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Epigenetic mechanical factors in the evolution of long bone epiphyses

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In developing vertebrate long bones in which endochondral ossification occurs, it is preceded or accompanied by perichondral ossification. The speed and extent of perichondral apposition relative to endochondral ossification varies in different taxa. Perichondral ossification dominates early long bone development in extinct basal tetrapods and dinosaurs, extant bony fish, amphibians, and birds. In mammals and lizards, perichondral and endochondral ossification proceed more synchronously. One of the most important epigenetic factors in skeletogenesis is mechanical loading caused by muscle contractions which begin *in utero* or *in ovo*. It has been previously shown that the stress distributions created perinatally in the chondroepiphysis during human skeletal development can influence the appearance of secondary ossification centres. Using finite element computer models representing bones near birth or hatching, we demonstrate that in vertebrates in which perichondral ossification significantly precedes endochondral ossification, the distribution of mechanical stresses in the ossifying cartilage anlagen tends to inhibit the appearance of secondary ossification centres in the ends of long bones. In models representing vertebrates in which endochondral ossification keeps pace with perichondral apposition, the appearance of secondary centres is promoted. The appearance of secondary centres leads to the formation of bony epiphyses and growth plates, which are most common in mammals and extant lizards. We postulate that genotypic factors influencing the relative speed and extent of perichondral and endochondral ossification interact with mechanical epigenetic factors early in development to account for many of the morphological differences observed in vertebrate skeletons.

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INTRODUCTION

The evolution of bone tissue types

Genotypic variations in vertebrate cartilage both permit and constrain the range of skeletal features that can appear in the vertebrate skeleton (Moss & Moss-Salentijn, 1983). The skeletal features that actually do appear, however, are greatly influenced by epigenetic factors (Hall, 1992). One of the most important epigenetic factors in skeletogenesis is the local tissue stress state that is created by physical activity. To understand the possible phylogenetic reasons for differences in skeletal tissue response to mechanical stimuli, it is important first to understand the evolution and development of three distinct bone types: (1) dermal membranous bone, (2) perichondral/periosteal bone, and (3) endochondral bone.

Fossil evidence from the Late Cambrian and Early Ordovician periods of the Paleozoic Era has provided the basis for our understanding of the skeletal structure of the first known vertebrates, the Heterostracans and Osteostracans. The most striking feature of these fossils is the presence of an extensive external cranio-pharyngeal armour that, in different species, consisted of various forms of dermal bone, an acellular bone-like tissue called aspidin, and mineralized tissue similar to enamel and dentin (McLean & Urist, 1968; Repetski, 1978; Romer & Parsons, 1986; Smith & Hall, 1990; de Ricqlès, 1991). In contrast, the endoskeletons of these early vertebrates were either entirely cartilaginous or consisted only of a notochord (Fig. 1).

If the first major event in the evolution of bone was the appearance of dermal cranio-pharyngeal exoskeletal armour, the next was the ossification of the postcranial endoskeleton. Ultimately, this adaptation made it easier for the vertebrates to move out of the water and onto land, where they were exposed to much greater gravitational forces in the absence of a buoyant aqueous environment.

In the evolution of postcranial endoskeletal ossification, perichondral ossification is generally thought to have preceded endochondral ossification (Romer, 1964)

