Mechanics Applied to Skeletal Ontogeny and Phylogeny^{*}, ^{**}

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Abstract. The musculo-skeletal system serves the mechanical function of creating motion and transmitting loads. It is made up mainly of four components: bone, cartilage, muscle and fibrous connective tissue. These have evolved over millions of years into the complex and diverse shapes of the animal skeleton. The skeleton, however, is not built to a static plan: it can adapt to mechanical forces during growth, it can remodel if the forces change, and it can regenerate if it is damaged. In this paper, the regulation of skeletal construction by mechanical forces is analyzed from both ontogenetic and phylogenetic standpoints. In the first part, models of biomechanical processes that act during skeletal ontogenesis – tissue differentiation and bone remodeling – are presented and, in the second, the evolution of the middle ear is used as an example of biomechanical change in skeletal phylogenesis. Because the constitutive laws for skeletal tissues are relatively well understood, and because the skeleton is preserved in the fossil record, application of mechanics to skeletal evolution seems to present a good opportunity to explore the relationships governing ontogenetic adaptations and phylogenetic change.

Key words: Biomechanics, Mechanobiology, Remodeling, Ontogenesis, Vertebrates, Optimization, Developmental mechanics, Palaeobiology.

1. Introduction

The skeleton is a machine whose construction is the net result of two processes – a phylogenetic process acting over millions of years and an ontogenetic process acting over the life of the individual. How have these processes created skeletons that fulfill biomechanical functions with such apparent efficiency? There are two main approaches to investigating this question:

Method I: Discovery of the optimality of skeletal elements. What features of a skeleton give an animal advantage over its competitors? Are bones minimum weight structures? Are trabeculae optimally oriented within spongy bone? Alexander's *Optima for Animals* [1] is a well-known work in this vein: for example he presents an analysis showing an optimal factor-of-safety for many bones. Similarly Biewener [2] found that larger animals tended to have larger moment arms for their muscles thereby needing relatively less muscle mass for locomotion than smaller animals.

Method II: Discovery of 'mechano-regulation rules' to predict the growth, adaptation, and decay of the musculo-skeletal frame. Achievements to date include algorithms to predict bone adaptation to mechanical loading. These algorithms extend the theories of continuum mechanics and material behavior to account for biological phenomena, as in adaptive-elasticity theory [3] and related models [4, 5],

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including recent anisotropic formulations [6, 7], the optimal response hypothesis [8] and damage-adaptive bone remodeling concepts [9–11], or approaches centered on modeling the net effect of individual cell responses [12, 13]. Advances have also been made on the problem how mechanical forces stimulate tissue differentiation. The problem was first defined by Pauwels [14]. More recently, Carter and co-workers [15] pursued Pauwels’ approach using stress and strain invariants, and predicted *inter alia* that hydrostatic stresses inhibited ossification in differentiating tissues such as growing bones and in fracture healing of long bones. Claes and co-workers [16] and Gardner and co-workers [17] have presented analogous approaches using more accurate descriptions of bone shapes. Research by the author and co-workers on the problem of mechano-regulation of tissue differentiation first proceeded with an analysis of stress/fluid flow changes in regenerating tissue around implants [18] and later considered actual *simulations* of bone regeneration [19] including most recently simulations of tissue differentiation during fracture healing [20–22].

Method I assumes something about the skeleton itself (that some aspect has been optimized) whereas Method II assumes something about the process forming the skeleton (that there are stimuli that drive it and rules that control it).

2. Purpose of This Paper

Gould [23] gives a general account of the nature of the relationship between the growth of the individual (ontogeny) and the development of the species (phylogeny). What is of interest in this paper is whether or not such a relationship can be studied using mechanics; in particular the author is interested in whether or not the mathematical relationships (in the form of algorithms or rules) for the growth, adaptation, and remodeling (as listed above [3–22]) can be used to better understand any aspect of the evolution of skeletal efficiency in the species. The connection between skeletal ontogeny and phylogeny can be inferred in three steps as follows:

- First, the phylogenetic process involves natural selection. Random genetic variation results in skeletal differences causing the musculo–skeletal systems of some animals to be better adapted to their environment than others. Such animals have a survival advantage and are more likely to reproduce, passing on their genes (and hence their skeletal form) to future generations.
- Second, ontogenetic processes act on the skeleton, following mechano-regulatory rules, so that the skeleton becomes adapted to the individual animal’s environment and activity level. For example, mechano-regulation of long bone growth by weight-bearing and muscle forces increases the second moment of area of the bone shaft thereby improving the strength-to-weight ratio [24].
- Finally, the mechano-regulatory rules themselves may be adapted by natural selection. For example, an animal that can increase muscle mass if strenuous tasks become required for survival will have an advantage over a competitor who is not able to do so. An animal with the most effective mechano-regulatory response will be ‘selected’ and, after many generations, even a slight appearance of the environmental stimuli may elicit the appropriate adaptive response in a non-linear and highly efficient fashion. In this respect, a species may be considered as partly characterized by its set of mechano-regulation rules.

