Bone microstructure and growth patterns of early mammals

ANUSUYA CHINSAMY and JØRN H. HURUM


We present results of the first studies of the bone microstructure of early mammals, based on the Early Jurassic *Morganucodon*, the Late Cretaceous multituberculates, *Kryptobaatar* and *Nemegtbaatar*, and the Late Cretaceous eutherians *Zalambdalestes* and *Barunlestes*. Our results show that the two eutherian taxa grew relatively slowly with periodic pauses in growth indicated by the presence of rest lines, while the multituberculates and *Morganucodon* had a faster rate of bone formation that suggests an overall rapid growth rate that slowed down later in ontogeny. Comparisons of the early mammalian bone microstructure with that of non-mammalian cynodonts, extant monotremes, and placentalts are also made, and significant differences in the rate of osteogenesis in the various groups are documented. Our findings suggest differences in the growth rate between the multituberculates and the Mesozoic eutherians, and moreover, both groups appear to have slower growth rates as compared to modern monotremes and placentals. Our results further suggest that the determinate growth strategy typical of extant mammals evolved early in the evolution of the non-mammalian therapsids. We speculate that the sustained, uninterrupted bone formation among the multituberculates may have been an adaptive attribute prior to the K-T event, but that the flexible growth strategy of the early eutherians was more advantageous thereafter.

Key words: Mammalia, Morganucodonta, Multituberculata, Eutheria, Cynodontia, bone microstructure, growth rate, Jurassic, Cretaceous.

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Introduction

A notable bias in the fossil record is that few Mesozoic mammalian taxa are represented by skulls and postcrania. As a consequence, much of the early evolutionary history of mammals presents an enigma since most of what is known is limited largely to tooth and jaw morphology (Lillegraven et al. 1979; Kielan-Jaworowska et al. 2004). Within these limitations, the phylogeny of early mammals is reasonably well understood (Luo et al. 2002). In contrast, however, the biology of Mesozoic mammals is poorly known (e.g., Kielan-Jaworowska 1970, 1979; Jenkins 1990; Novacek et al. 1997). Furthermore, although the morphological evolution of mammals from the non-mammalian cynodonts is reasonably well-documented, the physiological changes associated with this transition remain more elusive. For example, exactly when endothermy evolved in the mammalian lineage is uncertain, although there have been several suggestions that it evolved among the cynodont ancestors of mammals (e.g., Kemp 1982). Indeed, evidence for respiratory turbinals in the nasal cavities of Late Permian nonmammalian therapsids has been documented, and may be indicative of high ventilatory rates associated with endothermy in these taxa (Hillenius 1994).

Although bone microstructure has been studied to assess growth patterns in a diverse range of extinct vertebrates (e.g., Chinsamy 1997, 2002; Ricqlès et al. 2000; Sander 2000; Erickson et al. 2004; Ray and Chinsamy 2004), the microscopic structure of Mesozoic mammals has remained undocumented. The reason for this is probably the lack of adequate materials since Mesozoic mammals (except for those from the Late Cretaceous of the Gobi Desert and Early Cretaceous of China) are generally known only from teeth or fragments of jaws with teeth. The current study examines the bone microstructure of five early mammalian taxa, *Morganucodon*, two multituberculates (*Kryptobaatar* and *Nemegtbaatar*), and two eutherians (*Barunlestes* and *Zalambdalestes*) (see Kielan-Jaworowska et al. 2000, and Kielan-Jaworowska et al. 2004 for recent reviews), to deduce aspects of their growth and biology. The bone histology of these early mammals is compared with previous studies of nonmammalian cynodonts (e.g., Ricqlès 1969; Botha and Chinsamy 2000; Ray et al. 2004), as well as with a range of extant and extinct mammals (Enlow and Brown 1958; Klevezal and Kleinenberg 1969). For comparative purposes the bone microstructure of the Early Jurassic pleurodont lepidosaur, *Gephyrosaurus*, the nonmammalian cynodont, *Tritylodon*, an extant monotreme, *Ornithorhyn...
chus, and the extant eutherian Heterocephalus is also described. The cladogram (Fig. 1) illustrates the postulated phylogenetic relationship of the taxa studied.

Institutional abbreviations.—BPI, Bernard Price Institute for Palaeontological Research, University of the Witwatersrand, Johannesburg; UCL.ADB, University College, Department of Anatomy and Developmental Biology, London, United Kingdom; ZOO/UCT, University of Cape Town, Department of Zoology, Cape Town, South Africa; ZPAL, Institute of Paleobiology, Polish Academy of Sciences, Warsaw, Poland.

Other abbreviations.—ICL, inner (endosteal) circumferential layer; LAG, Line of arrested growth; OCL, outer circumferential layer; RBT, Relative bone wall thickness; t, average bone wall thickness; D, average cross-sectional diameter.

