

Bone lamina thickness, bone apposition rates, and age estimates in sauropod humeri and femora

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with 4 figures and 6 tables

Abstract: Laminar fibro-lamellar bone from the mid-shaft region of sauropod humeri and femora was analyzed quantitatively to understand variation in lamina thickness and apposition rate. The samples were derived from five taxa (*Apatosaurus*, *Barosaurus*, an indeterminate gracile diplodocid, *Brachiosaurus*, *Janenschia*) and two localities (Tendaguru beds, Tanzania, and Howe-Stephens Quarry, Morrison Formation, Wyoming, USA). Lamina thickness is rather constant at 4–6 laminae/mm (mean 4.8), independent of locality, taxon, bone, and ontogenetic age, suggesting structural constraints as a cause. Bone apposition rate was calculated in bones with annual growth lines (polish lines) by two methods to serve in age estimates of individuals without growth lines and unsampled individuals. Apposition rates are rather variable (1–20 $\mu\text{m}/\text{day}$), resulting in a wide bracket for the ages estimated. Our age estimates are compatible with recent estimates derived by other methods, however.

Keywords: fossil bone histology, fibro-lamellar bone, lamina thickness, apposition rates, age estimates, sauropod dinosaurs, *Apatosaurus*, *Barosaurus*, *Brachiosaurus*, *Janenschia*

Zusammenfassung: Laminarer fibro-lamellärer Knochen aus der Schaftmitte von Sauropoden-Humeri und -Femora wurde quantitativ analysiert, um die Dickenvariation der Laminae und die Anlagerungsrate des Knochens abzuklären. Untersucht wurde Material von fünf Taxa (*Apatosaurus*, *Barosaurus*, ein nicht näher bestimmter graziler Diplodocide, *Brachiosaurus*, *Janenschia*) aus zwei Fundstellen (Tendaguru-Schichten, Tansania, und Howe-Stephens Quarry, Morrison-Formation, Wyoming, USA). Die Lamina-Dicke ist ziemlich konstant bei 4–6 Laminae pro Millimeter (Durchschnitt 4,8). Sie ist unabhängig von Fundstelle, Taxon, Knochen und Individualalter, was strukturelle Gründe für ihre geringe Variabilität vermuten lässt. Mit zwei verschiedenen Methoden wurde die Anlagerungsrate des corticalen Knochens für Knochen mit jährlichen Wachstumsmarken (Politurlinien) berechnet. Sie konnte dann zur Abschätzung des Individualalters von Tieren benutzt werden, die keine Wachstumsmarken zeigen bzw. nicht histologisch beprobt wurden. Die Anlagerungsraten sind relativ variabel (1–20 μm pro Tag), woraus relativ ungenaue Altersabschätzungen resultieren. Trotzdem stimmen die Ergebnisse mit kürzlich mit anderen Methoden erzielten Abschätzungen überein.

Schlüsselwörter: Histologie fossiler Knochen, fibro-lamellärer Knochen, Laminadicke, Anlagerungsrate, Altersabschätzungen, sauropode Dinosaurier, *Apatosaurus*, *Barosaurus*, *Brachiosaurus*, *Janenschia*

Introduction

The quantification of the growth of different dinosaurs based on bone histology has received considerable attention lately. Sauropods, in particular, were investigated by SANDER (1999, 2000) and CURRY (1998, 1999) based on cyclical growth marks in bone. This technique, also known as skeletochronology (CASTANET 1986; CASTANET et al. 1993), was applied to populations of fossil tetrapods for the first time by SANDER (1989, 1990) and is increasingly used in dinosaur paleobiology (reviews in PADIAN et al. 2001; ERICKSON et al. 2001).

This study aims at quantifying sauropod long bone histology beyond the analysis of growth marks. The cortex of sauropod long bones is dominated by a type of primary bone tissue known as “laminar fibro-lamellar bone” (FRANCILLON-VIEILLOT et al. 1990) which is also the type seen in large mammals and in living birds. With regard to this bone type, we wanted to answer two main questions: first, what is the variation of the lamina thickness in different parts of the cortex, and is there a dependency on taxon, locality, bone, or ontogenetic age? In particular, does lamina thickness decrease during the growth of the individual, and is there a difference in lamina thickness between the fibro-lamellar bone formed in juvenile individuals (“juvenile” bone of SANDER 1999, 2000) and the fibro-lamellar bone formed in adult individuals (“adult” bone of SANDER 1999, 2000)?

The second question is: what are the rates of apposition of laminar fibro-lamellar bone in the cortex of sauropod humeri and femora? The rates can be computed by measuring cortical thickness in specimens that show cyclical growth marks such as lines of arrested growth (as defined e.g. by CASTANET et al. 1993), modulations (as defined by RICQLÈS 1983), and polish lines.

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Polish lines (as defined by SANDER 2000) are growth marks that are too subtle to be observed in thin section but that are brought out by polishing the surface of a bone section. Assuming that these growth marks are annual, the age of the individual at death can be estimated and cortical bone apposition rates can be calculated. For comparison with sauropod dinosaurs, we also studied apposition rates in recent ostriches (*Struthio camelus*).

In a further step, the cortical bone apposition rates derived from sauropod long bones with growth marks can then be applied to bones without growth marks of different individuals to estimate their age. This method was briefly introduced by CURRY (1998). The method is of interest because it would allow age estimates based on the type of bone tissue alone, independent of growth marks. It thus could be used to age long bones that lack growth marks as well as test ages derived from the analysis of growth marks. With the limited database at hand, the method will only yield rather tentative estimates of individual age due to various sources of error discussed below, but we feel that the benefits of pushing the data to their limit outweigh the disadvantages. Our approach to individual age calculation based on long bone apposition rates is rather to test the potential of the method than to obtain data for further deductions.

Materials and methods

The sample

For this study, we investigated samples from the long bones of several sauropod individuals pertaining to different taxa from the Upper Jurassic Tendaguru beds of Tanzania (JANENSCH 1914, 1961), kept in the Naturkundemuseum der Humboldt-Universität (Berlin, Germany; NHUB), and from the Morrison Formation of Wyoming (AYER 1999, 2000), kept in the Sauriermuseum Aathal (Aathal, Switzerland; SMA). The samples (Tab. 1) consisted of cores of the cortex of the mid-shaft region of humeri and femora.