The purpose of this paper is to apply both Method I and Method II described in Section 1 to address the question of how the skeleton is constructed by both ontogenetic adaptation and phylogenetic change.

3. Analyses of Mechano-regulation Rules

Biomechanicians find the idea of mechano-regulatory rules intriguing. If we knew them we could predict an individual's skeletal growth, adaptation, and degeneration under different activity levels and mechanical environments. Mechano-regulatory rules could be put to practical use in bioengineering to predict tissue response after implantation of medical devices [25]. In the derivation of mechano-regulation rules, biomechanicians (being mainly mechanical engineers) have typically considered the apparent-level stimuli as the driving force for adaptations, even though decision-making with respect to mechano-regulation occurs at a lower level in the microstructure – at the level of the cells. This simplification is acceptable so long as cell stimuli are directly related to the apparent-level (continuum) stimuli.

3.1. BONE REMODELING IN RESPONSE TO MICRODAMAGE

Theories to predict ontogenetic bone adaptation to mechanical forces hypothesise that the stimulus for adaptation is either strain or a related variable such as strain energy density. If it is assumed that the rate of change of mass in a region is proportional to the change of strain from a reference or homeostatic value denoted ϵ^{ref} , then

$$\frac{dm}{dt} = C(\epsilon - \epsilon^{\text{ref}}), \quad (1)$$

where m is the mass and C is a remodeling rate constant. Alternatively, if bone remodeling is stimulated by microdamage [9] then,

$$\frac{dm}{dt} = C_1 \Delta\omega, \quad (2)$$

where $\Delta\omega$ is the change in damage from the equilibrium level, which is given by the integrated difference between the damage formation rate and the damage repair rate, as

$$\Delta\omega(t) = \int_0^t \frac{d\omega}{dt} - \frac{d\omega_{\text{RE}}}{dt}. \quad (3)$$

The damage-adaptive remodeling rule is an integral one; this is because damage accumulates (whereas strain does not). In equation (3), $\dot{\omega}_{\text{RE}}$ is the repair rate which must be equal to the damage formation rate $\dot{\omega}$ at remodeling equilibrium. The damage formation rate can be calculated as a function of stress using the experimental data for fatigue of bone [26]. One such equation is

$$\frac{d\omega}{dt} = B\sigma^n, \quad (4)$$

where B and n are empirical constants. It has been hypothesized that a particular cell (the osteocyte) distributed throughout the bone matrix is responsible for sensing the stimulus in the tissue, and signaling to other cells to carry out the adaptation if necessary to return the

Figure 1. Effect of damage on the deformation of an osteocyte lacuna. As the crack opens under tension it reduces the strain felt by the cell.

stimulus to a homeostatic level [27]. Prendergast and Huiskes [28] investigated whether or not the damage in the bone matrix would affect the strain felt by the osteocytes because, if that were true, then the mechanisms of strain sensitivity proposed by others [27] could also serve as a mechano-transduction mechanism for damage-adaptive remodeling. A finite element model of a piece of osteonal bone was used to analyze the effects of various kinds of microdamage on the deformation of nearby osteocyte lacunae. One result shows that osteocyte deformation is reduced by the growth of a microcrack, in this case an interlamellar microcrack, see Figure 1. (A similar result was found for bone lining cells [29].)

In *An Analysis of Theories in Biomechanics* [30], the author listed five potentially falsifying hypotheses for damage-adaptive remodeling, that is, statements that, if true, would refute the theory. Three of these were: first, static loads cause the same degree of remodeling as dynamic loads; second, diffuse microdamage does not exist in bone at remodeling equilibrium; and third, experiments on living bone show no change in the microdamage burden when the load is changed. The first may be refuted using the experimental observation that cyclic daily loading is needed to stimulate remodeling [31], the second has been refuted by the findings of microscopic cracks in the bone of living animals after the load has been increased and a sample of the bone subsequently submitted to histological analysis [32, 33], and the third has been refuted by finding of more microscopic cracks in the radius of sheep that have undergone ulnar-osteotomy relative to sheep who did not [34].