Materials and methods

The earliest mammals appeared in the Late Triassic, but fossils of these are fairly incomplete. Adelobasileus (Lucas and Luo 1993) and Sinoconodon (Crompton and Luo 1993; Zhang et al. 1998) are recognized today as the most basal mammals. However, the first well-represented early mammals in the fossil record are the Morganucodontidae (Kielan-Jaworowska et al. 2004, but see McKenna and Bell 1997 for an alternative interpretation), which are known from Late Triassic to Early Jurassic sites worldwide. The Morganucodontidae were relatively small, shrew-like animals, having adult skull lengths measuring about 20–30 mm and total adult body lengths of about 150 mm (Kermack et al. 1973, 1981; Jenkins and Parrington 1976; Crompton and Luo 1993). Their relatively small body size and the presence of sharp, tricuspid teeth are suggestive of an insectivorous diet. Various unnumbered skeletal elements from UCL.ADB of Morganucodon watsoni Kühne, 1949 (sometimes also referred to as Eozostrodon; see Clemens 1979) from the Early Jurassic fissure fillings of the Pant Quarry, Wales, were sectioned in this study.

The multituberculates, which are rodent-like in external appearance, are the largest group of Mesozoic mammals, and begin to appear in the fossil record from about the Middle Jurassic (Kielan-Jaworowska and Hurum 2001). They are, however, best known from the Late Cretaceous and Early Tertiary of North America and Central Asia, though recent discoveries have extended their range to Africa and possibly South America (see Kielan-Jaworowska et al. 2004 for review). In this study, two femora of Late Cretaceous multituberculates, Kryptobaatar dashzevegi Kielan-Jaworowska, 1970 (ZPAL MgM-I/18) and Nemegtbaatar gobiensis Kielan-Jaworowska, 1974 (ZPAL MgM-I/81), from the Gobi Desert, Mongolia, were taken from diagnostic articulated specimens. The remains of Kryptobaatar were recovered from the Djadokhta Formation (?lower Campanian), while that of Nemegtbaatar are from the Baruungoyot Formation (?upper Campanian) (Kielan-Jaworowska et al. 2003).

In addition to these Gobi multituberculates, two eutherian species Barunlestes butleri Kielan-Jaworowska, 1975 (ZPAL MgM-I/77) from the Barungoyot Formation and Zalambdalestes lechei Gregory and Simpson, 1926 (ZPAL MgM-I/43) from the Djadokhta Formation were also studied. Both are placed in the family Zalambdalestidae. Mongolia is distinctly recognized for some uniquely complete eutherian specimens, such as Zalambdalestes, which has been reconstructed as a specialized jumping, hedgehog sized animal, with a long snout. Barunlestes is of approximately the same size but is more heavily built, with a more robust skull. A femur of Zalambdalestes, and a rib and femur of Barunlestes from diagnostic postcranial skeletons were thin sectioned in this analysis. Given that Mesozoic mammals are so rare, this small sample of Barunlestes also provided a rare opportunity to assess histological variation of two skeletal elements in a single individual.

Although detailed comparisons are made with the published literature on the bone microstructure of nonmammalian therapsids (e.g., Ricqlès 1969; Botha and Chinsamy 2000; Ray et al. 2004; Botha and Chinsamy 2004) to facilitate direct comparisons, we included in the current study a humerus (BPI/5671) and radius (BPI/5167) of the small non-mammalian cynodont, Triyloodon from the Early Jurassic Massospondylus range zone of the Elliot Formation, South Africa.

Several bones of the extinct pleurodont lepidosaur Gephyrosaurus sp. (UCL.ADB no number) (which probably looked like the small, heavily-built extant tuatara, Sphenodon) were also included in the current study. Gephyrosaurus sp. was specifically selected because it was contemporaneous with Morganucodon, and the studied bones were also re-

![Fig. 1. Cladogram showing the phylogenetic relationship of the taxa studied (simplified from Kielan-Jaworowska et al. 2004).](image-url)
covered from the Early Jurassic fissure fillings of the Pant Quarry in Wales. Indeed, since the animals are also similar-sized, several of the bones received were not positively assigned to either *Morganucodon* or *Gephyrosaurus* and were therefore labeled ambiguously.

