed to be one of the prime considerations of evolution of hydraulic architecture in modern plants (Ennos 1999). For example, a single cell water-transporting conduit called a “tracheid” found in primitive plants evolved into a multicellular “vessel” capable of transporting water over several feet. This multicellular “vessel” provided a higher factor of safety necessary for transporting water over larger distances (figure 7). Similarly, one finds amphibian bones to be devoid of vascular networks of Haversian and Volkmann’s canals as the canaliculi network suffices to transport fluid and solutes directly from periosteal and endosteal vascular bed to osteocytes over transport path of less than 150 μm .

Relatively simple hydraulic networks found in primitive organisms were efficient only for small distance transportation. Thereafter, branched, series and parallel transportation networks developed in higher plants and animals depending upon the type of evolutionary demand. This hypothesis (figures 7 and 8) is supported by the comparative study of canaliculi structure of dinosaurs, birds, and mammals by Rensberger and Watabe (2000) where it has been suggested that more irregular, random

canaliculi organization found in a type of dinosaurs (coelurosaurs) and birds is a condition derived from the radially directed canaliculi network that was primitive condition for tetrapods. The evolutionary force behind the random canaliculi network is believed to be the high rate of growth of bones of birds (Rensberger and Watabe 2000). However, Chinsamy-Turan (2005) has shown that different depositional rates of bone formation exist among vertebrates and these are further influenced by ontogeny, location in the skeleton and local growth conditions.

The evolutionary advantage for allometry for osteon and Haversian canal size as described in the previous section appears to be related to optimizing efficiency of nutrient and metabolite transport in the lacunocanaliculi network. Due to the differences in SE (figures 4 and 5), animals with lower weights tend to have a relatively higher ratio of osteon diameter to Haversian canal diameter (figure 6); this may be advantageous for survival. On the other hand, heavier skeletons tend to have a constant ratio (4 to 6) between osteon and Haversian canal diameter, suggesting

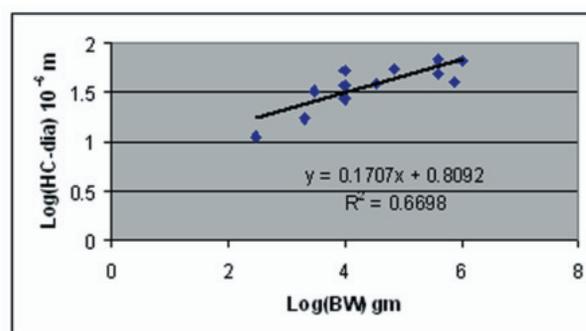


Figure 5. Plot of Haversian canal diameter vs. body weight for the animals. (Adopted from Mishra and Knothe Tate 2008)

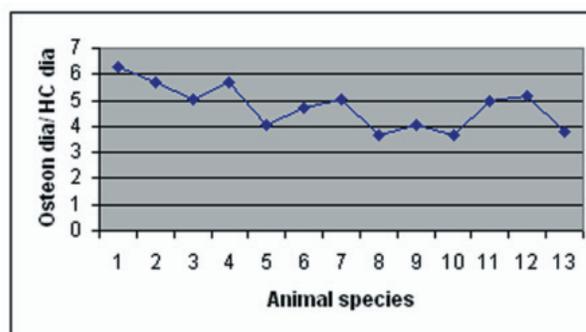


Figure 6. Ratio between osteon diameter and Haversian canal diameter for the different animals considered in this study, ¹rat, ²rabbit, ³cat, ⁴dog, ⁵rhesus monkey, ⁶monkey, ⁷pig, ⁸man, ⁹man, ¹⁰cow, ¹¹*Diadectes*, ¹²horse, ¹³*Iguanodon*. The data points 1,2,3,4,5,8,10,11 and 13 were after Jowsey (1966) and the data points 6,7,9 and 12 were after Tarach (1973). (Adopted from Mishra and Knothe Tate 2004.)