These experimental results provide evidence that damage plays an important mechano-regulatory role in bone remodeling. The results suggest that the next step in biomechanical modeling of bone adaptation should be to combine equations (1) and (2) to recognize bone as 'adaptive' to both strain and microdamage.

3.1.1. Strain-adaptive and damage-adaptive remodeling combined

Following the principles of strain-adaptive remodeling, it is assumed that an increase of strain above the homeostatic level stimulates the BMU¹ to deposit more bone than it resorbs thereby

¹ BMU is an acronym for bone multicellular unit. A BMU consists of osteoclast cells resorbing bone and osteoblast cells depositing bone. At remodeling equilibrium, bone formation rate = bone resorption rate.

Figure 2. Bone may remodel in response to strain and damage simultaneously. In that case a non-linear mechano-regulation rule is found for bone remodeling, with the atrophy rate greater than the hypertrophy rate, and a pathological atrophy rate found at high strains. Estimated values for the strains are: $\epsilon_{\min} \approx 50\text{--}200 \mu\epsilon$ atrophy occurs; $\epsilon_{\max} \approx 2000\text{--}3000 \mu\epsilon$ hypertrophy occurs, $\epsilon_{\text{MDx-thresh}} \approx 4000 \mu\epsilon$ atrophy due to damage begins.

increasing bone mass whereas a decrease in strain causes a BMU to resorb more bone than it deposits leading to a decrease in bone mass.² In a previous paper [9], the idea that increases of homeostatic damage would stimulate deposition of new bone, and decreases of homeostatic damage would stimulate bone resorption, was introduced. However, if the net resorption stimulated by decreases in homeostatic damage is assumed negligible then the resulting mechano-regulatory rule based on the combined strain-damage stimulus may be written as

$$\frac{dm}{dt} = C(\epsilon - \epsilon^{\text{ref}}) + xC_1(\Delta\omega), \quad (5)$$

where $x = 1$ if $\Delta\omega$ is positive else $x = 0$, where $\Delta\omega$ is given in equation (3). This addition of strain and damage stimuli is shown graphically in Figure 2, including the lazy-zone for strain as proposed by Carter [35]. The resulting expression for the rate of change of mass in response to a mechanical stimulus is highly non-linear. Furthermore it

- (i) predicts more rapid bone resorption than deposition, something which has been found in experiments [36] and is a common clinical observation [37],
- (ii) predicts bone resorption at pathologic overload strain levels, such as is found at implant/bone interfaces.

The possible phylogenetic consequences of such a remodeling rule are addressed in Section 5.

² A decrease in bone mass could be due to either a decrease in osteoblast activity (deposition) or an increase in osteoclast activity (resorption).

3.2. TISSUE DIFFERENTIATION SIMULATIONS

Tissue differentiation occurs when tissue in a region changes from one type to another (say from cartilage to bone). Tissue differentiation is regulated by both genetic factors and by epigenetic factors, such as mechanical stimuli. In the case of bone formation, the template tissue is either a soft connective tissue membrane (intramembraneous ossification) or cartilage (endochondral ossification). The objective of biomechanical studies of tissue differentiation is to predict how mechanical environments stimulate or inhibit the formation of the different tissue types.

Previous work by Carter and colleagues [38, 39] have described tissue differentiation as a function of the stress invariants calculated in a model of the tissue as an elastic solid. An alternative approach pursued by the author and his colleagues requires modeling the porous fluid-filled nature of the tissues to obtain a mechano-regulation theory of tissue differentiation [18, 19]. According to the theory, the mesenchymal stem cells (precursor cells) differentiate into either fibroblasts, chondrocytes, or osteoblasts depending on the combination of shearing strain and fluid flow acting on them. Specifically, a phase space of the solid phase stimulus (shear strain, denoted γ) and the fluid phase stimulus (in this case fluid velocity, denoted v) is divided into four regions; fibrous connective tissue at high (γ, v) , cartilage at intermediate (γ, v) and bone at low (γ, v) , and a resorption field where the tissue will be resorbed at very low (γ, v) . The phase space may be represented graphically (Figure 3). Referring to the notation in Figure 3, if it is assumed that $n_1 = n_2 = n$ and $m_1 = m_2 = m$ then the combined stimulus can be written as

$$S = \frac{\gamma}{a} + \frac{v}{b} \quad (6)$$

and the conditions for the formation of differentiated tissues is given as

$$\begin{aligned} 0 \leq S \leq n, & \quad \text{bone-resorption,} \\ n \leq S \leq 1, & \quad \text{bone,} \\ 1 \leq S \leq m, & \quad \text{cartilage,} \\ m \leq S, & \quad \text{fibrous-connective-tissue.} \end{aligned} \quad (7)$$

Figure 3. A mechano-regulation diagram based on tissue strain and fluid flow.