The bone microstructure of the extinct mammals was compared with published histological descriptions of mammalian taxa. In particular, comparisons were made with that of the medium size, arboreal, Paleocene multituberculate, *Ptilodus* (Enlow and Brown 1958), as well as with a range of similar, small-sized extinct and extant mammalian taxa (Enlow and Brown 1958; Klevelzal and Kleinenberg 1969). Again, to enable direct comparisons we included in our analyses, femora of an extant monotreme, the duck-billed platypus, *Ornithorhynchus anatinus* Shaw, 1799 (ZOO/UCT no number), and the small eutherian, the naked mole-rat, *Heterocephalus glaber* Rüppell, 1842 (ZOO/UCT no number) (Rodentia, Bathyergidae). A rib and femur of *Ornithorhynchus* have previously been described by Enlow and Brown (1958), but the bone microstructure of *Heterocephalus glaber* has not been described previously. This rather atypical modern eutherian taxon was selected specifically because of its unusual life style and small size. As far as possible, long bones, particularly femora and humeri, were selected for histological study since these generally preserve the best ontogenetic record of osteogenesis, particularly when sectioned in the midshaft region of the bone (Chinsamy and Dodson 1995; Chinsamy 2005). Furthermore, assessment of variation in the bone microstructure of various skeletal elements of nonmammalian synapsids (e.g., Ray and Chinsamy 2004; Ray et al. 2004) agrees with experimental findings that long bones, such as humeri and femora, have the fastest bone depositional rates in a skeleton (e.g., Starck and Chinsamy 2002). The histology of these elements in extinct animals therefore provides a reliable indication of growth during life. In the current study, other skeletal elements were also included when available (see Table 1).

Thin sections were prepared according to the methodology outlined in Chinsamy and Raath (1992). Because of the small size of the early mammal bone samples, and the rarity of the material, the histological sections were manually ground down on frosted sheets of glass with progressively finer grit of carborundum powder. Final polishing was done using a velvet polishing cloth and a diamond polishing suspension (Struers OP-U).

The relative bone wall thickness (RBT) of each of the thin sections is the ratio of average bone wall thickness, \( t \), to the average cross-sectional diameter of the bone, \( D \), expressed as a percentage, i.e. \( \% \text{ RBT} = t/D \times 100 \) (Bühler 1986).

http://app.pan.pl/acta51/app51-325.pdf

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1 The red beds cropping out at the locality Hermiin Tsav, are regarded on the basis of the fauna as equivalent of the Barunungoyot Formation. Here we refer to them as Barunungoyot Formation for the sake of brevity.
Results

Tritylodontian

*Tritylon sp.*—The *Tritylon* humeral section depicted in Fig. 2 shows a thick cortical bone wall consisting of fibro-lamellar bone with abundant primary and secondary osteons distributed in the cortex. A cross section of a radius shows a similar bone tissue (Fig. 2B), but here a change in bone depositional rate is observed in the peripheral region, where lamellar bone with several closely spaced rest lines occurs.

Lepidosaurian

*Gephyrosaurus sp.* (Fig. 3A).—Like the *Morganucodon* samples, several sections of *Gephyrosaurus* bones showed diagenetic alteration. However, there were some sections in which the histology could be clearly assessed. Thin sections of the femur revealed a more or less elliptical cross section with an average bone wall thickness of about 79 microns (RBT of 20%). The compacta consists of essentially parallel-fibred bone tissue interrupted by several lines of arrested growth (LAGs). Several other sections also showed distinct LAGs. A maximum of nine LAGs were recorded in a transverse section of a femur (ZOO/UCT/196). The first LAG visible from the medullary cavity appears to be a hatching line (e.g., Castanet and Baez 1991) since the bone formed earlier seems to be “embryonic bone” with its more haphazardly oriented, globular shaped, osteocyte lacunae. Following the hatching line is a wide region of parallel fibred bone tissue, and then a series of fairly closely spaced LAGs. The next five LAGs are quite closely spaced, while the subsequent three are more widely spaced i.e., wider zonal regions are present.

Morganucodontan

*Morganucodon watsoni* (Fig. 3B, C).—The majority of the *Morganucodon* bone sections examined showed extensive diagenetic alteration. Only a few sections preserved microstructural details. Of these, an ulna of *Morganucodon* showed distinct woven bone tissue with large, irregularly oriented osteocyte lacunae and several primary osteons (Fig. 3B1). No secondary osteons were visible, though several enlarged erosion cavities are evident in the compacta (Fig. 3B2). In the same section, it appears that substantial endosteal resorption had occurred (Fig. 3B1), and parallel-fibred bone tissue is evident only in a localized area peripherally. This area includes several rest lines, which indicate pauses in the rate of bone formation, and hence, pauses in growth. In a humeral section (not figured) a distinct region of parallel fibred bone tissue is also preserved, though no assessment of earlier bone tissue can be made. Bone wall thickness can be measured in a number of the skeletal elements, and for comparison with other specimens sectioned, the RBT of the femur measured 26%. In the femur, although microstructural details of the mid-cortical area are not discernible because of diagenesis, a fairly thick layer of endosteal bone is visible around the medullary cavity (Fig. 3C).