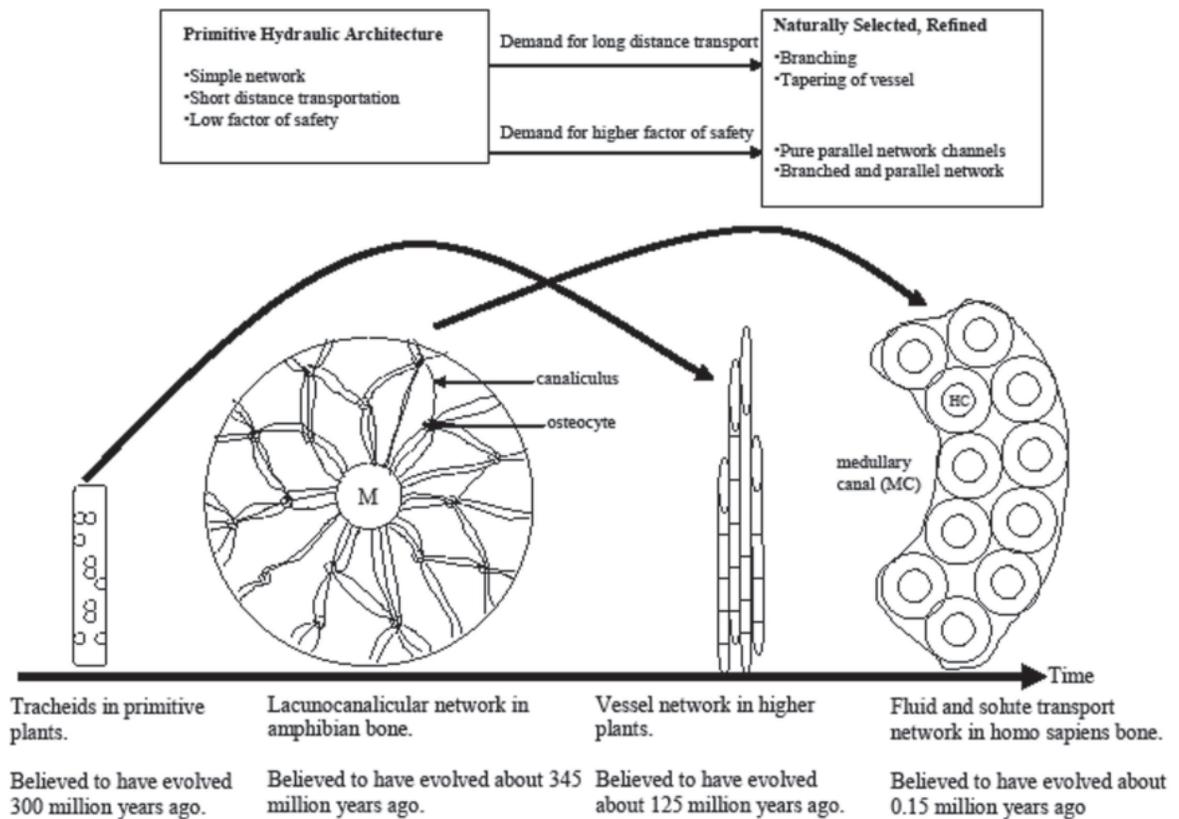


Figure 7. Schematic diagram describing the proposed evolution of transport networks in biology. (Adopted from Mishra and Knothe Tate 2003.)

an optimum value to satisfy the conflicting requirements for lower hydraulic resistance in lacunocanicular network and increased nutritional demand due to increase in the size of osteon. Other mechanical factors such as optimal fibre size for maximal composite strength may have influenced this evolutionary trend as well.