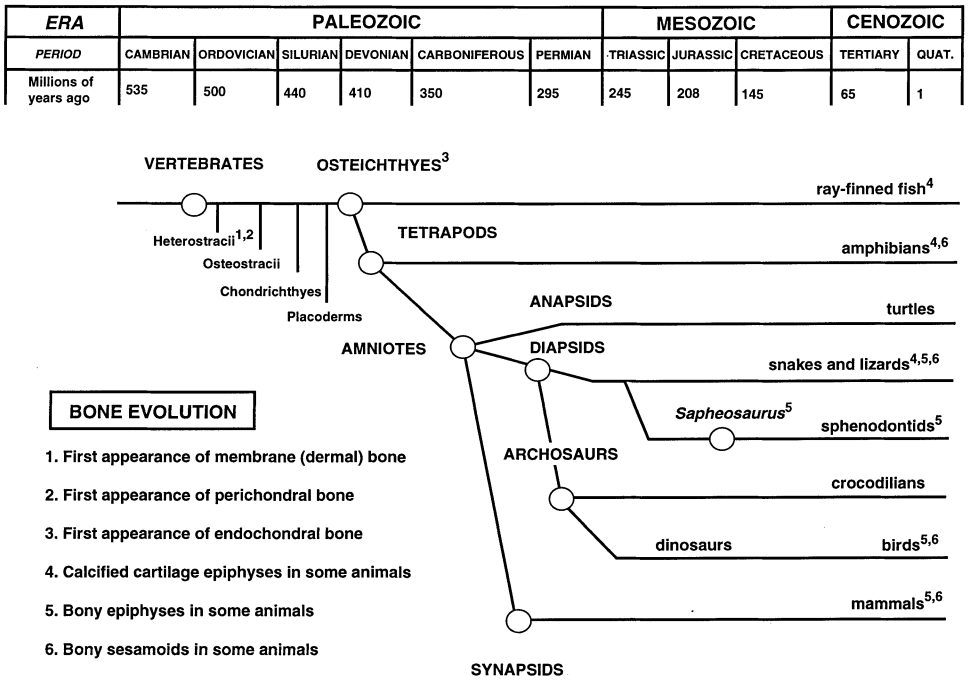

Figure 1. Cladogram depicting the evolution of bone tissue types. For phylogenetic details and chronology see *Tree of Life* (http://phylogeny.arizona.edu/tree/phylogeny.html). Note that only a very small sample of fossil and extant vertebrates has been studied to date, so the distribution of some character states may require adjustment in the future.

(Fig. 1). Perichondral ossification appears in some Heterostracan fossils of the Ordovician (Hall, 1975). Although evidence of endochondral ossification has been observed in some Placoderms of the Silurian, it did not become widespread until the Devonian Osteichthyes (bony fish) (Rosen *et al.*, 1981; Maisey, 1988) (Fig. 1).

By the time the first tetrapods appeared in the Devonian, perichondral bone apposition was established as the primary mechanism for forming the shafts of long bones, and the basic process of endochondral ossification was also present. These early tetrapods possessed rather heavy, well-ossified endoskeletons (Haines, 1938, 1942; Enlow, 1969; Radinsky, 1985; de Ricqlès, 1991; Kent, 1992). In addition, the dermal membrane bone armour had evolved in these creatures to a form of internal intramembraneous ossification that is crucial to the formation of the skull bones and shoulder girdle in most vertebrates (Smith & Hall, 1990).

We refer to the first tetrapods which emerged from the water during the Devonian and Carboniferous as basal tetrapods. These animals provide the phenotypic legacy of all extant terrestrial vertebrates as well as marine mammals and birds. Although the process of endochondral ossification was present in basal tetrapods, it later became more organized and efficient in some subgroups (lizards and mammals, for example), and secondarily lost in others (some frogs and other amphibians).

In contrast to the better developed endochondral ossification process of extant lizards and mammals, the long bones of basal tetrapods are thought to have developed like those of dinosaurs and extant crocodiles, turtles, and birds (Haines,

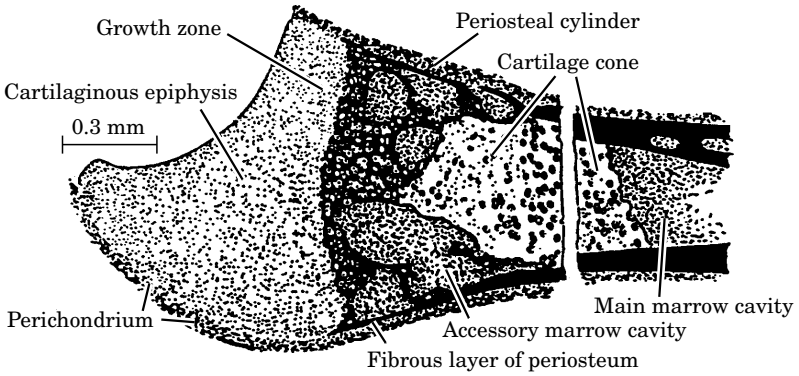

Figure 2. Proximal ulna of a hatchling turtle (3 cm carapace length) demonstrating cartilage cones which will disappear during subsequent growth, like those of all animals in which cartilage cones are formed (adapted from Haines, 1969, with the permission of Academic Press).

1938, 1942, 1969; Reid, 1984, 1996; de Ricqlès, 1991; Barreto *et al.*, 1993). During *in ovo* development of these animals, perichondral ossification advances toward the bone ends faster than endochondral ossification. Consequently, large areas of cartilage, referred to as cartilage cones, become temporarily encased within the shaft during early development (Fig. 2). During later development, erosion of this cartilage proceeds in a poorly organized manner as endochondral bone is deposited on the surfaces of the erosion bays. Because there is little secondary remodelling of the endochondrally derived cancellous bone in these animals, the trabecular bone orientation is primarily defined by the surface geometry of the cartilage erosion bays, even in the adult animal (Haines, 1938, 1942; Enlow, 1969). Some fragments of calcified cartilage may become isolated in the marrow. As the endochondral ossification front approaches the bone end, it becomes more organized with the formation of columns of proliferating chondrocytes. Endochondral ossification proceeds directly under the joint surface, leaving a rather compact layer of articular cartilage. Eventually, the fragments of calcified cartilage isolated in the marrow are completely eroded, and the only calcified cartilage remaining in the adult animal is directly beneath the articular surface. The joint surfaces are evenly curved and without complexity, and secondary ossific nuclei rarely form at the bone ends (Haines, 1942; 1969).