Monotreme

*Onithorhynchus anatinus* (Fig. 4).—Transverse thin sections of a femur of the extant monotreme *Onithorhynchus* reveal a fairly thick, richly vascularized, compact bone wall. The bone wall measures about 2.3 mm in some areas, and consists of a mixture of bone tissue types. The overall texture of the bone appears to be a woven bone matrix, although in some areas this is interrupted by lamellar deposits of bone with osteocyte lacunae oriented in parallel (Fig. 4). The type of vascularization and the orientation of the vascular channels varies from simple blood vessels with longitudinal, circumferentially and radial orientations, to primary osteons with longitudinal and reticular arrangements. Some isolated secondary osteons are
also present. Large parts of the compacta consists of compacted coarse cancellous bone, and in some instances, the entire bone wall is made up of this tissue. In parts of the midshaft section of the femur (ZOO/UCT/193), a narrow outer circumferential layer occurs, which consists of poorly vascularized lamellar bone. The inner medullary margin of the bone is highly resorptive, and consequently large trabecular spaces open into the medullary cavity, although the central part of the cavity is vacant. No inner circumferential layer of bone was evident.

**Multituberculates**

*Kryptobaatar dashzevegi* (Fig. 5).—Thin sections in the midshaft region of the *Kryptobaatar* femur revealed a fairly thin-walled compact bone region with a large medullary cavity (Fig. 5A). The average relative bone wall thickness is about 10% of the cross sectional diameter of the bone. The bone appears to be a mixture of parallel-fibred and woven textured type of bone tissue. Several channels occur within the bone, which appear to be at an early stage of primary bone formation.
osteon formation. The peripheral and medullary margins of the bone are uneven, and are distinctly resorptive in nature (Fig. 5B).

*Nemegtbaatar gobiensis* (Figs. 6, 7).—The serially sectioned femur of *Nemegtbaatar* provided a good assessment of the microstructural changes associated with the growth in length and girth of the proximal part of the femur. Unfortunately, none of the sections were in the midshaft region, i.e., the area least affected by remodeling. The regions B1, B2, B3, and B4 in Fig. 6A show exactly where the cross sections were taken from along the femoral shaft.

All cross sections of the bone show distinct changes in the histological composition of the compacta in response to medullary cavity expansion, metaphyseal remodeling, and drift. Distinct changes in the cross sectional shape of the bone is also observed. Section B4 shows a complex, stratified cortex, with variable microstructure across the section (Figs. 6B4, 7A). The compacta is largely made up of compacted, fine cancellous bone (Enlow 1962). Superficially this tissue looks like woven fibro-lamellar bone, however, upon closer inspection it is evident that this bone lies below a distinct tide line (= erosion line), which indicates its endosteal nature (Fig. 7A). In places, the lamellae have a more convoluted appearance. The endosteal bone varies from the compacted fine cancellous bone tissue described above, to a type with more convoluted lamellae, and also to a more lamellated type of bone tissue. The periosteal bone deposits mainly consist of parallel fibred bone with a distinct line of arrested growth interrupting the bone deposition (Fig. 7A). No cancellous bone is observed in the medullary cavity. The RBT is about 15%.

In general, the cortices of the other three sections have a similar, complex organization of bone. In Fig. 6, drifting in the medial direction is clearly evident. In section B3, resorption occurs on the medullary side of the region that is drifting. This trend is observed in section B2 as well, and here there are increased deposits on the lateral margin of the bone, as well as peripherally. A small trabecular extension is observed in both section B2 and B1. The drifting is most pronounced in section B1, and the compacta in this section, and in section B2, largely comprises of a periosteal deposits of lamellar bone with some radially and longitudinally oriented channels. A small patch of bone appears to be true periosteally deposited woven bone, below which a tide line is present (Fig. 7B). Under the tide line deposition of endosteally formed lamellated bone occurs. The opposite side of the section shows resorption, and the more globular, haphazardly oriented, osteocyte lacunae are visible (Fig. 6B3). At the level B1 of the femur, in the region of drifting (Fig. 6B1), there also appears to be resorption along the peripheral margins of the bone. Sharpey’s fibres are abundant in this region, and canaliculi appear to be highly branched.

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Fig. 4. *Ornithorhynchus anatinus* (Shaw, 1799); Recent, ZOO/UCT no number, femur, section catalogue number, ZOO/UCT/194. Transverse section showing the extensive fibro-lamellar bone and the episodically formed lamellar deposits of bone.

Fig. 5. *Kryptobaatar dashzevegi* Kielan-Jaworowska, 1971 ?early Campanian, Djadokhta Formation, Bayan Zak, Gobi Desert, Mongolia, femur (ZPAL MgM-I/18; Section catalogue number, ZOO/UCT/184). A. Low magnification of the femur showing the overall structure of the cross section. Note the woven bone nature of the thin bone wall. B. A higher magnification of the same section, illustrating the channels in the bone, and the irregular peripheral and medullary margins of the bone wall.
Eutherians

Zalambdalestes lechei (Fig. 8).—The RBT of the femoral cross section is about 17%. The compacta in Zalambdalestes consists of parallel-fibred bone tissue (Fig. 8). The compacta is rather poorly vascularized, except in a localized region where several simple blood vessels are located. A few poorly defined primary osteons as well as isolated secondary osteons are observed. In the peripheral region, the osteocytes appear to be more flattened and less abundant (Fig. 8B). A single LAG is visible near the peripheral margin of the bone wall. The medullary margin is irregular, and it appears that endosteal resorption was underway at the time of death.