Comparison of absolute size of the osteon and Haversian canal of *Diadectes* of Permian age (299–251 Ma) and *Iguanaodon*, a dinosaur of Jurassic age (199–145 Ma), shows a relatively large increase in the size of Haversian canal (from 47 to 64 μm ; 36%) and a small increase in the size of the osteon (236 to 246 μm ; 4%). The morphological data of *Iguanaodon* (osteon diameter 246 μm and Haversian canal diameter 64 μm) are more similar to those of modern mammals such as the cow (osteon diameter 250 μm and Haversian canal diameter 68 μm) than to *Diadectes*. If bone microarchitecture is related to its function, then the present results indicate that Jurassic dinosaur bones were functionally similar to modern mammals. However, other biomechanical variables need to be explored to support this finding.

The study suggests that osteon domain that can be nourished efficiently by its Haversian canal is approximately

4 to 6 times of the size of Haversian canal, irrespective of the animal species (figure 6). Therefore, the relative area (volume) of the nutrient reservoir in Haversian canal to the supply area (volume) in an osteon is similar regardless of species. Only the morphometric data from the rat shows a higher ratio (>6) between osteonal diameter and Haversian canal diameter, which may be due to non-closure of growth plates and a different mechanical environment imbued in the rat femur, as described by Mullender *et al.* (1996).

Limitations of the study: The actual three-dimensional geometry of the osteon is neither perfectly circular nor spherical. Furthermore, canaliculi that were idealized as hollow cylinders actually contain osteocyte processes, which would be expected to increase the hydraulic resistance within. In addition, all the canaliculi were assumed to have identical dimensions ($\sim 30 \mu\text{m}$) in the model although there is variation in dimensions of canaliculi in a real osteon. Finally, the canaliculi are assumed to be radial and branched in parallel, a general but not absolute trend in mammalian bone (Marotti *et al.* 1995; Rensberger and Watabe 2000). Nonetheless, these idealizations affect neither the order of magnitude of the results nor the final conclusions of this study.