The midshaft cross section of sauropod long bones shows a number of morphological and histogenetic

characteristics that make the investigation of growth based on histology in small core samples possible: the shaft is circular to elliptical, it experienced little lateral drift during growth, the medullary cavity is small, and the cortex shows very little to moderate remodelling. The degree of remodelling is taxon-specific in the sauropods from the Tendaguru beds (SANDER 1999, 2000). In ideal cases, therefore, nearly the entire growth history of an individual is recorded in a single core sample (e.g. NHUB NW 4 and Nr. 22, see SANDER 2000). The cores were bisected along the cross sectional plane of the bone, and one half was ground into a thin section while the other was prepared into a polished section (for details of sampling and sample preparation, see SANDER 2000). In this study, we mainly relied on polished sections but consulted the thin sections to confirm the type of tissue observed.

Of particular interest were four samples with well developed growth marks, expressed as polish lines (Fig. 1, Tab. 1). Two of these pertain to *Barosaurus africanus* from Tendaguru, a large femur (NHUB NW 4, 135 cm long) and a small humerus (NHUB R.2625, 61 cm long). SANDER (1999, 2000) observed two different bone types in *B. africanus* which may represent the different sexes. Type A is represented in this study by NHUB NW 4 while NHUB R.2625 belongs to type B which is less common than type A. The third sample derives from a large *Janenschia* femur from Tendaguru (NHUB Nr. 22, 127 cm long). This specimen was already analyzed in some detail by SANDER (1999, 2000). *Janenschia* was recently recognized to be related to the Camarasauridae (BONAPARTE et al. 2000). The fourth sample is from a large *Apatosaurus* femur (SMA "Jaques", 164 cm long) from the Howe-Stevens Quarry (AYER 1999, 2000). Two samples (*Barosaurus* NHUB NW 4 and *Janenschia* NHUB Nr. 22) of the four are from fully grown individuals because the outermost cortex consists of a very thin layer of lamellar bone with closely spaced growth marks.

An additional four core samples (Tab. 1) from Tendaguru and Morrison sauropods were used to quantify lamina thickness. The comparative ostrich sample

Tab. 1. Material sampled. The upper set of specimens was used to measure lamina thickness and apposition rate, while the lower set was only used to measure lamina thickness. – "growth marks?" refers to the presence and number of growth marks (polish lines).

genus	spec. no.	bone	r/l	length (in mm)	growth marks?	locality
<i>Barosaurus</i>	NHUB R.2625	humerus	l	610	yes/7	Tendaguru
<i>Barosaurus</i>	NHUB NW 4	femur	l	1350	yes/14	Tendaguru
<i>Janenschia</i>	NHUB Nr. 22	femur	l	1270	yes/26	Tendaguru
<i>Apatosaurus</i>	SMA "Jaques"	femur	r	1640	yes/25	Morrison
<i>Barosaurus</i>	NHUB XVI 64I	humerus	l	730	no	Tendaguru
<i>Brachiosaurus</i>	NHUB T 8	humerus	l	1280	no	Tendaguru
<i>Brachiosaurus</i>	NHUB t 7	humerus	r	1530	no	Tendaguru
diplodocid	SMA "Max"	humerus	l	850	no	Morrison

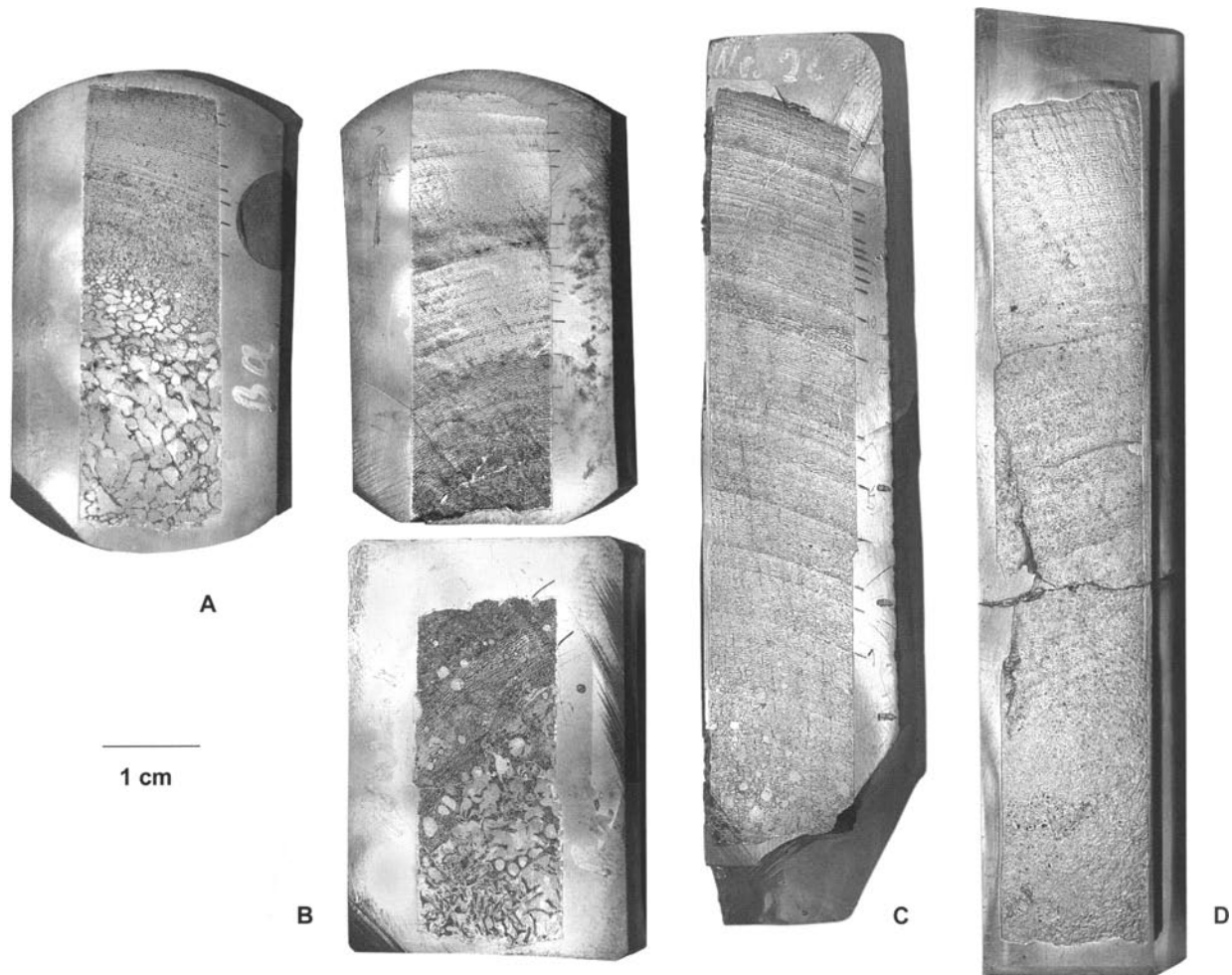


Fig. 1. Polished sections of samples with growth marks imaged in surface reflection (bright-field illumination). The sections are embedded in polyester resin for support. The bone surface is at the top of the images. Growth marks are variously expressed as polish lines (A, B, C) or modulations (D). – **A:** *Barosaurus africanus* NHUB R.2625, humerus, 61 cm. – **B:** *Barosaurus africanus* NHUB NW 4, femur, 135 cm. Note that the inner and the outer part of the section are in separate resin blocks. – **C:** *Janenschia robusta* NHUB Nr. 22, femur, 127 cm. – **D:** *Apatosaurus* SMA “Jaques”, femur, 164 cm.