Barunlestes butleri (Fig. 9)
Rib.—A complete transverse section of a rib was available. The bone wall is fairly thick, with a RBT of about 31%. It largely comprises of parallel-fibred bone tissue, in which several rest lines are recognizable. The bone is generally poorly vascularized, except for a localized area in the region of drift, the osteocytes tend to be haphazardly oriented, and a few channels for blood vessels are visible in the compacta (Fig. 9A).
Femur.—The Barunlestes femoral sample examined comprised only a small fragment. The section shows an outer parallel-fibred bone tissue with several LAGs (Fig. 9B1, B3). In a localized part of the section, there appears to be a bone tissue with more globular haphazardly oriented osteocyte lacunae...
Fig. 7. Nemegtohantu gobiensis Kielan-Jaworowska, 1974, ?late Campanian, red beds of Khermeen Tsav, Khermeen Tsav II, Gobi Desert, Mongolia. ZPAL MgM-I/81, section catalogue number, ZOO/UCT/183. A. Higher magnification of framed region in Fig. 6B, showing the complex nature of the compacta. Region 1 is a periosteal deposition of parallel-fibred bone, region 2 is a compaction of fine cancellous bone. Arrow indicates the tide line. B. Higher magnification of framed region in Fig. 6B, (section catalogue number, ZOO/UCT/181), illustrating the true woven bone. Arrow indicates the tide line.

(Fig. 9B), which may be correlated with a faster rate of bone deposition.

Discussion

As early as 1947, Amprino recognized that the type of bone tissue formed is directly related to the rate at which it was deposited. In recent years, several experimental studies on extant vertebrates have quantified the rates at which different bone tissues form (e.g., Margerie de et al. 2002, 2004; Starck and Chinsamy 2002), and although there is considerable overlap in the types of bone tissues that result at particular rates (Starck and Chinsamy 2002), qualitatively “Amprino’s rule” still holds. Thus, when analyzing the bone microstructure of extinct animals, the type of bone tissue present provides a qualitative assessment of the rate at which the bone formed i.e., depending on the type of bone tissue, one can reasonably deduce whether it formed at a “fast” or “slow” rate. For example, fibro-lamellar bone with its characteristic woven bone matrix and haphazardly-oriented osteocyte lacunae is typically deposited at a faster rate than lamellar bone tissue with its more organized matrix and osteocyte lacunae. However it must be emphasized that deducing an actual (i.e. quantitative) rate at which the bone formed is imprecise and speculative. Histological characteristics of the bone, such as the presence of annuli or LAGs, provide further information regarding the continuity or discontinuity of growth, while other microstructural details (e.g., the particular type of bone tissue) provide a reasonable assessments of various other aspects of the life history of the animal (e.g., Chinsamy 1997, 2005).

The analysis of the bone microstructure of the Late Jurassic Morganucodon and the Late Cretaceous multituberculates and eutherians reveals evidence pertaining to their growth strategies, especially when compared to their ancestors, the non-mammalian cynodonts, and to modern mammals (eutherians and monotremes).

Implications of the observed bone histology in the studied taxa

Non-mammalian therapsid Tritylodon.—The predominance of uninterrupted fibro-lamellar bone in the Tritylodon sections studied, suggests that these tissues were formed at a sustained, relatively rapid rate. A change to a slower rate of bone formation during later ontogeny is indicated by the presence of lamellar bone in the peripheral part of the cross section of the radius (Fig. 2B). This suggests that overall body growth was slowing down, and that Tritylodon had a determinate growth strategy. Closely spaced rest lines that
interrupt the peripheral lamellar layer of bone indicate that during late ontogeny, a slow rate of bone deposition occurred periodically.

Fibro-lamellar bone predominates in the cortical bone tissues of several nonmammalian therapsids, e.g., Bienotherium, and Oligokyphus (Ricqlès 1969), Diademodon and Cynognathus (Botha and Chinsamy 2000), Trirachodon (Botha and Chinsamy 2004), as well as in a range of traversodontids (Ricqlès 1969). However, in some non-mammalian cynodonts, such as Diademodon (Botha and Chinsamy 2000), Trirachodon (Botha and Chinsamy 2004), and Procynosuchus (Ray et al. 2004), periodic pauses in growth (represented by annuli and/or LAGs) interrupt the deposition of fibro-lamellar bone. Thus, among the nonmammalian cynodonts, both sustained and episodic (interrupted/periodic) growth strategies occur (e.g., Botha and Chinsamy 2000; Ray et al. 2004).