Bony epiphysis and sesamoid bone evolution

The first published fossil evidence of a secondary ossification centre in a long bone appears in the Jurassic rhynchocephalian *Sapheosaurus* (Haines, 1942) (Fig. 1). These centres probably developed in a manner similar to that of its living relative, *Sphenodon*, which most likely represents the most basal living vertebrate lineage possessing secondary centres (Haines, 1942). In *Sphenodon*, the long bones ossify like those of turtles and crocodiles. However, as the ossification front approaches the bone end, a region of calcified cartilage appears in the chondroepiphysis. This secondary calcified centre expands throughout the cartilage, forming a calcified cartilage epiphysis analogous to the bony epiphyses observed in mammals. In the

adult, the calcified cartilage is resorbed and replaced by marrow and cancellous bone.

Bony and calcified cartilage epiphyses evolved independently in many vertebrate lineages (e.g. some fishes, frogs, reptiles, birds, and mammals, Fig. 1). The coincidental appearance of sesamoid bones and secondary ossification centres suggests an evolutionarily acquired intrinsic capacity for endochondral ossification in many taxa (Haines, 1969), or a change of environmental conditions that promotes the expression of an existing capacity for this type of bone formation. Sesamoid bones tend to develop within tendons in areas that wrap around bony prominences and experience large hydrostatic compressive stresses (Giori *et al.*, 1993). Crocodiles, turtles, and dinosaurs have neither secondary ossification centres nor sesamoids. Cartilagenous and bony sesamoids are rare in amphibians but have been observed in some frogs (Nussbaum, 1982). In some extant and extinct amniotes (e.g. crocodiles and the mammal-relative *Dicynodon*), secondary centres of calcified cartilage (not bone) are found, and mineralized metaplastic tissue may also be present in areas where sesamoids would form (Haines, 1969). Many extant lizards form both bony epiphyses and sesamoids, although their formation is usually delayed by a period of diffuse cartilage calcification before ossification (Haines, 1969). Birds form sesamoid bones, and occasionally form secondary ossific centres (Haines, 1942). These observations suggest that relatively minor genotypic changes in vertebrate cartilage may be responsible for changes in the efficiency of endochondral ossification in different taxa (Haines, 1969). Indeed, recent discoveries of specific factors involved in the regulation of cartilage maturation (Indian hedgehog and parathyroid hormone-related protein and its receptor) present clear candidates that may regulate endochondral ossification and that may additionally influence the relative rates of endochondral and perichondral bone formation in different species (Lanske *et al.*, 1996; Vortkamp *et al.*, 1996).

Endochondral ossification in mammals

The role of endochondral ossification is most prominent in mammalian long bone development. Immediately after the first perichondral bone appears, the endochondral ossification of the cartilage core begins. Cancellous bone is immediately deposited and, over time, is remodelled as the marrow cavity is formed. A well-organized endochondral ossification front consisting of columns of proliferating chondrocytes is formed. Ossification proceeds within the cartilage toward the bone ends at roughly the same pace as the adjacent perichondral bone is deposited (Fig. 3). Cartilage cones do not appear. The cancellous bone that is formed is secondarily remodelled while it adjusts its porosity and trabecular orientation according to the local tissue stresses (Haines, 1942; Carter, 1987). As the ossification front approaches the bone ends, secondary ossification centres often appear in the chondroepiphyses. Local tissue stresses play an important role in the appearance of these centres (Carter *et al.*, 1987, 1991). In mammals, there are numerous secondary centres in the long bones, and sesamoid bones are frequently seen in tendinous regions exposed to high compressive stresses. Thus, in mammals, the evolution of endochondral ossification appears to be sufficiently refined that the formation of secondary centres and sesamoid bones is easily achieved. It is not known whether this is because mammals have evolved more efficient molecular biological factors, because their chondrocytes

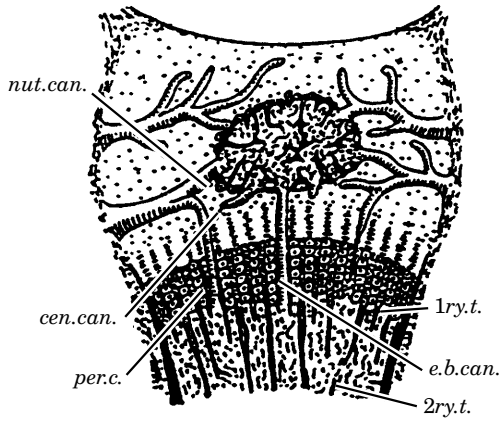

Figure 3. Schematic of a developing secondary centre in a eutherian mammal. Note the well-organized endochondral front that is at the same level as the perichondral front (adapted from Haines, 1942, with the permission of Cambridge University Press).

have evolved to be more sensitive to mechanical stimuli, or because of some as yet unrecognized combination of factors.