consisted of cross sections of the entire mid-shaft of one femur and three tibiotarsi, all from different yearling individuals raised on an ostrich farm.

Quantification of lamina thickness

The polished sections were analyzed with a binocular microscope in incident light (same as dark-field illumination in a compound microscope) and reflected light (same as bright-field illumination), and the observations were recorded in camera lucida drawings. Where present, polish lines were observed by tilting the polished surface to create a reflection (same as bright-field illumination) and were recorded in the drawings. The number of laminae between successive polish lines was counted. Lamina thickness was compared in different parts of each sample, irrespective of the presence or absence of polish lines. It is important to note that lamina thickness was measured in this study from the center of one vascular canal to the center of the next vascular canal. This differs from the correct histological definition

of a lamina (FRANCILLON-VIEILLOT et al. 1990) in which the lamina extends from the center of one zone of fibrous bone to the next. Measuring from one canal center to the next has the advantage of greater accuracy. Also for practical reasons, lamina thickness is expressed as the number of lamina per millimeter (Tabs. 2, 3).

Lamina thickness was also recorded separately in fibro-lamellar bone formed in juvenile individuals and fibro-lamellar bone formed in adult individuals (Tabs. 2, 3). Bone formed in adult individuals is distinguished from bone formed in juvenile individuals mainly by distinctly thinner vascular canals due to more complete infilling of the primary osteons, flattened osteocyte lacunae, fewer radial vascular canals, and the predominantly lamellar arrangement of the bone fibers in the fibro-lamellar complex, also due to more complete infilling of the primary osteons (for a detailed description see SANDER 2000: 472). Presumably, the transition from bone formed in juvenile individuals to bone formed in adult individuals is an expression of sexual maturity in sauropods. This is supported by the observation that in

mark number	<i>Barosaurus</i> NHUB R.2625	<i>Barosaurus</i> NHUB NW 4	<i>Janenschia</i> Nr. 22 NHUB Nr. 22	<i>Apatosaurus</i> SMA "Jaques"
1–2	4.0	4.4	5.0	–
2–3	5.0		5.0	–
3–4	4.1	4.7	5.1	–
4–5	5.6	4.7	5.5	–
5–6	5.2	5.0	4.7	3.3
6–7	5.1	7.6	4.8	3.3
7–8		6.1	4.4	2.8
8–9		6.1	4.2	3.8
9–10		4.3	4.2	3.6
10–11		4.6	5.1	4.1
11–12		5.3	6.0	3.4
12–13		5.3	6.0	3.0
13–14		5.4	6.0	3.8
14–15			6.0	5.2
15–16			8.0	4.6
16–17			4.5	4.4
17–18			6.0	4.6
18–19			5.3	5.3
19–20			4.5	5.1
20–21			6.0	4.1
21–22			5.3	4.3
22–23			3.9	4.1
23–24			6.0	6.1
24–25			3.6	6.1
25–26			4.8	
mean	5.1	5.3	5.0	4.2

Tab. 2. Variation of lamina thickness with ontogenetic age in bones with regularly spaced growth marks delimiting appositional cycles. Lamina thickness is expressed in laminae per mm. The combined mean of all four samples is 4.8. – Bold numbers refer to values for "juvenile" bone, normal print denotes values for "adult" bone. Missing values are due to heavy remodelling or diagenetic alteration. "mark number" refers to the number of the growth marks delimiting the cycle measured.

bones with growth marks, the transition from bone formed in juvenile individuals to bone formed in adult individuals is correlated with a sudden decrease in growth mark spacing (see also SANDER 2000). The best specimen to show this is the *Janenschia* femur NHUB Nr. 22 which was used in this study to compare lamina thickness as well as apposition rate in bone formed in juvenile individuals and bone formed in adult individuals. In addition, in three specimens that lack growth lines

(Tab. 3), a five millimeter thick layer of typical bone formed in juvenile individuals of the inner cortex was compared with a five millimeter thick layer of typical bone formed in adult individuals of the outer cortex. In one specimen (NHUB XVI 641), consecutive measurements were obtained throughout the entire unremodelled cortex, including the transition zone from bone formed in juvenile individuals to bone formed in adult individuals (Fig. 3, Tab. 3).

genus	spec. no.	"juvenile" bone	"adult bone"	mean
<i>Janenschia</i>	NHUB Nr. 22	4.8	5.5	5.1
<i>Brachiosaurus</i>	NHUB T8	4.2	4.6	4.4
<i>Brachiosaurus</i>	NHUB t7	5.0	5.6	5.3
diplodocid	SMA "Max"	4.0	5.0	4.5
<i>Barosaurus</i>	NHUB XVI 641	4.4	4.1	4.25
<i>Apatosaurus</i>	SMA "Jaques"	–	4.2	4.2
<i>Barosaurus</i>	NHUB R.2625	–	5.1	5.1
<i>Barosaurus</i>	NHUB NW 4	–	5.3	5.3
grand mean				4.8

Tab. 3. Comparison of lamina thickness in "juvenile" versus "adult" bone tissue and comparison of lamina thickness in all bones studied. Lamina thickness is expressed in laminae per mm. – Missing values are due to juvenile bone not being preserved.

Bone apposition rates

In the computation of rates of cortical bone apposition, the distance between polish lines in the fibro-lamellar bone is the basic measurement because it represents the amount of bone laid down in a single year. It allowed us to study variation in apposition rate with ontogenetic age, i.e. apposition rate per growth cycle (Tab. 4). To compare the values obtained with those measured in living animals, we transformed the annual rate to the daily rate (Tab. 4) which was expressed in μm per day, using a year consisting of 365 days. Although there apparently were more days in the Mesozoic year due to the slow down of the Earth's rotation since then, the length of the year probably was the same.