The growth pattern of rapid, sustained early growth, and later slower, episodic growth observed in Tritylodon has also been observed in the gorgonopsid, Aelurognathus (Ray et al. 2004), the dicynodont, Diictodon (Ray and Chinsamy 2004) and the therocephalian, Pristerognathus (Ray et al. 2004). Early Jurassic Morganucodon and Gephyrosaurus.—Although a large number of Morganucodon and Gephyrosaurus bones were diagenetically altered, some sections preserved unaltered bone tissue, and therefore permitted an assessment of the bone microstructure of these taxa.

Morganucodon formed fibro-lamellar bone during early ontogeny, but later in life formed a more parallel-fibred bone tissue that was periodically interrupted by growth lines. Although a clearly defined outer circumferential lamella (OCL) is lacking, the presence of parallel-fibred bone with LAGs suggests that later growth was slowed and intermittent (Fig. 3B). The bone histology of Gephyrosaurus is similar to that of extant squamates (e.g., Castanet and Baez 1991; Chinsamy et al. 1995) (Fig. 3A). The distinctive LAGs present in the bone suggest that overall growth of the animal was affected episodically, possibly by seasonally fluctuating ambient conditions. Remnants of “embryonic bone” preserved around the perimedullary region suggest that medullary remodeling was not extensive, and that diametric bone growth generally occurred in a slow, appositional manner. This type of growth has been observed in the lizard Gallotia stehlini where 90% of all specimens studied preserved embryonic bone below the first LAG (Castanet and Baez 1991). In such cases, skeletochronology is simplified as there is no need to account for resorption of earlier growth marks. In the compacta of a femoral thin section of Gephyrosaurus, 9 LAGs (including the hatching line) can be counted, which (assuming 1 LAG is formed per year) suggests a minimum age of nine years for the individual. The first zonal region after the hatching line is distinctly wider than the subsequent ones, which could be interpreted as signaling the attainment of sexual maturity in the first year post hatching (this would need to be verified by studying a larger sample of Gephyrosaurus). It is possible
that the wider spacing of the outer 3 zonal regions (as compared to the preceding 5) is a reflection of favorable environmental conditions during those particular seasons (Fig. 3A).

The distinctive characteristics of the bone microstructure of *Morganucodon* and *Gephyrosaurus* permitted a clear distinction of bones of each taxon. This was especially useful in the instances where isolated postcranial bones of *Morganucodon* and *Gephyrosaurus* could not be taxonomically distinguished previously (see methods).

**An extant monotreme Ornithorhynchus.**—Femoral thin sections of *Ornithorhynchus* revealed a fairly thick bone wall comprising a mixture of bone tissues types that formed as a result of various episodes of growth and remodeling. Secondary osteons are fairly abundant in the compacta, and large trabecular spaces were present in the medullary cavity. This contrasts with the findings of Enlow and Brown (1958), who noted the absence of Haversian (secondary) reconstruction, and the presence of a non-cancellous central medullary cavity in the femur and rib of *Ornithorhynchus*. The differences between the present *Ornithorhynchus* samples and those of Enlow and Brown’s (1958) study could represent intra-bone differences in bone morphology. A further point worth noting is that Enlow and Brown (1958) also described *Ornithorhynchus* bones as having unusual primary osteons with small lumina that were surrounded by a ring of acellular, perivascular, non-lamellar tissue. However, although we recognized a mixture of different types of vascularization i.e., from simple vascular canals to primary and secondary osteons, we did not notice anything unusual about the osteonal structure of the primary osteons in our sample.

The overall structure of the bone suggests that it was formed rapidly. However, evidence of a slowing down in the rate of bone deposition is suggested in the peripheral parts of the bone by the presence of poorly vascularized lamellar bone tissue with osteocyte lacunae organized in parallel. Although not visible in the micrograph figured by Enlow and Brown (1958: plate XXVIII: 3), they describe the presence of “unorganized, non-lamellar osteoid tissue” between regions of lamellar bone. It is possible that the lamellar bone deposits (described by Enlow and Brown 1958, and also observed in the current study) represent annuli, but in the absence of life history data, it is uncertain whether they are true annuli (Fig. 4) or simply a reflection of localized slow rates of bone deposition.