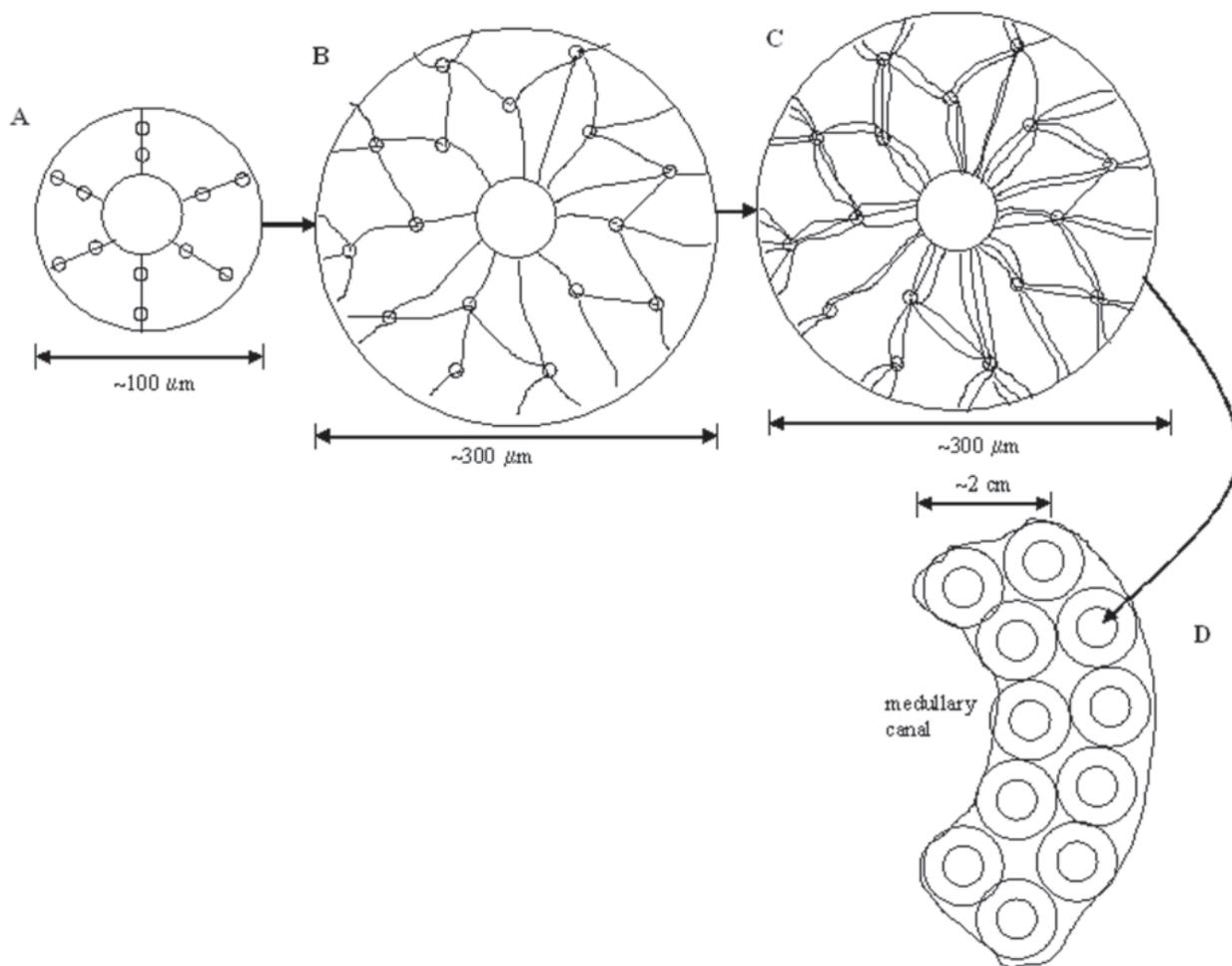


Figure 8. Hypothesized evolution of transport networks in bones (not to scale) including (A) simple networks for small distance transport. (B) With little branching and parallel networks, transportation is possible over a longer distance but the *factor of safety* of such network is low. (C) The same network as in (B) but with numerous parallel conduits exhibits a higher factor of safety, similar to canalicular network found in amphibians. (D) In modern mammals such as homo sapiens, the demand for fluid and solute transport over much larger distances has resulted in branched vascular system i.e. Volkmann's and Haversian canal. (Adopted from Mishra and Knothe Tate 2003.)

There are limitations in the allometric scaling study. Firstly, the weights of *Diadectes* and *Iguanodon* were estimates. Secondly, morphometric measurements in the study of Tarach and Czaja (1973) did not account for weight of animals, and minimum and maximum diameters of osteon and Haversian canal were measured instead of mean \pm SD as reported by Jowsey (1966). The methodology used by the two previous studies to measure the dimensions of the osteon and Haversian canal may not be similar. Therefore, estimates applied in this analysis may deviate from actual values for the man, monkey, pig and horse specimens reported by Tarach and Czaja (1973). Hence, combining these two sets of morphometric data to calculate allometric equations may have introduced a degree of error. Furthermore, limited data sets from secondary sources, undue weighting for some

species coupled with application of linear regression lines may not be appropriate to draw conclusions. Nonetheless, this artifact is not expected to alter the trend demonstrated with the allometric equations and qualitative findings. Further studies with large sample size of primary data with stereological methods will be required to further refine this preliminary study.

7. Conclusion

In summary, the lacunocanicular architecture of bone tissue is optimised for transport of fluid and solutes between the blood supply and bone cells. This intrinsic means to increase hydraulic conductance has not been explored previously. The dimensions of the Haversian canal and

canaliculi appear to be optimized for efficient utilization of this transport mechanism. Furthermore, it appears that hydraulic architecture in bone has evolved not only for efficient biotransport between the blood supply and cells but also to provide redundant means for transport and communication between cells. Similar to the evolution of increasingly complex water transport networks in plants, the hierarchical organization of the vascular and lacunocanicular network appears to be increasingly complex with increasing size and metabolic requirements in vertebral bones. The biomechanical aspects of bone microarchitecture can be easily used by palaeontologists, for making ontogenetic and phylogenetic inferences, as a thin section of a fossil bone under a microscope will reveal the microstructures such as the shape and size of lacuna and canaliculi