It is assumed that apparent level biophysical stimuli can be calculated according to biphasic poroelasticity theory [40], whereby the stress in the fluid and solid phases is given by

$$\sigma_s = \phi^s pI + \lambda e^s I + 2\mu \varepsilon^s, \quad (8a)$$

$$\sigma_f = -\phi^f pI, \quad (8b)$$

where λ and μ are Lamé constants, e^s in the dilational strain and ε^s in the deviatoric strain, and the superscripts s and f refer to the solid and fluid phase, respectively. The fluid velocity can be calculated using D'Arcy's equation

$$\vec{v} = K \vec{\nabla} p, \quad (9)$$

where K is a constant related to the permeability of the tissue. From equations (8) and (9) the controlling parameters of equation (6) can be determined and hence the differentiated cell of the relation 'precursor cell $\rightarrow$ differentiated cell' can be determined. To compute the amount of extracellular matrix formed by the differentiated cells in a region, and hence the material properties for the element, the number of precursor cells present in a region must be found. The calculation of cell spreading can be calculated according to

$$\frac{dn}{dt} = D \nabla^2 n + ns(c) - kn, \quad (10)$$

where n denotes the number of cells, D is a diffusion co-efficient, $s(c)$ denotes the rate of cell mitosis (division to create new cells) as a function of a mitosis inducing factor c , and k is a apoptosis rate (or death rate) [41]. In the present work, it was assumed that $s(c) = 0$ and $k = 0$.

To test this theory, a simulation of bone regeneration during fracture healing of a long bone was carried out and predictions of tissue types were compared to observations, see [20, 21]. The predicted patterns of tissue formation were found to depend on the origin of the precursor cells (i.e. whether they came from the marrow, the periostium, or the bone surface, see [20]). The model also predicted the correct influence of loading and gap size (see [21]). An example of the results is shown in Figure 4 where the following tissue differentiation

Figure 4. Simulation of fracture healing in a long bone external callus using the mechano-regulation diagram shown in Figure 3. Light gray = soft tissue, dark gray/black = bone.

steps were predicted:

- (i) Early direct bone formation (intramembraneous ossification) at sites distant from the fracture site, fibrous connective tissue in the interfragmentary region and cartilage elsewhere.
- (ii) The cartilage adjacent to the intramembraneous bone begins to be resorbed and replaced by bone – that is, endochondral ossification occurs.
- (iii) Process (ii) continues until ‘bridging’ of the external callus occurs; after this event the load transfer path is via the external callus thereby unloading the fibrous connective tissue in the interfragmentary gap.
- (iv) Because of process (iii), biophysical stimuli reduce in the interfragmentary gap and ossification can commence there. The load transfer path then switches to the more direct one; as a consequence the external callus is unloaded and it resorbs.
- (v) Remodeling returns the bone to a healed condition.

It is predicted, therefore, that the tissue in the fracture gap cannot differentiate *directly* into bone because, when soft tissues of increasing stiffness appear there, the biophysical stimuli are largely unaffected. The opposite is the case in the external callus; soft tissues can reduce the local biophysical stimuli so that the differentiation of new, stiffer, tissues is favored.

It is often hypothesized that fracture repair uses the mechano-regulatory tools provided for the more general processes of skeletal growth. If this is true then mechano-regulation of tissue differentiation should have some consequences for how the skeleton has evolved – this issue is examined in Section 5.

4. Analysis of Skeletal Elements: the Case of the Middle Ear

In humans, the middle ear consists of the tympanic membrane connected to three ossicles (the malleus, incus, and stapes³) commonly called the hammer, anvil, and stirrup. The tympanic membrane itself has the shape of a cone with curved sides, and the sides are reinforced by radial and circumferential fibres. The ossicles are connected to several ligaments, called the suspensory ligaments, and two muscles: the tensor tympani muscle and the stapedius muscle. A pressure fluctuation in the air (sound wave) enters the external auditory meatus (ear canal) and impacts on the tympanic membrane causing vibration of the ossicles which in turn causes motion of the perilymph of the inner ear. The perilymph vibrates the cilia of specialized mechano-sensitive cells resulting in an electrical stimulus to the brain.