In addition to the single-cycle growth rate analysis, we also computed average rates for the entire ontogeny and their differences between samples and between taxa (Tab. 5). Two approaches were taken in the calculation of average rates for the entire ontogeny. In the first, the distance between the first and the last observable line was measured and then divided by the number of observed cycles (presumably annual) between the first and the last line. The result is the average annual apposition

rate for the time span observed. Of course, growth marks developed in the outermost lamellar-zonal bone of the two specimens that show it were not included in this calculation because the aim was to calculate apposition rate in one tissue type only, fibro-lamellar bone.

Because apposition rate generally decreases during ontogeny (Tab. 4), the rate represents an average rate for the entire documented life history of the animal, overestimating deposition rate late in ontogeny and underestimating the rate in early ontogeny. In addition, the apposition rate computed this way is a minimal apposition rate because it neglects the inner, remodelled part of the cortex and the bone deposited earliest in ontogeny and later resorbed to form the medullary cavity. However, the error introduced this way is not too great because the innermost region of the cortex grew fastest as it was formed early in ontogeny and thus does not represent much time.

In the second method for computing apposition rates, the radius of the bones at the sample site was divided by the number of polish lines. Despite diagenetic crushing, it can be recognized that the cross section of the bones was not circular but elliptical in the fresh bone. The samples were taken from the region with the

Tab. 4. Variation of apposition rates of laminar fibro-lamellar bone with age in samples with cyclical growth. Apposition rate is expressed in μm per day for each cycle. The rate was computed by dividing the thickness of a growth cycle by the number of days per year (using the value of 365 days per year). – “mark number” refers to the numbers of the growth marks delimiting the growth cycle measured. Bold numbers refer to values for “juvenile” bone, normal print denotes values for “adult bone”. Values in parentheses were omitted from the calculation of the mean because they probably are due to the lumping of several undetected growth cycles. The mean of all values of one specimen is the minimal apposition rate.

mark number	<i>Barosaurus</i> NHUB R.2625	<i>Barosaurus</i> NHUB NW 4	<i>Janenschia</i> Nr. 22 NHUB Nr. 22	<i>Apatosaurus</i> SMA “Jaques”
1–2	7.2	11.2	10.5	5.0
2–3	4.9		5.5	4.5
3–4	2.7	8.1	11.9	6.7
4–5	5.4	7.6	10.5	7.2
5–6	9.0	4.9	8.7	9.9
6–7	2.7	1.8	4.6	5.8
7–8		1.8	8.7	(20.7)
8–9		3.1	10.5	5.8
9–10		4.5	9.6	5.4
10–11		5.4	6.4	(15.3)
11–12		(13.0)	1.8	4.0
12–13		3.6	0.9	3.6
13–14		4.0	2.3	3.6
14–15			2.3	3.1
15–16			1.4	3.6
16–17			1.8	3.1
17–18			0.9	3.6
18–19			3.7	3.6
19–20			1.8	2.7
20–21			1.8	4.0
21–22			3.7	4.5
22–23			6.4	4.0
23–24			0.6	2.7
24–25			2.3	3.6
25–26			2.3	
mean	5.2	5.1	4.7	3.9

Tab. 5. Age estimates derived from bone dimensions and number of growth marks. Bone dimensions are in mm, while apposition rates of lamellar fibro-lamellar bone are in μm per day. See text for further details. The intermediate ages were calculated using the grand mean of the mean minimal apposition rate of all four specimens and the mean maximal apposition rate of all four specimens. This mean is 7.7 $\mu\text{m}/\text{day}$.

genus	spec. no.	bone	length	circumference	short radius (b)	growth marks	min. rate	max. rate	int. age in years	remarks
<i>Barosaurus</i>	NHUB R.2625	humerus	610	240	31	7	5.2	12.1	10.2	type B
<i>Barosaurus</i>	NHUB NW 4	femur	1350	600	76	14	5.1	14.8	26.8	type A
<i>Janenschia</i>	NHUB Nr. 22	femur	1270	570	73	26	4.7	7.6	24.3	
<i>Apatosaurus</i>	SMA "Jaques"	femur	1640	730	93	25	3.9	8.2	33.1	
mean							4.73	10.68		

minimal radius of the ellipse which coincides with the anteroposterior direction in the animal. We approximate the ellipse by using an ellipse in which the minimal radius is 40 % of the sum of the minimal and the maximal radius. Because of the moderate crushing of the bones having deformed their original cross section, the minimal radius could not be measured directly but was computed from the measured circumference at the sample site by dividing the circumference by π (3.142) which gives the sum of the minimal and the maximal radius. The minimal radius was then computed by subtracting 60% of this sum. The minimal radius was divided by the number of cycles to obtain the annual apposition rate which again was transformed into daily apposition rates. Again, the cycles documented in the outermost lamellar-zonal bone of the two specimens that show it were not included in this calculation. Although nearly negligible, the thickness of the outermost layer of lamellar-zonal bone was subtracted from the bone radius as well.

This method produced the maximal apposition rate because it assumes that the entire thickness of the bone from the center to the first growth lines was deposited in one year when, in fact, much of this bone was resorbed and remodelled and may actually have taken a few to several years to form. Although the maximum growth rate thus may significantly overestimate the actual growth rate, together with the minimum growth rate it still can be used to bracket the actual growth rate and thus is worth computing.

We also computed the cortical apposition rate of ostrich long bones with the intention of applying them to sauropod dinosaurs. This was only done in one year old individuals at which age farm-raised ostriches stop increasing in size and have reached harvesting size. Ostrich bones have an approximately circular shaft cross section, making it possible to calculate mid-shaft radius directly from circumference at this location. We then divided the radius by the number of days in a year to obtain apposition rate. The result may slightly overestimate actual apposition rates because at hatching the ostrich chick already has long bones several millimeters in diameter.

Age estimates based on apposition rates

To obtain the individual age of sauropods from the thickness of their humeri and femora, we first had to decide which apposition rates to use – only those calculated for the same taxon or the average rates of all sauropod taxa studied. We opted for the latter approach because apposition rates were broadly similar in all samples and were thus taken to be valid for sauropods in general. This allowed us to apply them to taxa not studied here, such as *Dicraeosaurus*. We used three different rates in the age calculations: the mean of the minimal apposition rate of all four samples (mean minimal rate), the mean of the maximal apposition rate of all four samples (mean maximal rate), and the grand mean of these two rates which we termed the **mean apposition rate**. The age estimates based on mean minimal and mean maximal apposition rates diverge widely but provide a general bracket for the true age of the individual.