It is noteworthy that several fossils of global importance have been found in India in recent years. However, no or little biomechanical study has been carried out until now. For example, a recent study on Indian dinosaur eggshell structure (Srivastava *et al.* 2005) was perhaps the first application of FEA to plot stresses on dinosaur eggshells. FEA needs to be more often used in palaeobiological studies. The other remarkable study is by Thewissen *et al.* (2007) on whale origins, based upon unique fossils of *Indohyus*, the closest land ancestor of whales found in approximately 47 million year old rocks in the Jammu and Kashmir region of India. This study has changed the cladistic definition of Cetacea and proposes a new hypothesis for the origin of whales based upon the new dental, cranial and postcranial material. In this study several biomechanically important parameters such as tympanic wall thickness, tooth pattern in the jaw were studied along with bone histology to find the presence of osteosclerosis. Further biomechanical studies (e.g. FEA of bones to estimate loading regimens, computer simulation of chewing to understand its dietary habits) of these fossils will provide additional evidence about its skeletal strength, adaptability in water and food habits.

Acknowledgements

The author is thankful to Prof Melissa Knothe Tate, Department of Biomedical and Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH, USA for providing financial assistance and academic guidance to carry out the above work. Furthermore, thanks to anonymous reviewer for giving suggestions to improve the manuscript.

References

Aarden E M, Burger E H and Nijweide P J 1994 Function of osteocytes in bone; *J. Cell Biochem.* **55** 287–299

- Alexander R M 1989 Mechanics of fossil vertebrates; *J. Geol. Soc., London* **146** 41–52
- Alexander R M 2006 Dinosaur biomechanics; *Proc. R. Soc. London* **B272** 1849–1855
- Bajpai S and Thewissen J G M 2000 A new, diminutive Eocene whale from Kachchh (Gujarat, India) and its implications for locomotor evolution of Cetaceans; *Curr. Sci.* **79** 1478–1482
- Carter D and Beaupre G 2001 *Skeletal function and form* (Cambridge University Press)
- Cooper R R, Milgram J W and Robinson R A 1966 Morphology of the osteon: An electron microscopic study; *JBJS* **48A** 1239–1279
- Chinsamy-Turan A 2005 *The microstructure of dinosaur bone* (Baltimore and London: John Hopkins University Press).
- Copenhaver W M 1964 *Baily's textbook of histology* (eds) W M Copenhaver, R P Bunge and M B Bunge (Baltimore: Williams and Wilkins Co) pp 122–123
- Currey J D 2002 *Bones: Structure and mechanics* (Princeton: Princeton University Press)
- Darwin C 1859 *On the origin of species* (ed.) J Murray (London)
- Doty S B 1981 Morphological evidence of gap junctions between bone cells; *Calcif. Tissue Int.* **33** 509–512
- Ennos A R 1999 The aerodynamics and hydrodynamics of plants; *J. Exp. Biol.* **302** 3281–328
- Fox R W and McDonald A T 1985 *Introduction to fluid mechanics* (John Wiley)
- Guo E 2001 Mechanical properties of cortical bone and cancellous bone tissue; in *Bone mechanics handbook* (ed.) S C Cowin (CRC Press)
- Jowsey J 1966 Studies of Haversian systems in man and some animals; *J. Anat.* **100** 857–864
- Kanis JA 1996 *A textbook of osteoporosis* (Oxford: Blackwell Sci.) pp 11–12
- Knapp H F, Reilly G C, Stemmer A, Niederer P and Knothe Tate M L 2002 Development of preparation methods for and insights obtained from atomic force microscopy of fluid spaces in cortical bone; *Scanning* **24** 25–33
- Knothe Tate M L 2003 Whither flows the fluid in bone? An osteocyte's perspective; *J. Biomech.* **36** 1409–1424
- Marotti G, Ferretti M, Remaggi F and Palumbo C 1995 Quantitative evaluation on osteocyte canalicular density in human secondary osteons; *Bone* **16** 125–128
- Mishra S and Knothe Tate M L 2003 Effect of lacunocanicular architecture on hydraulic conductance in bone tissue: Implications for bone health and evolution; *Anat. Rec. Part A Discov. Mol. Cell Evol. Biol.* **273A** 752–762
- Mishra S and Knothe Tate M L 2004 Allometric scaling relationships in microarchitecture of mammalian cortical bone; in *Proceedings of 50th Annual Meeting of the Orthopaedic Research Society*, 7–10 March, San Francisco, CA, USA
- Mishra S and Knothe Tate M L 2008 Comparative study of bone micro-architecture of some mammalian bones; *Palaeobotanist* **57** 299–302
- Mullender M G, Huiskes R, Versleyen H and Buma P 1996 Osteocyte density and histomorphometric parameters in cancellous bone of the proximal femur in five mammalian species; *J. Orthopaed. Res.* **14** 972–979