In his work on hearing, Helmholtz [42] proposed that the tympanic membrane was curved so that the fibres, when straightened, could force the umbo to move against a large force and therefore small acoustic pressures could be magnified sufficiently to displace the ossicles and the perilymph. Dahmann [43], von Békésy [44] and others proposed that the ossicles operate as a lever transforming small forces of low magnitude and high displacement at the tympanic membrane to small displacements and high forces at the incudo-stapedial joint. Considering Figure 5, a small force F_1 is magnified by a simple lever arrangement into $F_2 = F_1 l_1 / l_2$ at the incudo-stapedial joint. The lever ratio theory is the currently accepted explanation of human middle ear morphology.

³ The Sicilian physician Gian Filippo Ingrassia discovered the stapes in 1546, see O'Malley, C.D., Clarke, E. ‘The discovery of the auditory ossicles’, *Bull. Hist. Med.* **35** (1961) 419–491.

Figure 5. The three middle-ear ossicles: the malleus, incus, and stapes. The lengths l_1 and l_2 are the distances from the point of application of the force and the center of rotation of the malleus–incus complex. According to the hypothesis of the lever ratio, the force F_1 is magnified so that $F_2 = F_1 l_1 / l_2$.

4.1. FINITE ELEMENT MODELING

Various finite element models were created to investigate the vibratory motion in the middle ear. The geometry of the outer ear canal was determined using magnetic resonance imaging and the geometry of the ossicles was determined using μ CT scanning. The material properties, damping, and other attributes of the finite element model are taken partly from Wada *et al.* [45], see Kelly *et al.* [46]. The finite element model of the outer and middle ear was confirmed to behave well by comparing laser Doppler vibrometry measurements of the tympanic membrane with predictions of the model [46]. The center of rotation of the malleus/incus complex was calculated and it was found to vary with frequency. Furthermore, we calculated the force exerted by the tympanic membrane on the long process of the malleus and it was also found to vary with frequency.

By resolving forces about the axis of rotation, the lever ratio could be calculated across the frequency range. It was predicted that the lever ratio depends on frequency. Furthermore, if F_1 (see Figure 5) is defined as acting, not at the end of the malleus (as depicted in Figure 5 and as is assumed by the lever ratio theory) but from the position along the tympanic membrane/malleus connection where the net force acts, then the lever ratio is predicted to be so close to unity as to be negligible (see Figure 6). Hence our model could not confirm the biomechanical theory that the middle-ear ossicles have a shape optimized to form a lever.

Figure 6. A finite element model was used to calculate the lever ratio as a function of frequency under two assumptions: the force was exerted at the umbo (center of the tympanic membrane) as assumed by the lever ratio theory (Figure 1) and when the force was assumed to be distributed along the length of the malleus–tympanic membrane connection.

Figure 7. A comparison of the impedance of a three-ossicle middle-ear and a single ossicle (columella) middle-ear. The three-ossicle system allows lower frequency hearing.

An alternative hypothesis is that the ossicles create a linkage between the tympanic membrane and the inner ear in the form of a spring. Comparing a columella (one ossicle; the columella is an extended stapes) with the ossicular chain (three ossicles) shows that better low frequency response is achieved with three ossicles as they create a lower stiffness connection than the one-ossicle columella, see Figure 7.

4.2. EVOLUTION OF THE MIDDLE EAR AND THE POSSIBILITY OF AN OPTIMUM

Aquatic animals crawled onto land some 410 million years ago. These ancient amphibians could only sense vibrations of the ground; they could hear seismic signals or possessed ‘hearing by body vibration’ [47] (as do extant newts and snakes). The stem reptiles evolved from these ancient amphibians (Figure 8) and, although it seems that some of them had an ‘ossicle’, they did not evolve ‘air-sensitive’ hearing. The stem reptiles evolved into the mammal-like reptiles and the Archosaurs (Dinosaurs formed part of this group). The Archosaurs evolved a tympanic middle-ear sensitive to airborne vibrations [48] which consisted of one ossicle and a tympanic membrane – it could have given them considerable advantage over the mammal-like reptiles with whom they coexisted. The dinosaurs, and their descendents (extant birds and crocodilians), maintained this single-ossicle middle ear. The three-ossicle middle-ear (assumed to be superior because it has a lever mechanism) evolved between the Therapsids and mammals of today (Figure 8), perhaps with the morganucodontids of the early Triassic about 210 mya [49].