In mammals, the primary endochondral growth front is extremely well organized into very fine columns of proliferating and hypertrophying cells oriented in the direction of ossification (Fig. 3). At this front, each cell column is separated from its neighbor by a columnar resorption space in which a bony trabecula forms. All or nearly all of the cartilage matrix is resorbed, and a firm, well-ordered, interconnected cancellous bone framework is created directly under the ossification front (Haines, 1938, 1942; Barreto *et al.*, 1993). In non-mammalian species, the marrow erosion spaces in which bone is formed are often irregularly shaped and separated from adjacent spaces by several columns of cartilage cells that are not as well organized as those found in mammals (Haines, 1938, 1942; Reid, 1984, 1996; Barreto *et al.*, 1993). This histomorphologic arrangement leads to the formation of a coarse trabecular architecture in which remnants of calcified cartilage are sometimes observed (Fig. 2).

The poorly organized and inefficient endochondral ossification in many non-mammalian taxa is probably related to the prominence of perichondral ossification in long bone development (Haines, 1942; Barreto *et al.*, 1993). In early development, perichondral growth simply proceeds faster than endochondral ossification in these animals, unless long bone growth is relatively slow (as in lizards, for example). Later in development, however, perichondral ossification slows and endochondral ossification proceeds at a rate that overcomes this initial lag. As a result, in the older animals cartilage cones are no longer present. In the two juvenile crocodilians shown by Haines (1969), for example, endochondral ossification had progressed as far as perichondral ossification. In mammals, by contrast, during *in utero* development endochondral ossification proceeds with perichondral ossification so that cartilage cones are never present. At the perinatal stage of development secondary ossific centres often appear at the ends of long bones. The bone morphology and mechanical loading in have been shown to influence the presence or absence of these centres and also the timing of their appearance (Carter *et al.*, 1991).

Developmental mechanics in different taxa

As we have discussed, the skeletal features that appear in vertebrate long bones are greatly influenced by epigenetic factors such as mechanical loading during ontogeny. Carter *et al.* (1987) proposed that in human skeletogenesis, the endochondral cartilage normally tends to undergo a process of tissue growth and ossification. Local intermittent tissue stresses caused by physical activity can influence the speed at which this process occurs throughout the skeleton. Specifically, it is thought that regions that experience high intermittent shear stresses (stresses that induce distortion of the cells) will tend to have accelerated growth and ossification. Areas in which large intermittent hydrostatic pressures are imposed (i.e. regions where the stresses maintain the spherical cell shape) will tend to have reduced growth rates and maintain the cartilage phenotype (Carter *et al.,* 1987). These rather simple mechanical cues influence the geometry and velocity of the ossification fronts, determine the appearance of secondary ossification centres, and the establishment of the thickness of articular cartilage at the bone ends (Carter, 1987; Carter & Wong, 1988; Carter *et al.*, 1991). The mechanical epigenetic cues that influence human endochondral ossification rates probably also influence the process of cartilage growth, cartilage calcification, and endochondral ossification in other taxa, although the response of the skeleton to these cues may not necessarily be the same across all taxa.

We postulate that differences in the relative rates of perichondral and endochondral ossification in different animals cause important differences in the bone morphology, and therefore in the distribution of cartilage stresses during the critical time in development when secondary centres would be formed. The formation or failure of formation of these centres determines whether a true growth plate will develop and thus influences bone formation throughout the rest of ontogeny. To investigate this hypothesis, we performed finite element computer analyses of basal tetrapod and mammalian bones using models of bone and cartilage morphology at the time of birth or hatching. The two models that were analysed represent the long bones of vertebrates that form (1) cartilage cones with little cancellous bone (basal tetrapods, crocodiles, turtles, dinosaurs, and birds) (Fig. 2), and (2) well-ossified cancellous bone that keeps pace with perichondral bone development (mammals) (Fig. 3). In discussing these analyses, the term 'basal tetrapod model' will henceforth be used to represent all vertebrates (both extant and extinct) that retain their basal-tetrapod-like bone growth and ossification characteristics (e.g. crocodiles, turtles, birds and dinosaurs, but not lizards). By examining the cartilage stresses in the basal tetrapod and mammal models, we can assess the potential interaction of genetic and epigenetic mechanical factors on the appearance of secondary centres of calcification and ossification.