**Multituberculates.**—*Nemegtochoides* sections (Figs. 6, 7) all show complex cortical stratifications associated with structural remodeling of the growing femur. The presence of compacted, fine-cancellous bone in the cortex indicates that this region was once close to the epiphyseal growth plate (Enlow 1962). As the femur grew in length this region was relocated from its original medullary location into the cortical region of the growing bone. Such mixed bone tissues have been noted in the bones of young rapidly growing individuals, and occurs in the bones of white rats at various stages of ontogeny (Enlow 1962).
Consequently, it is not known whether bone formation suggests that the bone is from a young, immature individual. bone margins, and early stages of osteonal development bone formation. The uneven peripheral and perimedullary tissue with primary osteons, which suggests a rapid rate of growth early on, which slowed down during later ontogeny. The well-vascularized compacta of the Mesozoic multituberculate \textit{Kryptobaatar} is distinctive in having the entire cortical bone consisting of a woven bone tissue with primary osteons, which suggests a rapid rate of bone formation. The uneven peripheral and perimedullary bone margins, and early stages of osteonal development suggest that the bone is from a young, immature individual. Consequently, it is not known whether bone formation slowed down in later stages. The small patch of periosteally formed woven bone tissue in \textit{Nemegtbaatar} (Fig. 7B) suggests that during early ontogeny, the initial periosteal cortical bone tissue formed rapidly. However, in the sections we studied, most of this early formed bone tissue was already resorbed, and mainly the later, periosteal lamellar bone with longitudinally and radially oriented channels is evident. Thus, given the overall structure of the bone (the occurrence of compacted fine cancellous bone in the cortical bone tissues, the presence of a small localized region of woven bone, as well as vascularized lamellar bone), it appears that \textit{Nemegtbaatar} had a fast rate of growth early on, which slowed down during later ontogeny. The small patch of periosteally formed woven bone tissue in \textit{Nemegtbaatar} (Fig. 7B) suggests that during early ontogeny, the initial periosteal cortical bone tissue formed rapidly. However, in the sections we studied, most of this early formed bone tissue was already resorbed, and mainly the later, periosteal lamellar bone with longitudinally and radially oriented channels is evident. Thus, given the overall structure of the bone (the occurrence of compacted fine cancellous bone in the cortical bone tissues, the presence of a small localized region of woven bone, as well as vascularized lamellar bone), it appears that \textit{Nemegtbaatar} had a fast rate of growth early on, which slowed down during later ontogeny. The overall nature of the Mesozoic eutherian bone tissue suggests a slow rate of bone deposition, with \textit{Barunlestes} having an even slower growth rate than \textit{Zalambdalestes}. Small extant eutherian \textit{Heterocephalus glaber}.—The woven texture of the inner layer of the compacta of \textit{Heterocephalus glaber} suggests that it was deposited at a faster rate than the outer circumferential layer (OCL), which consists of lamellar bone. Klevezal (1996) noted that although diametric (appositional) growth decreases in extant mammals once adult body size is attained, it generally does not stop, and often accretionary deposits of bone occur in the peripheral part of the compacta. Thus, the avascular, lamellar bone of the OCL in \textit{Heterocephalus} indicates that although the animal had reached adult size, appositional growth still continued at a slow rate. In several extant mammals, particularly those from temperate areas with strong seasonality, the OCL is often interrupted by LAGs (Klevezal 1996). No LAGs are recorded in the OCL of the \textit{Heterocephalus} specimens in this study; this could be because the animal was from a captive colony (housed indoors), and would not have had to endure seasonal fluctuations. (This may also occur naturally, as \textit{Heterocephalus} in the wild live in burrows, therefore experiencing minimal ambient fluctuations.) It is worth noting that the simple blood vessels in the compacta are radially oriented and are embedded in a woven bone matrix, which suggests that the bone was formed at a relatively faster rate than the bone in the OCL (Margerie de et al. 2004). The lack of intensive secondary reconstruction is consistent with observations made in several small mammals (e.g., bats, moles, squirrels) (Enlow and Brown 1958; Klevezal 1996). The relatively thick bone wall of \textit{Heterocephalus glaber} appears to be an adaptation for its fossorial lifestyle (Botha and Chinsamy 2004). General implications of the observed bone microstructure It is evident that the mammals and the non-mammalian cynodont studied have a bone microstructure distinct from that of the pleurodont lepidosaur \textit{Gephyrosaurus}. Given that the growth rate of bone roughly corresponds with the overall stages, the Mesozoic eutherians \textit{Zalambdalestes} (Fig. 8) and \textit{Barunlestes} (Fig. 9) both show a relatively slow rate of growth with periodic pauses indicated by the presence of rest lines. The \textit{Zalambdalestes} specimen appears to have been a young individual with parallel fibred bone tissue and mainly simple blood vessels evident in its compacta. Although the \textit{Barunlestes} rib preserves a fairly thick cortical region, only parallel fibred bone tissue with rest lines is present. However, in the femoral fragment studied it looks as though an earlier tissue with more haphazardly organized osteocytes (suggesting a faster depositional rate) formed during early ontogeny. This suggests that a slower rate of growth soon replaced an earlier faster rate of growth. The overall nature of the Mesozoic eutherian bone tissue suggests a slow rate of bone deposition, with \textit{Barunlestes} having an even slower growth rate than \textit{Zalambdalestes}.
growth of the whole animal (Klevezal 1996), it is reasonable to deduce that the pleurodont lepidosaur and mammalian taxa experienced different growth trajectories. As indicated by the interrupted parallel-fibred nature of the compacta, the pleurodont lepidosaur growth was seasonally affected: it grew relatively fast during the favorable season, and growth ceased during the unfavorable season.