Fleischer [50] proposed that the middle-ear in all extant mammals evolved from an ‘ancestral case’ where the malleus was U-shaped with one arm connected to the circumference of the tympanic membrane, followed by an intermediate case where the one arm of the U-shaped malleus became a ligament, to the present case where it has almost disappeared to form the anterior malleolar ligament. Concomitant with these adaptations, the axis of rotation of the ossicles changed. If the middle-ear bones fulfill the function of a lever then evolution for an improved lever-ratio should be expected from these adaptations. The likely position of the axis of rotation is shown in Figure 9 where no improvement in the lever ratio is evident. On the contrary, the studies of Fleischer [50] indicate that the center of mass is moving towards the axis of rotation, which would minimize the inertial forces generated during vibration rather than cause any increase in the lever ratio effect. Therefore the idea that the middle-ear is

Figure 8. An attempt to illustrate middle-ear evolution. The system is characterized by great variation.

evolving to a more optimal lever is not supported by phyletic analyses. What then might be the biomechanical driving force behind middle-ear evolution?

Given that the stem-reptiles had a middle-ear that included an ossicle but no tympanic membrane, as described above and noted in Figure 8, it might be proposed that the first ossicle evolved to provide an inertial mass relative to which body vibration could be sensed. Evolution in the late Archosaurs linked it to a membrane giving them, fortuitously, air-sensitive hearing. Because of its first purpose as an inertial mass, this ossicle would have been heavy allowing only low frequency hearing according to the well-known relation $\omega = \sqrt{k/m}$. Early mammals, being very small shrew-like animals [49], could not have 'fit' a large ossicle and

Figure 9. Radiation of the malleus-incus complex in mammals. (A) is the ancestral type; (B) is a transitional type; (C) is the 'freely-mobile' type found in man and other primates; (D) is a specialization of the ancestral type called the microtype (found in bats, shrews, and many rodents); (E) is a specialization of the transitional type found in Sirenia (Manatees, Dugongs). In each case the approximate center of mass and axis of rotation is shown. Adapted from Fleischer, *Adv. Anat. Embryol. Cell Biol.* 55 (1978) Fasc. 5.

therefore, it is proposed, developed low frequency hearing only by reducing the stiffness k of the ossicular chain by interposing two bones from the mandible to create a three-ossicle system. The external ear canal then may have evolved to provide high frequency hearing as the mammals got larger. (It is noteworthy that monotremes, who it is believed entered the mammalian state independently, have no pinna (fleshy part of the ear) [51] and differently shaped ossicles [50].)

That the mammalian three-ossicle plus external ear canal extends the frequency range of hearing to lower frequencies is shown in the predictions of the finite element model (Figure 7) – this is achieved, as already stated, only because the three bones constitute a not-so-stiff spring (like pressing the ends of a tweezers). If this is true, the evolutionary advantage of the three-ossicle system is the ability to hear lower frequencies while having light middle-ear bones-in this way mammals escaped the constraints of the single ossicle system of their therapsid ancestors (Figure 8). The conclusion is that the force magnification of a lever, if it exists at all (and our analyses indicates it does not), is only an accidental side-effect.

The abundance of 'degenerate' adaptations in the middle ear also supports the conclusion that optimizations are not achieved because of the great number of 'local-minima'. Two examples are the complete loss of the tympanic membrane and middle-ear in the reptile *Sphenodon* and the closure of the ear canal in aquatic mammals. These degenerations have served to adapt hearing to local circumstances from initial conditions of an air-sensitive middle-ear. The ears of whales seem to be efficient for underwater communication, but this efficiency has not been achieved by convergence to an optimum but instead it is driven by a rapid adaptation to environmental exigencies. If a particular middle-ear is examined, it cannot be assumed that it has had time to reach an optimum. We can be more certain that that adaptations have occurred as a series of adaptive steps each of which is carried out according to some mechano-regulation rule than we can be about reaching towards an optimum.

5. Discussion

The first sentence in this paper is a rather raw statement – a statement that the skeleton is a machine. Thoughts of this kind go back, one must presume, to antiquity, but their influential formulation by Descartes in his *Treatise on Man* brought the idea of ‘mechanism in the human body’ out into the Enlightenment. Some years later *On the Movement of Animals* by Borelli, the Neapolitan anatomist and mathematician and sometime student of Galileo, was published (posthumously and with a grant from the Queen of Sweden), and it marked the first application of modern statics to the understanding of biological function. Although the idea that the human body can be viewed as a machine is under continuous attack (e.g. [52]), it is an idea that has had great success in solving real problems. The Russian scientist Bernstein⁴ was, it seems, the first person to write a book explicitly about biomechanics [53] – it treated the subject of neuromuscular control of human motion [54]. Although his topic is very different from the one presented in this paper, it also applied ‘the principles of mechanics to the understanding of living systems’.