METHODS

Model geometry

Two two-dimensional plane strain finite element models were constructed representing comparable developmental stages in basal tetrapods (Fig. 4, left) and

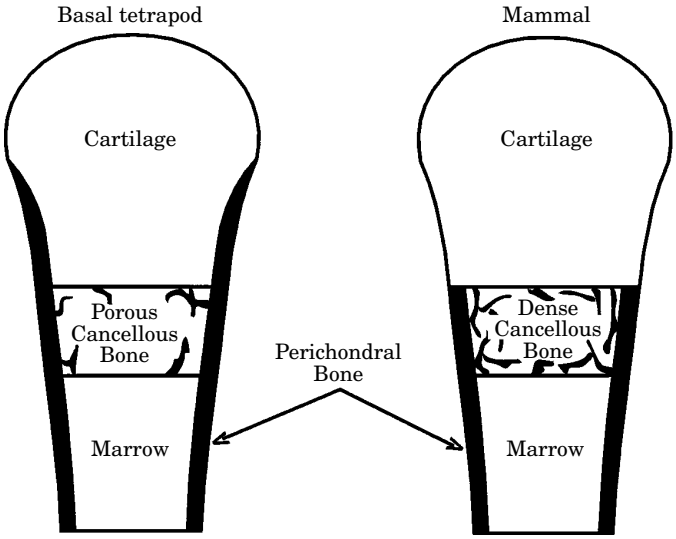

Figure 4. Material regions of basal tetrapod and mammalian models. The basal tetrapod model represents both extinct and extant vertebrates whose bones form in a similar manner (e.g. crocodiles, turtles, dinosaurs, and birds).

mammals (Fig. 4, right). Recognizing the wide range of bone size and morphology within as well as among the different taxa represented by these two models, we chose not to model specific long bones in these animals, but rather to model a generalized long bone. Model geometry and load cases were kept constant between the two models to isolate the effects of two parameters: (1) the compliance (or stiffness) of the endochondral bone supporting the chondroepiphysis, and (2) the presence or absence of cartilage cones.

The geometry of the models used in this analysis is similar to the proximal portion of the human humerus at 190 mm crown-rump length with a length/diameter ratio of 3.5 (Haines, 1947; Klein-Nuelend *et al.*, 1986; Carter & Wong, 1988). The width of the cortical bone collar was taken as one-sixth the diaphyseal radius, as measured from slide preparations in Haines (1947). Note that the models do not represent one half of the bone rudiment, but only the end of the long bone. The base and height of each model were 25 mm and 87 mm, respectively, and each model consisted of 3682 plane strain quadrilateral elements (Fig. 5).

Material properties

Both models consisted of four distinct material regions (Fig. 4). As a first approximation, all four tissues were assumed to be single-phase, linearly elastic, homogeneous, and isotropic. The top third was modeled as cartilage with a Poisson‘s ratio of 0.47 and a shear modulus of 2.04 MPa, yielding an elastic modulus of 6.0 MPa. The middle third directly beneath the chondroepiphysis was modelled as endochondral bone. In the mammalian model, a Poisson‘s ratio of 0.20 and a shear modulus of 208 MPa were chosen, yielding an elastic modulus of 500 MPa. These properties correspond to an apparent bone density of 0.57 g/cc and are consistent

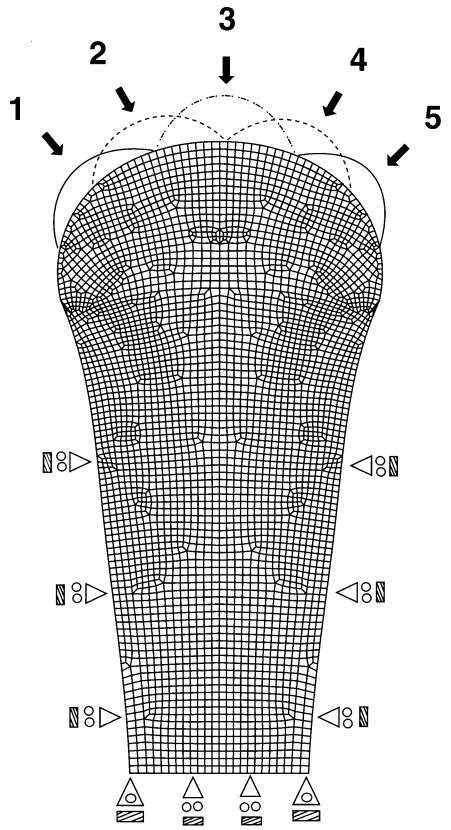

Figure 5. Plane strain finite element mesh with five load cases.

with values for newly mineralized endochondral mouse bone (Huiskes *et al.*, 1995). In the basal tetrapod model, the middle third was modeled as a homogeneous mix of calcified cartilage and endochondral bone with an apparent density of 0.24 g/cc, corresponding to a Poisson's ratio of 0.14 and a shear modulus of 26 MPa, yielding an elastic modulus of 60 MPa (an order of magnitude less than mammalian endochondral bone as observed by Mente & Lewis (1994)).

In both models, the bottom third of the shaft was modeled as empty marrow space with a Poisson's ratio of zero, a shear modulus of 500 Pa, and an elastic modulus of 1 kPa. The perichondral bone sleeve was modelled as new cortical bone with a Poisson's ratio of 0.40 and a shear modulus of 1786 MPa, yielding an elastic modulus of 5000 MPa as extrapolated from the data of Currey and Butler (1975). In the mammalian model, the endochondral and perichondral fronts were at the same level, whereas in the basal tetrapod model, the perichondral bone collar extended beyond the endochondral front to create a cartilage cone in the chondroepiphysis (Fig. 4).