Age calculation starts off with the calculation of the minimal radius of the long bone at mid-shaft from its circumference at this location, as described above. This radius is equal to the entire thickness of cortical bone deposited (some of which actually was destroyed by resorption) during the life of the animal at this location. The radius is then divided by a particular apposition rate (Tab. 5) which gives the age of the individual in days (because apposition rate was expressed in μm per day). Finally, the age in days was transformed into the age in years (Tab. 6). Although some bones, the age of which was calculated, are from fully grown individuals and therefore have an outermost thin layer of lamellar-zonal bone which increased the circumference of the bone, this increase is so slight because the layer is so thin (< 200 μm) that the error introduced this way is negligible.

Results

Quantification of lamina thickness

First, we will present the results on lamina thickness: independently of taxon, there are between 3 and 8 laminae

Tab. 6. Age estimates for all NHUB Tendaguru sauropod humeri and femora sampled by SANDER (2000) using cortical thickness and mean bone apposition rates. Bone length and circumference are in mm, ages are in years. The mean bone apposition rates for the age calculations are taken from Tab. 5. The mean minimal apposition of 4.73 $\mu\text{m}/\text{day}$ was used to calculate the maximum ages. The mean maximal apposition of 10.68 $\mu\text{m}/\text{day}$ was used to calculate the minimum ages. The intermediate ages were calculated using the grand mean of 7.7 $\mu\text{m}/\text{day}$ (see Tab. 5). – “marks” refers to the number of observed growth marks.

genus	spec. no.	bone	left/right	length	circumf.	min. age	int. age	max. age	marks
<i>Brachiosaurus</i>	XX 19	humerus	l	690	235	7.7	10.1	14.5	
<i>Brachiosaurus</i>	cc 2	humerus	r	1080	380	12.4	16.2	23.4	
<i>Brachiosaurus</i>	T 8	humerus	l	1280	390	12.7	16.7	24.0	
<i>Brachiosaurus</i>	t 7	humerus	r	1530	540	17.6	23.1	33.2	
<i>Brachiosaurus</i>	J 12	humerus	r	1700	550	18.0	23.5	33.8	
<i>Brachiosaurus</i>	II 28e	humerus	l	1760	620	20.2	26.5	38.1	
<i>Brachiosaurus</i>	g 1	femur	r	690					
<i>Brachiosaurus</i>	St 134	femur	r	740	275	9.0	11.7	16.9	
<i>Brachiosaurus</i>	IX 1	femur	l	880	340	11.1	14.5	20.9	
<i>Brachiosaurus</i>	dd 452	femur	l	1350	620	20.2	26.5	38.1	
<i>Brachiosaurus</i>	Nr. 305	femur	r	1560	620	20.5	26.8	38.6	
<i>Brachiosaurus</i>	St 291	femur	r	1830	750	25.5	32.0	46.1	
<i>Brachiosaurus</i>	XV	femur	l	2190	820	26.8	35.0	50.4	
<i>Barosaurus</i>	G 91	humerus	r	435	220	7.2	9.4	13.5	
<i>Barosaurus</i>	R.2625	humerus	l	610	240	7.8	10.2	14.8	7
<i>Barosaurus</i>	IX 94	humerus	l	640	270	8.8	11.5	16.6	
<i>Barosaurus</i>	XVI 64	humerus	l	730	330	10.8	14.1	20.3	
<i>Barosaurus</i>	XI a7	humerus	l	805	370	12.1	15.8	22.8	
<i>Barosaurus</i>	A1	humerus	r	990	480	15.7	20.5	29.5	
<i>Barosaurus</i>	XVI 5	femur	l	790					
<i>Barosaurus</i>	Ki 71 a	femur	l	1020	330	10.8	14.1	20.3	
<i>Barosaurus</i>	k 10	femur	r	1100	400	13.1	17.1	24.6	
<i>Barosaurus</i>	Ki 2	femur	r	1190	420	13.7	17.9	25.8	
<i>Barosaurus</i>	Ki 4	femur	l	1200					
<i>Barosaurus</i>	Nr. 76	femur	l	1350	560	18.1	23.7	34.1	
<i>Barosaurus</i>	NW 4	femur	l	1350	600	20.5	26.8	38.6	14
<i>Dicraeosaurus</i>	ab 10	humerus	l	580	250	8.2	10.7	15.4	
<i>Dicraeosaurus</i>	O 3	humerus	r	610	270	8.8	11.5	16.6	
<i>Dicraeosaurus</i>	ab 2	humerus	r	620	240	7.8	10.2	14.8	
<i>Dicraeosaurus</i>	O 2	femur	r	980	350	11.4	14.9	21.5	
<i>Dicraeosaurus</i>	dd 3032	femur	r	1140					
<i>Janenschia</i>	Nr. 22	femur	l	1270	570	18.6	24.3	35.1	26
<i>Janenschia</i>	P8	humerus	l	890	420	13.9	18.1	26.1	

per mm and the mean is 4.8 laminae per mm (Fig. 2, Tab. 2). The standard deviation is rather low, with most values for lamina thickness being between 4 and 6 laminae per mm. There appears to be no dependency on taxon, bone, or ontogenetic age. Only the early growth cycles of *Apatosaurus* SMA “Jaques” have rather thick laminae. The number of laminae per growth cycle does not show a distinctive trend during ontogeny, only a slight one towards decreasing lamina thickness.