- Padian K 1997 Physiology; in *Encyclopedia of dinosaurs* (eds) P J Currie and K Padian (Academic Press London) pp 552–557
- Piekarski K and Munro M 1977 Transport mechanisms operating between blood supply and osteocytes in long bones; *Nature (London)* **269** 80–82
- Polig E and Jee W S 1990 A model of osteon closure in cortical bone; *Calcif. Tissue Int.* **47** 261–9
- Prange H D, Anderson J F and Rahn H 1979 Scaling of skeletal mass to body mass in birds and mammals; *Am. Nat.* **113** 103–122
- Rayfield E J 2007 Finite Element Analysis and understanding the biomechanics and evolution of living and fossil organisms; *Annu Rev. Earth Planet. Sci.* **35** 541–576
- Rayner J M V and Wootton R J 1991 *Biomechanics in evolution* (Cambridge University Press)
- Rensberger J M and Watabe M 2000 Fine structure of bone in dinosaurs, birds and mammals; *Nature (London)* **406** 619–622
- Remaggi F, Cane V, Palumbo C and Ferretti M 1998 Histomorphometric study on the osteocyte lacuno-canalicular network in animals of different species. I. Woven-fibered and parallel-fibered bones; *Ital. J. Anat. Embryol.* **103** 145–55
- Swartz S M and Biewener A A 1992 *Biomechanics – Structures and systems* (ed.) A A Biewener (Oxford University Press)
- Srivastava R, Sahni A, Jafar S A and Mishra S 2005 Microstructure dictated resistance properties of some Indian dinosaur eggshells: finite element modelling; *Paleobiology* **31** 315–323
- Tarach J and Czaja M 1973 Statistical analysis of some size parameters of Haversian systems in femoral, ground transverse sections in man and animals (Translated from Polish); *Ann. Univ. Marie Curie-skłodowska Lublin-Polina* **28** 99–105
- Thewissen J G M, Cooper L N, Clementz M T, Bajpai S and Tiwari B N 2007 Whales originated from aquatic artiodactyls in the Eocene epoch of India; *Nature (London)* **450** 1190–1194
- Thompson D 1936 *On growth and form* (Cambridge: Cambridge University Press) (Reprinted 1963)
- West G B, Brown J H and Enquist B J 1997 A general model for the origin of allometric scaling laws in biology; *Science* **276** 122–126
- Weiss L 1988 *Cell and tissue biology; A textbook of histology* (Baltimore: Urban and Schwart Zenberg)
- Zimmermann M 1983 *Xylem structure and the ascent of sap* (Berlin: Springer Verlag)

ePublication: 29 October 2009