The world’s population has aged considerably since Bernstein’s book appeared in 1926; because of this the topic of the present paper has become an important one in biomechanics; if we are to maintain the functionality of the ageing musculo–skeletal system then we should develop a better understanding of the mechanobiology of skeletal function, adaptation, and degeneration. Comprehensive theories relating mechanical forces to biological behavior will underlie this understanding [55]. Such theories, to be useful, should be testable. One of the main reasons for applying advanced mechanics in biology is to more rigorously test theories by experiment and by computer simulation [56]. Computational mechanics, in particular, has great potential to create strong tests of causation in both orthopaedic [57] and cardiovascular [58] biomechanics.

5.1. OPTIMISATION (METHOD I) VERSUS MECHANO-REGULATION (METHOD II)

Method I is a problematic methodology as it invites hypotheses that often cannot be subjected to rigorous tests: one practical reason for this is that an assumption must be made about the initial state from which optimization occurs. For example, Alexander [59] explains the relatively inefficient swimming behavior of a squid as: ‘A squid clearly is not the best possible swimmer, but it may be close to the best than can be evolved from a mollusc ancestor’. How far back the phylogenetic tree should an ancestor be so that its descendent can be considered optimized with respect to it? (Computational analyses predicting optimization of, say, the femur suffer from an analogous difficulty because the assumed initial pattern largely determines the final outcome [60, 61].) On the subject of optimization, it is perhaps salutary to relate the story of a former Senior Fellow of Trinity College Dublin, a one Rev. Dr. Samuel Haughton. In the 1860s, he attempted to refute Darwin’s theory of natural selection by the argument that the musculo–skeletal structures of animals are optimized. In his view, if animals were optimized, then no mutation (he called it a ‘peculiarity’) could lead to an improved musculo–skeletal

⁴ Nikolai Alexandrovich Bernstein (1906–1966). His paper ‘Studies of the biomechanics of stroke by means of photo-registration’, *Journal of the Central Institute of Work (Moscow)* **1** (1923) 19–79, may be the first use of the word ‘biomechanics’ in its scientific sense. Pers. comm., Prof. Y. Nyashin, Perm, Russia, 21 July 2001.

structure – in other words, if it were true that animals were optimized then the driving force for natural selection should vanish [62]. So he set about proving optimization. He dissected many animals and, by various calculations, showed their muscles to be optimal [63] – and the more beautiful the animal (tigers and albatrosses were beautiful) the more optimized they were.

Method II is technically demanding because it requires the calculation of biophysical stimuli assumed to act at a cellular level. Iterative computations to simulate the time-course of mechanobiological processes in response to these stimuli also need to be set up. However, simulations provide better tests that could lead to theories of great predictive power. For example, with the simulations of fracture healing presented above, once the simulation begins there is no control of the process other than the hypothesized mechano-regulation rule. In studies of fracture healing, to test the hypothesis that ossification is driven by a combination of various invariants of the stress or strain tensors, analyses are carried out only at specific instances in the process – time is not explicitly included in these models. Although such approaches can serve to identify potential mechano-regulatory signals, simulations are stronger tests of the hypothesis that such signals can *regulate* the tissue differentiation process.

5.2. MECHANO-REGULATION RULES AND PHYLOGENESIS

This section addresses the issue of how mechano-regulatory rules can make themselves felt at a phylogenetic level. As yet it is not possible to give a general discussion of this problem except to emphasize that one constant factor in evolution is the physical laws of mechanics [64]. Carter *et al.* [65] have demonstrated that epigenetic mechanical effects operating on the evolutionarily acquired capability for endochondral ossification may account for morphological diversity in different taxa. What follows here is an attempt to describe how skeletal phylogenesis may be related to the mechano-regulation rules described in Sections 3.1 (bone remodeling) and 3.2 (tissue differentiation) and, conversely, how mechano-regulation rules may have constrained the phylogenetic change observed in the middle-ear.

5.2.1. *Damage-adaptive bone remodeling*

Bones sufficiently strong to prevent microdamage *could* have evolved; however such bones would be large and have a high energy cost in terms of maintenance and movement [1, 66]. The disadvantage of damage-adaptive bones is that a repair process is required to prevent damage from causing failure. Damage-adaptive mechano-regulation therefore ensures a balance between mechanical strength of a bone on the one hand and the energetic cost of maintaining and using it on the other. That this mechano-regulation rule is successfully incorporated into the genetic code is supported by the finding of bone repair processes in many species [67].