In comparing bone formed in juvenile individuals and bone formed in adult individuals, we noted that there is a slight trend towards thinner laminae in the bone formed in adult individuals as compared to the bone formed in juvenile individuals but the differences are rather small (Tab. 3). The transition from the bone formed in juvenile individuals to bone formed in adult individuals, which was tracked in specimen NHUB XVI 64l, is not characterized by a significant decrease in lam-

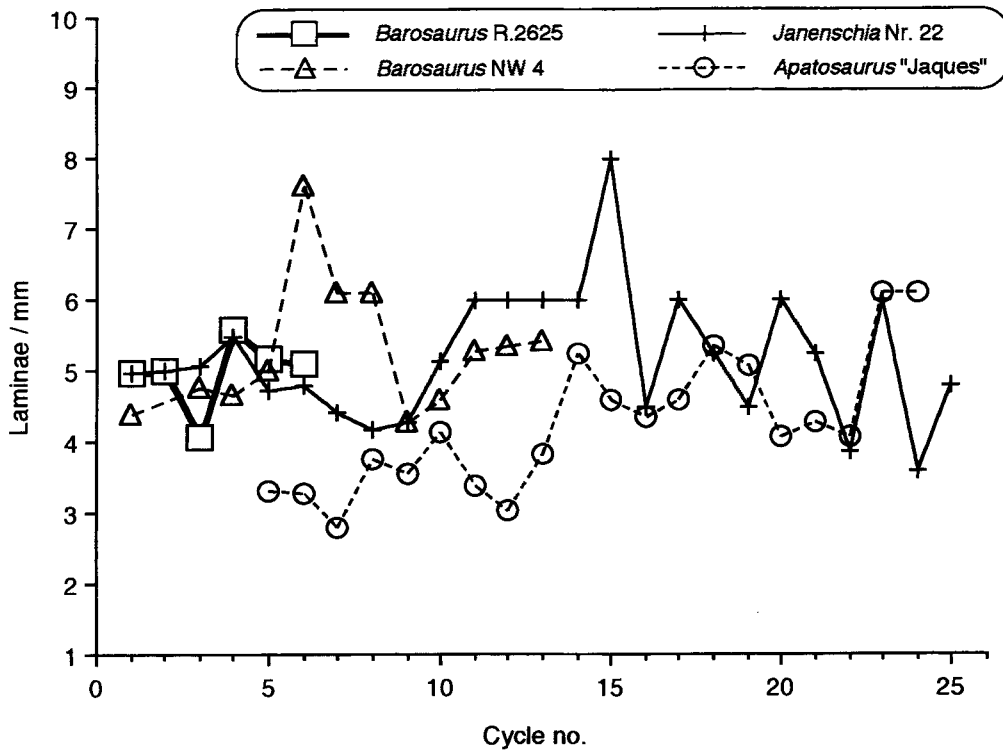


Fig. 2. Variation of lamina thickness with ontogenetic age in four sauropod long bones with cyclical growth marks. Lamina thickness is expressed in laminae per mm. The combined mean of all four samples is 4.8 laminae/mm. Note that the first five recognizable cycles of *Apatosaurus* "Jaques" where too heavily remodelled for lamina thickness to be measured.

ina thickness either (Fig. 3). This is in contrast with the marked decrease across this transition in vascular space, i.e. vascular canal diameter, in the same specimen (SANDER 2000: fig. 6).

Bone apposition rates

In the four samples with polish lines, we calculated the apposition rates of the laminar fibro-lamellar tissue per

cycle (Fig. 4, Tab. 4) in a single-cycle growth rate analysis. The highest rates are generally around 10 $\mu\text{m/day}$ and are found in bone formed in juvenile individuals. Occasionally, rates as high as 20 μm per day were obtained which are not limited to bone formed in juvenile individuals. These high values presumably are caused by the lumping of several cycles into one because of poorly developed growth marks. This is suggested by a wide gap in otherwise regularly and closely spaced

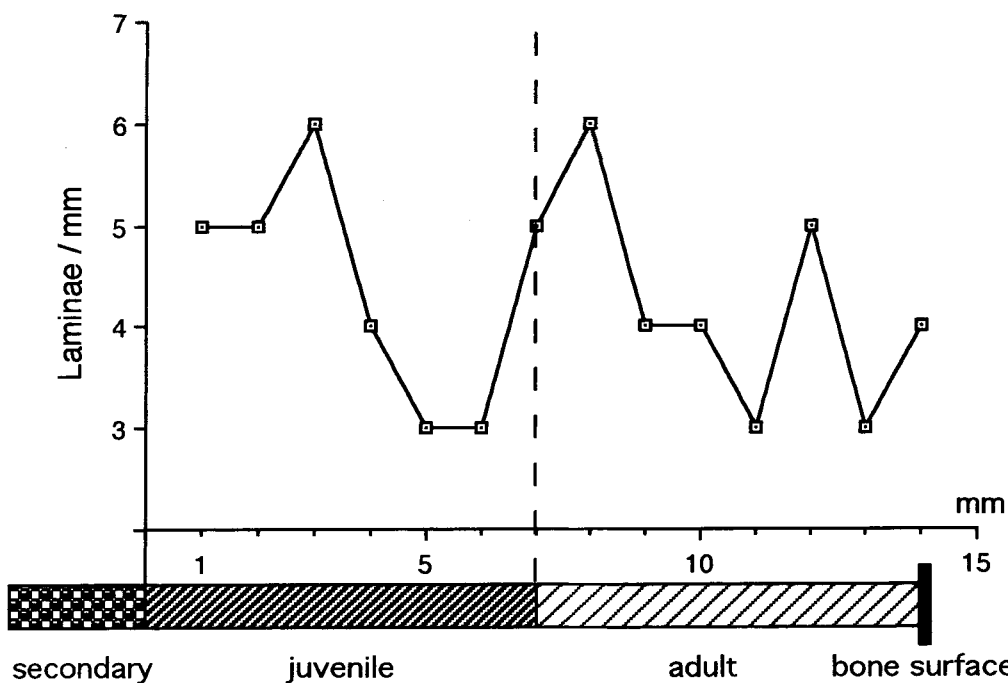


Fig. 3. Variation of lamina thickness with ontogenetic age in a single specimen, *Barosaurus* humerus XVI 641. The specimen shows bone formed in juvenile individuals in the inner cortex and bone formed in adult individuals in the outer cortex (see also SANDER 2000: fig. 6). There is only a slight trend towards thinner laminae in the bone formed in adult individuals. Lamina thickness is expressed in laminae per mm.

growth marks in the outer cortex. These high rates were excluded from the calculation of averages.

The lowest apposition rates are 2 $\mu\text{m}/\text{day}$, rarely 1 $\mu\text{m}/\text{day}$, which are usually found in the region of bone formed in adult individuals (Tab. 4). There is a general decrease of apposition rate with increasing age of the individual. The minimal apposition rate in *Barosaurus* NHUB R.2625 was 5.2 $\mu\text{m}/\text{day}$, in *Barosaurus* NHUB NW4 it was 5.1 $\mu\text{m}/\text{day}$, in *Janenschia* NHUB Nr. 22 it was 4.7 $\mu\text{m}/\text{day}$, and in *Apatosaurus* SMA "Jaques" it was 3.9 $\mu\text{m}/\text{day}$. The mean of these values is 4.73 $\mu\text{m}/\text{day}$.