Boundary conditions

Because the models are two-dimensional representations of a three-dimensional structure, additional constraints were imposed along the outer edges of the peri-

chondral sleeve to capture the out of plane stiffness of the cortices, preventing motion transverse to the long axis of the bone. To validate the plane strain models, we constructed a series of axisymmetric models and found the axisymmetric results to be similar to the results of the plane strain analyses.

Loading conditions

Quantifying the muscular activity of any animal *in ovo* or *in utero* presents a difficult problem and has yet to be achieved experimentally. Both before and shortly after birth, the loading history is determined largely by the muscular contractions and physical activity of the animal. *In ovo* and *in utero*, involuntary muscle contractions of the embryo create forces in muscles which cross joints. Regardless of the orientation of the limbs, these muscle forces result in compressive joint forces which are directed primarily down the shafts of the developing bone rudiments, although some differences in force orientations may exist. After birth or hatching, the magnitudes of the forces may increase. It is worth noting, however, that regardless of the posture of the animal or the orientation of the bones after birth, joint forces remain primarily directed along the shaft of the bone since they are created by the force vectors of the muscles which cross the joint. In our analysis, therefore,the loading history is characterized by five joint contact pressure distributions, corresponding to five different joint forces which could be created with the limb in different orientations (Fig. 5). This basic pattern of joint force direction is applicable, in the first approximation, to joints *in ovo* or *in utero*. It also applies after birth in tetrapods that stand erect, in which the proximal limb bones are held parasagittally, and in sprawlers, in which the same bones are often held horizontally or sloped upward distally.

Experimental work on fatigue of articular cartilage in shear indicates that fatigue damage begins to occur as shear strains approach 10% (Simon *et al.*, 1989). Consequently, we chose the magnitudes of the assumed joint contact pressures to maintain a maximum shear strain in the cartilage of the mammalian model at or below 6%. In the plane strain models, the axial loading condition (load case 3) consisted of a quadratically applied pressure distribution of 300 kPa across 18 elements (Fig. 5). Load cases 2 and 4 were 75% of load case 3 (275 kPa) applied over 19 elements, and load cases 1 and 5 were 50% of load case 3 (150 kPa) applied over 22 elements.

Analysis

The stress state for each of the five load cases was analysed in the chondroepiphysis of each model using the pre-and postprocessing capabilities and finite element solver of IDEAS (SDRC, Milford, OH). The cartilage theory of Carter and Wong (1988) states that intermittent shear stresses accelerate cartilage maturation and ossification, while intermittent compressive hydrostatic stresses inhibit this process. To assess the

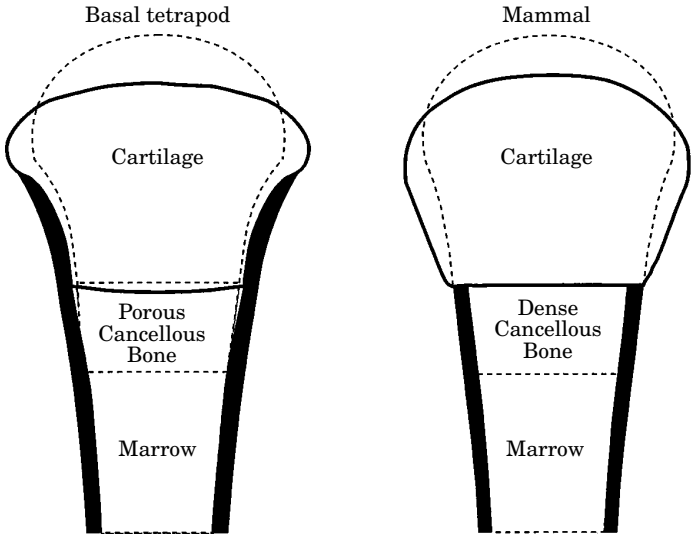

Figure 6. Magnified deformations of a centrally applied joint force in the basal tetrapod and mammal models under identical loading conditions.

likelihood of ossification, a parameter called the osteogenic index can be calculated as

$$I = \sum_{i=1}^{5} [S_i + kD_i], \tag{1}$$

where i indicates a specific loading case, S is the peak cyclic octahedral shear stress (which always has a positive value), D is the peak hydrostatic stress (compression has a negative value and tension a positive value), and k is an empirical constant taken as 0.5 in previous analyses (Carter & Wong, 1988). High values of I indicate a greater likelihood of ossification while lower values of I indicate regions of cartilage maintenance.

RESULTS

The magnified deformations predicted from loading the models with a centrally applied joint force (load case three) demonstrate the fundamental differences in cartilage deformation at the ends of the basal tetrapod and mammal models (Fig. 6). The poor mechanical support under the cartilage of the basal tetrapod model allows the chondroepiphysis to be pushed into the sleeve of surrounding perichondral bone. The cartilage is squeezed into the diaphysis and is compressed like a cork being pressed into a bottle. The magnified deformation plot of the mammal model, however, is quite different. Since the chondroepiphysis is mechanically supported by dense bone and is not surrounded by a diaphyseal sleeve, the cartilage tends to bulge outward and is not squeezed into the diaphysis.