Recalling the bone remodeling rule depicted in Figure 2, if a certain population of animals begin to overload their skeletons because of new environmental circumstances or activity levels, then their bones will adapt ontogenetically, but only if their $\varepsilon_{\text{MDx-thresh}}$ is high enough. If the $\varepsilon_{\text{MDx-thresh}}$ is too low then microdamage will accumulate to cause fracture and the animal will likely die. Therefore some animals will survive the overload environment (because their $\varepsilon_{\text{MDx-thresh}}$ is high enough) – the skeletal shape of these animals will be the result of ontogenetic adaptation. A selection pressure will favor those members of the population with the appropriate mechano-regulatory rule – and the appropriate response to overload is not just to grow the largest possible bone but to grow an *appropriately* large bone (too large would be disadvantageous for the reasons give above). The result of this process will be a

skeletal change, a change which could not happen if it were not facilitated by the working of mechano-regulation during ontogeny.

A similar situation exists if environmental change caused a population of animals to underload their skeletons, as might have happened when mammals became aquatic. However, there is a difference with respect to the overload case. An asymmetry exists because underloading will not cause bone fracture in animals that have too low an $\varepsilon_{MDx-thresh}$ – therefore the selection pressure in underloading is only driven by the metabolic cost of maintenance and movement. Therefore a slower phyletic change is predicted when mechanical loading is reduced compared to when it is increased.

5.2.2. *Tissue differentiation*

In Section 3.2, it was concluded that the stiffening of the collagenous soft tissues should cause the magnitude of the biophysical stimuli to reduce if ossification is to eventually occur. Could the same mechano-regulatory rule have an influence in skeletal phylogeny? Animals develop muscles, tendons, ligaments, and bones simultaneously during growth. In the appendicular skeleton, cartilaginous rudiments differentiate to form bone. If this differentiation event is to proceed successfully then, according to the theory given in Section 3.2, motion-control must give way to force-control wherever bone formation occurs. This would suggest that endochondral ossification would not occur at sites where the deformations within the tissues caused by muscle contractions could not be reduced by mineralization. Such situations might arise in rudiments forced to stretch a given amount due to geometric factors, such as those tissues that later differentiate into ligaments of joints. In this respect it is important that muscle contractions do not create forces that are too large [68]. Perhaps it may be found that only certain mechanical combinations of tissues can evolve into mechanisms under this constraint.

5.2.3. *Mechano-regulation in middle-ear evolution*

The malleus in the mammalian middle-ear has evolved from the ‘U-shape’ of Figure 9(A) to the ‘hammer-shape’ of Figure 9(C). We could ask what mechano-regulation rule would have permitted such an adaptation. Ossification of the malleus begins *in utero* with the head ossifying first and the long process remaining cartilaginous until later [69]. At some step during evolution, the strain during growth would have to be kept sufficiently high that ossification in one of the long processes of the ‘ancestral’ malleus would not occur (Figure 9(B)). Perhaps this could be consequent on a developmental rearrangement. To make the step from A to B (see Figure 9); some animals would have had to evolve a situation that put the template soft tissue under higher strain during growth preventing ossification. There would be a selection pressure to maintain this because of the advantages for low frequency hearing (Figure 7).

6. Conclusions

1. In general terms, there are two methods for the mechanical analysis of skeleton; one attempts to discover mechano-regulation rules for the response of tissues to mechanical forces and the other attempts to discover how a particular skeletal part has achieved some kind of optimal performance.
2. Mechano-regulation rules for two mechanobiological processes in skeletal ontogeny are reviewed; the first concerns bone remodeling and the second concerns tissue differentiation. If the proposed mechano-regulation rules hold, then inferences about skeletal

phylogeny can be made. It is proposed that the biomechanical aspects of skeletal evolution can be further investigated in this manner.

3. Optimization of the middle-ear to form a lever is not confirmed. Instead it is proposed that the three-ossicle middle-ear is a spring mechanism. This can, perhaps, also be argued by examining middle-ear phylogeny. The evolution of the mammalian middle-ear is possibly consistent with proposed mechano-regulation rules for tissue differentiation.
4. It is interesting to speculate that the governance of skeletal adaptation to epigenetic mechanical factors in both ontogeny and phylogeny could be the result of a unified set of mechano-regulatory rules.

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