Except for the *Kryptobaatar* and *Zalambdalestes* bones, which seem to be from individuals that have not yet attained adult body size, all other mammalian bone studied, irrespective of whether they are extant or extinct, show a change to a slower rate of bone deposition later in ontogeny. This indicates that although growth had slowed down appreciably, small amounts of slowly formed bone continued to be deposited (e.g., Klevezal 1996; Chinsamy and Dodson 1995). The fact that the Early Jurassic mammal *Morganucodon*, the cyodontid *Triplyodon*, as well as other, more basal nonmammalian therapsids, e.g., *Aetouraughus*, *Psisterognathus* (Ray et al. 2004), and *Dictodon* (Ray and Chinsamy 2004), all show a tendency for this type of growth pattern, suggests that a determinate growth strategy evolved early in the ancestry of mammals.

Perhaps in response to selection for fast growth most extant mammals generally grow with sustained rapid rates (as reflected by uninterrupted bone tissue) i.e., most have relatively reduced developmental plasticity (Smith-Gill 1983). However, it appears that in response to unfavorable environmental conditions, some mammals e.g., small forms living in temperate conditions, can form periodically interrupted bone (Klevezal and Kleinenberg 1969; Chinsamy and Dodson 1995; Chinsamy 1998). Extensive work by Klevezal (1996) suggests that strong seasonality usually involves growth marks in the OCL of mammalian bone. However, Klevezal (1996) cautions that the LAGs in the OCL are not reliable indicators of age since in mammals these lines often do not form in the early years of an individual’s life.

It is interesting to note that although *Heterocephalus glaber* is generally considered to have a low basal metabolic rate and with body growth rates lower than most mammals of similar size (see Jarvis and Bennett 1991), its compacta nevertheless appears to have formed at a more rapid rate than that of the two Mesozoic eutherian genera, and indeed, more rapidly than those of many other extant small eutherians (e.g., moles, bats, marmots, squirrels), which tend to have rather poorly vascularized compacta (Enlow and Brown 1958). Thus, size alone cannot explain the differences observed among the extant mammalian taxa. It is also worth noting that, although not conclusive, the periodically interrupted nature of *Ornithorhynchus* bone suggests a flexible growth strategy similar to that observed in the bone formed in the nonmammalian therapsids, *Diademodon* (Botha and Chinsamy 2000) and *Trirachodon* (Botha and Chinsamy 2004).

The overall nature of the bone tissue of the two Mesozoic eutherians suggests a much slower rate of bone deposition than that of the extant eutherians, as well as the multituberculates, *Morganucodon*, and the nonmammalian therapsids. Indeed *Barunlestes* appears to have an even slower growth rate than *Zalambdalestes*, *Zalambdalestes* and *Kryptobaatar* are from the Barunlestes Formation, while *Barunlestes* and *Nemegtbaatar* are from the Barunungoyot Formation, but these formations are considered to represent similar, semi-desert-like environmental conditions (Jerzykiewicz 2000). Other than the fact that eutherians are generally considered to be insectivores, whereas the multituberculates are considered omnivores/ herbivores, these four taxa are relatively similar in size, and occupied similar habitats. Therefore, from a paleoecological perspective, it may be significant that the multituberculates apparently had faster growth rates than the eutherians.

The flexible growth strategy indicated by the cyclical pattern of bone deposition (Starck and Chinsamy 2002) in several nonmammalian therapsids suggests that a flexible growth strategy, even after reaching “adult” size, is plesiomorphic for the mammalian lineage (Ray et al. 2004). Furthermore, it seems that the multituberculates grew at a faster rate than the contemporary eutherians which retained the plesiomorphic condition of flexible growth rates.

Such differences in growth strategy may account for some of the observations in the fossil record. During the Late Cretaceous, multituberculates dominated both central Asian and North American mammal faunas, comprising on average 70% of recovered specimens in central Asia and 50% or more in North America. Western Asia represents an exception since here unguilatomoform eutherians dominated while multituberculates comprise only 1% of collected mammal specimens (Kielan-Jaworowska and Nessov 1992; Nessov et al. 1998). Perhaps the fast growth rate of multituberculates, as suggested by their bone microstructure, provided an adaptive advantage, and may account for their abundance in the Late Cretaceous of the Gobi region. Being of similar size to the Eutheria described, but with a faster growth rate, they may have reached sexual maturity earlier than the eutherians and therefore reproduced at a faster rate.

Many authors (Van Valen and Sloan 1966; Hopson 1967; Kielan-Jaworowska and Gambaryan 1994) mention the apparent competitive inferiority of multituberculates relative to eutherians during the Tertiary, which may have led to multituberculate extinction at the end of the Eocene. It is possible that the flexible growth strategies of the eutherians may have given them an adaptive advantage after the Cretaceous–Tertiary extinction event, by enabling them to invoke faster (energetically costly) or slower (energetically less costly) developmental regimes in response to increased environmental variability and seasonality, thus enabling them to radiate and become the dominant mammals.

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