The differences in these predicted cartilage deformations directly correspond to

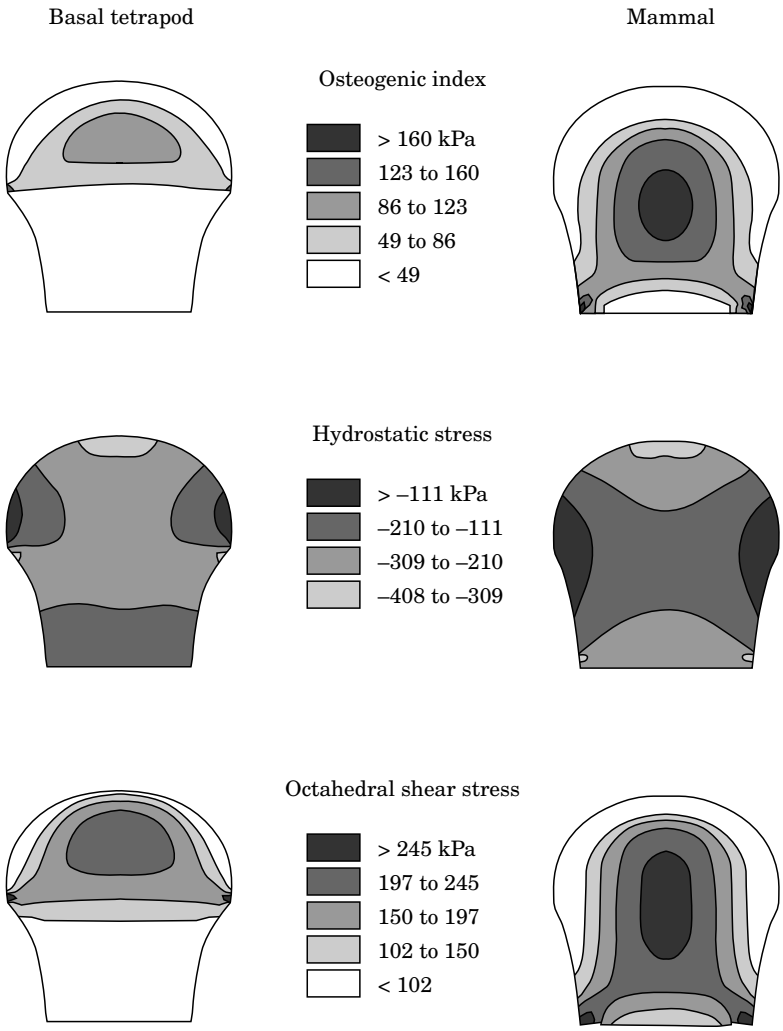

Figure 7. Hydrostatic stress, shear stress, and osteogenic index contour plots for the cartilage of the basal tetrapod (left) and mammal (right). Endochondral ossification is promoted in areas of high osteogenic index.

the differences in the hydrostatic stresses, shear stresses and osteogenic indices for all five load cases combined (Fig. 7). In the mammal model, the unconstrained chondroepiphysis is free to bulge radially under loading (Fig. 6). This lateral expansion causes high shear stresses, which promote ossification in the centre of the chondroepiphysis (Fig. 7). Especially high compressive hydrostatic stresses (negative is compression, positive is tension) are produced at the articular surface and in the presumptive growth plate area. The osteogenic index plot summarizes these data by predicting that there is a tendency in the mammal model for the secondary ossific nucleus to form in the centre of the chondroepiphysis while ossification is inhibited in the articular cartilage and growth plate regions.

In the basal tetrapod model, however, the more compliant endochondral bone

as well as the constraints of the perichondral envelope produce stresses that do not necessarily lead us to predict the formation of a distinct secondary ossific nucleus (Figs 6, 7). Although the region of highest osteogenic index in the basal tetrapod model does lie in the chondroepiphysis, it is more diffuse, closer to the joint surface, and has a magnitude approximately 50% lower than the maximum osteogenic index predicted for the mammal. While most bird long bones do not form secondary centres, when they do form, they are often found more superficially in the chondroepiphysis than are mammalian centres, with a shape that reflects the morphology of the joint surface, as our model predicts.

Since chondrodystrophic birds produce numerous secondary centres, Haines (1942) proposed that "the mechanism for the development of these centres must be present in birds, though it usually remains latent". He speculated that the ability to form secondary centres was secondarily lost in birds. Another perspective, based on our computer results, is that although birds have acquired the genetic machinery to form centres, the bone growth dynamics and morphology in normal birds around the time of hatching does not result in stress states in the developing cartilage which are favourable to their formation.