The maximal rate in *Barosaurus* NHUB R.2625 was 12.1 $\mu\text{m}/\text{day}$, and in *Barosaurus* NHUB NW 4 it was 14.8 $\mu\text{m}/\text{day}$. In *Janenschia* NHUB Nr. 22 the maximal rate was 7.6 $\mu\text{m}/\text{day}$, and in *Apatosaurus* SMA "Jaques" it was 8.2 $\mu\text{m}/\text{day}$. The mean of these four values is 10.68 $\mu\text{m}/\text{day}$. The difference in the maximal apposition rate in the two *Barosaurus* specimens could be related to the different histologic types (type A in NHUB NW 4 and type B in NHUB R.2625) which possibly represent different sexes (SANDER 2000). The grand mean of the mean minimal and mean maximal rates was 7.7 $\mu\text{m}/\text{day}$.

The apposition rates calculated for the Recent ostrich long bones are much higher than those calculated for the sauropods. Their average growth rate based on all four individuals is 40.3 $\mu\text{m}/\text{day}$.

Age estimates based on apposition rates

The cortical apposition rates calculated from the two *Barosaurus* bones, the *Janenschia* bone, and the *Apto-*

saurus bone (Tab. 5) were then applied to Tendaguru sauropod bones lacking growth lines (the sample of SANDER 2000) to obtain individual age estimates (Tab. 6). For example, among the six humeri of *Barosaurus* to which the rates were applied, the estimated age ranged from 7–13 years in the smallest (43.5 cm long, G 91) to 16–30 years in the longest bone (99 cm long, A1). For the three *Barosaurus* femora, we obtained estimates of 11–20 years (102 cm long, Ki 71a) to 20–38 years (135 cm long, NW 4).

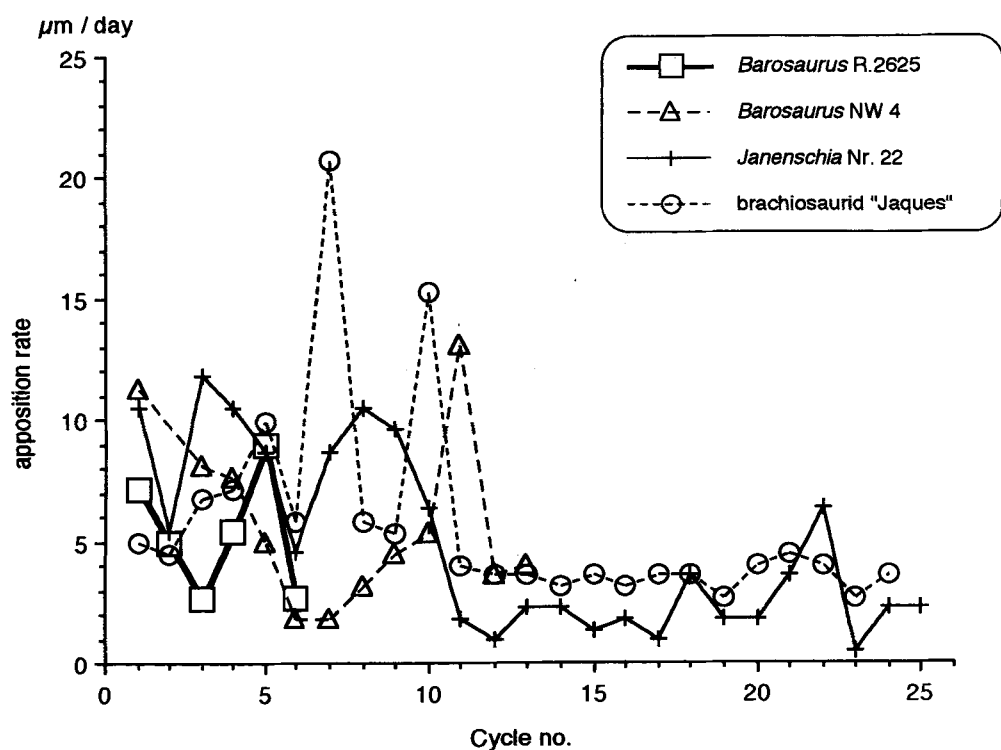
The ostrich bone apposition rates were not applied to the sauropod bones because they were so much higher than those obtained for sauropods with cyclical growth marks. If ostrich apposition rates were applied, obviously rather low age estimates of a fraction of those presented in Tab. 6 would result. The application of the apposition rates observed in laminar bone of young raptites by CASTANET et al. (2000) would yield similarly low age estimates.

Discussion

Quantification of lamina thickness

The investigation of the lamina thickness indicates that there are no significant differences between the bones sampled. The thinner laminae that might have been expected to be present in adult bone were not detected. It is indeed remarkable how little lamina thickness varies as it is almost always between 4 and 6 laminae/mm, independent of locality, systematic affinity, type of bone, and type of bone tissue (Tab. 3). However, taxon-specific variability in lamina thickness may yet prove to be

Fig. 4. Variation of apposition rates of laminar fibro-lamellar bone with ontogenetic age. Apposition rate is expressed in μm per day. The rate was computed by dividing the thickness of an appositional cycle by the number of days per year. The high outliers in the curves for *Barosaurus* NW 4 and *Apatosaurus* "Jaques" are probably due to unrecognized growth marks, resulting in the lumping of several cycles into one and accordingly very high apposition rates.



smaller than the variability observed in all sauropods once enough samples of one taxon are analyzed.

With lamina thickness being constant but growth cycle thickness (thickness of bone between two consecutive growth marks) rather variable, it becomes immediately obvious that the time of formation of an individual lamina must have greatly varied as well, and very little temporal significance can be attached to the number of lamina in the cortex of a bone. The rather uniform lamina thickness must be linked to the mode of formation of laminar fibro-lamellar bone in which a framework of vascular canals is established first which then is filled up by bone tissue. The dimension of this framework is apparently independent of apposition rate of the laminar fibro-lamellar bone. Little experimental comparative data are available in the literature, but they bear out the same observation: a lamina took about one month to lay down in a young *Bos* (RICQLÈS et al. 1991) while in a young duck (*Anas*) it formed in as little as 2.5 days (CASTANET et al. 1996). Such short times can be deduced as well for young ostriches based on the work of CASTANET et al. (2000: fig. 2c).