DISCUSSION

By performing finite element analyses on models of the developing chondroepiphyses of a generalized basal tetrapod and mammal, we examined the cartilage stress distributions caused by the presence or absence of cartilage cones and compliant *vs.* dense supporting endochondral bone. These different 'boundary conditions' created during morphogenesis in mammals and basal tetrapods/basal-tetrapod-like vertebrates result in a stress state that promotes the formation of a secondary ossific nucleus in the mammal, but not in the basal tetrapod/basal-tetrapod-like vertebrate. These results suggest that underlying genetic differences in the relative rates of endochondral and perichondral bone formation in different lineages result in altered cartilage stresses and that, consequently, the presence or absence of secondary sites of ossification in different lineages is most likely due to a combination of both genetic and epigenetic mechanical factors.

Numerous genetic and epigenetic factors most likely help to establish the extent and relative rates of endochondral and perichondral bone formation in different taxa. The recent discovery that Indian hedgehog and parathyroid hormone-related protein act in concert to slow the endochondral process of cartilage maturation suggests that these factors may reflect important intrinsic molecules modulating both the rates and efficiency of long bone ossification processes in vertebrates (Lanske *et al,*. 1996; Vortkamp *et al.,* 1996).

It is important to emphasize that these models isolate the effect of differences in the relative rates of endochondral and perichondral ossification, irrespective of the absolute rates of these processes and how they compare among taxa. In fact, the same structure can be caused by several combinations of absolute rates of ossification that give rise to similar relative rates. For example, birds show extremely rapid perichondral ossification and slightly less rapid endochondral ossification, whereas crocodiles have perichondral growth that proceeds at a moderate rate and endochondral growth that proceeds slowly. Nevertheless, these two taxa possess the

similar morphological feature of cartilage cones because the relative rates of these two growth processes are comparable. Similarly, mammalian bones can show a range of absolute rates of perichondral and endochondral ossification, but in all cases, these processes keep pace with one another as ossification toward the bone ends proceeds. Lizards have extremely slow bone growth, and their metabolic rates are generally lower than those of mammals, but because there is little difference in their relative rates of endochondral and perichondral bone formation, lizards and mammals develop similar chondroepiphyseal morphologies.

A limitation of these models is that they represent a single snapshot in time of the extremely time-dependent process of cartilage maturation and ossification. This process is not only influenced by the stresses from a single day's activity, but from the entire stress history applied to a particular joint over the lifetime of the animal. Despite these limitations, as a first examination into the various factors influencing phylogenetic differences in secondary ossific nucleus formation, these models provide a new approach to understanding possible interaction of genetic and epigenetic factors that result in the observed phylogenetic differences in ossification patterns.

In addition to differences in relative ossification rates, we have noted that basal tetrapods, dinosaurs, crocodiles, birds, and turtles exhibit less efficient endochondral bone formation than mammals. Further, secondary ossific nucleus formation and sesamoid bone formation, as well as fracture healing response, are all associated with the differentiation of connective tissue in response to particular mechanical stresses (Carter, 1987; Giori *et al.*, 1993). Not only are sesamoids and secondary bone centres often absent or calcified (rather than ossified) in the vertebrates mentioned above, but fracture healing response (speed and size of callus formation, as well as the efficiency of callus replacement by bone) differs among species (Pritchard & Ruzicka, 1950). Because it is known that endochondral ossification is sensitive to mechanical loading environment, it is intriguing to hypothesize that chondrocyte sensitivity to mechanical stimuli may depend on phylogenetic legacy. In addition to variations in bone biology that may exist among vertebrates, different epigenetic cues such as mechanical loading environment or differences in sensitivity to mechanical stimuli may affect the relative rates of bone formation in these different taxa.

Regardless of the cause, variations in the relative rates of perichondral and endochondral ossification create different 'boundary conditions', such as the presence or absence of cartilage cones. Under comparable loading conditions, these different boundary morphologies result in quite different stress states within the cartilage, and these stresses, in turn, influence the patterns of secondary bone formation in the chondroepiphysis. In mammals, the spatial stress distribution in the chondroepiphysis promotes the formation of a secondary center, whereas in basal tetrapods (and extant vertebrates such as crocodiles and birds), a distinct secondary centre is not predicted. We conclude that, to fully explain the observed variations in patterns of bone formation among different animal groups, it is not sufficient only to consider genetic/phylogenetic factors. In addition to inherited biologic differences among taxa, it is also necessary to consider how the genetic factors that influence the relative speed and extent of perichondral and endochondral ossification interact with epigenetic factors induced by the local mechanical environment. Only by considering both genetic and epigenetic mechanical factors in skeletogenesis can a complete understanding of different ossification patterns be obtained. As we learn more about the phylogenetic distribution of cartilage cones and secondary ossification

centres among fossil tetrapods, we will be able to work toward a clearer evolutionary explanation of the origin of these structures in various vertebrate lineages.

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