The observation of a lamina thickness of 4–6 laminae/mm begs comparison with other vertebrates possessing fibro-lamellar bone such as living mammals and birds. However, lamina thickness is almost never recorded in the literature, one exception being the study of CURREY (1962) who compared the lamina thickness in a prosauropod dinosaur vertebra with that in *Bos* and *Sus* bones and noted that laminae were much more closely spaced in the prosauropod vertebra than in the mammal long bones. The *Bos* femur had 5.3 laminae/mm, the *Sus* femur 10 laminae/mm, the *Bos* vertebrae 6.7 laminae/mm, and the prosauropod vertebrae 12.5 laminae/mm. Thus, there is obviously some variability in dinosaur and mammal lamina thickness, making the low variation in the sauropod long bones even more remarkable.

Bone apposition rates

The investigation of apposition rates of the laminar fibro-lamellar bone indicated that they are rather variable, ranging from under 1 μm to over 20 μm per day. Generally high rates of 4.5–11.9 $\mu\text{m}/\text{day}$ were obtained in the inner cortex, deposited early in ontogeny (Fig. 4, Tab. 4), while the lowest rates were found in the outer cortex, formed late in ontogeny. Although there are apparent deviations from this generalization with regions in the outer cortex having the highest rates of over 20 $\mu\text{m}/\text{day}$ (see Fig. 4, Tab. 4), these are probably due to unrecognized or weakly expressed growth lines, leading to inflated cycle thickness, as already discussed.

Only very few data on bone apposition rates in sauropod dinosaurs are available in the literature. RIMBLOT-BALY et al. (1995), studying a humerus, calculated an average rate of 7 μm per day in the Middle Jurassic sauropod *Lapparentosaurus* from Madagascar. This value is in general agreement with those obtained in this study.

In another study, CURRY (1998) obtained values of around 10.5 $\mu\text{m}/\text{day}$ for *Apatosaurus*. However, the bones studied by her were radii, ulnae, and scapulae which are smaller than humeri and femora, indicating that apposition rates in these larger bones of *Apatosaurus* would have been higher. The reasons for the differences in apposition rates obtained by CURRY and by us for the same genus are unclear at present.

Experimental studies of apposition rates of fibro-lamellar bones in birds shows an even greater range of rates. In the duck *Anas platyrhynchos* (MARGERIE et al. 2002, see also CASTANET et al. 1996), the rates vary from less than 20 $\mu\text{m}/\text{day}$ to up to 110 $\mu\text{m}/\text{day}$. The rates depend on the age of the individual, which bone in the skeleton was observed, and which region in the bone was studied. In young ostriches (*Struthio camelus*) and emus (*Dromaius novaehollandiae*), rates as high as 80 $\mu\text{m}/\text{day}$ were observed in the long bones of the leg (CASTANET et al. 2000), with the highest rates found in reticular fibro-lamellar bone tissue and somewhat lower rates in laminar fibro-lamellar bone tissue (around 25 $\mu\text{m}/\text{day}$). These values are thus compatible with the value of 40.3 $\mu\text{m}/\text{day}$ obtained by us for the ostriches. The faster apposition of reticular bone compared to laminar bone in ratites differs from the observation by MARGERIE et al. (2002) in *Anas* in which there was no correlation between the vascular architecture of the fibro-lamellar bone and its apposition rate. The observation for ratites by CASTANET et al. (2000) is in agreement, however, with the observations of SANDER (2000) in sauropod long bones that the fastest growing bone, formed in hatchling individuals, is of the reticular to plexiform type while the slower growing bone formed in juvenile individuals and in adult individuals is of the laminar type.

The apposition rate of laminar fibro-lamellar bone in living mammals is also poorly studied, only one value being cited by RICQLÈS et al. (1991): 2.5–4.5 $\mu\text{m}/\text{day}$ in the femoral shaft of a juvenile *Bos*. This value does compare favorably with the minimal average apposition rates observed by us in sauropods.

The rates obtained for sauropod long bones are thus compatible with those seen in living mammals and birds, with the exception the very low rates of under 1 $\mu\text{m}/\text{day}$ observed in the sample of *Janenschia* (Tab. 4). These may be due to the systematic error that the time represented by the growth lines themselves cannot be estimated reliably. Our computations are based on a negligibly short time because polish lines are a rather subtle expression of a decrease in growth rate. However, if the time of non-deposition in a polish line was in the range of weeks or month, the apposition rate of the intervening fibro-lamellar bone would have been in the range of that observed in living animals. Nevertheless, until experimental studies of polish lines clarify their duration, the apposition rates calculated for sauropods should be viewed as minimal rates.

Age estimates based on apposition rates

Are we justified in applying minimal, maximal, and intermediate apposition rates (Tab. 5) from one taxon to another, possibly only distantly, related taxon? And what about the validity of averaging apposition rates from different taxa to estimate age? These concerns are valid as indicated by the differences in histology observed in the different sauropod taxa (SANDER 2000). The ages obtained for humeri and femora from the Tendaguru beds (Tab. 6) can, of course, only be viewed as first rough estimates and not as accurate calculations. For example, in one sample (*Barosaurus* NHUBNW 4), the age suggested by growth marks is significantly lower than that estimated by apposition rate.

As in many other tetrapods, growth plateaued in sauropods well before the end of their life span, as evidenced by the closely spaced growth lines in the outermost cortex in some large individuals. Thus, once growth plateaued, the long bones ceased to increase in diameter. Age estimates based on bone circumference alone are thus minimum estimates, at least in large individuals, because the individual may have attained the growth plateau already but continued to live. This kind of error can only be avoided, if the histology of the outermost cortex of the bone in question is known.

We feel justified in producing the age estimates presented above by the complete lack of any estimates of age in sauropods based on long bone apposition rates. In addition, our results are compatible with recent work on sauropod overall growth rates and individual ages based on cyclical growth marks (CURRY 1998, 1999; SANDER 1999, 2000), suggesting ages measured in years and decades, not in centuries as assumed earlier by CASE (1978).

Conclusions

Of the two parameters investigated in this study of laminar fibro-lamellar bone in the humeri and femora of sauropod dinosaurs, the first, lamina thickness, could well be quantified. Lamina thickness is rather constant at about 5 laminae/mm (one lamina being about 200 μ m thick), independent of locality, taxon, and ontogenetic age. The only trend observable is a slight thinning of laminae with ontogeny.

The second parameter, cortical bone apposition rates, shows a range of 1–20 μ m per day with the highest rates being due to unrecognized growth lines. Apposition rate decreases with ontogenetic age. The application of these apposition rates to bones from individuals of unknown age from the Tendaguru beds only allowed a rather wide bracketing of age. However, the age estimates obtained are compatible with those derived by other methods and support the view that sauropods had growth rates comparable to those of large mammals.

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