The Journal of Experimental Biology 207, 869-879
Published by The Company of Biologists 2004
doi:10.1242/jeb.00841

Assessing a relationship between bone microstructure and growth rate: a ßuorescent labelling study in the king penguin chick (*Aptenodytes patagonicus*)

E. de Margerie[1,*], J.-P. Robin[2], D. Verrier[2], J. Cubo[1], R. Groscolas[2] and J. Castanet[1]

[1]*Adaptation et Evolution des Syst•mes OstŽo-Musculaires, FRE CNRS 2696, 2 place Jussieu, 75251 Paris Cedex 05, France* and [2]*Centre dÕEcologie et Physiologie EnergŽtiques, UPR CNRS 9010, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France*

*Author for correspondence (e-mail: margerie.e.de@club-internet.fr)

Accepted 15 December 2003

Summary

MicrostructureÐfunction relationships remain poorly understood in primary bone tissues. The relationship between bone growth rate and bone tissue type, although documented in some species by previous works, remains somewhat unclear and controversial. We assessed this relationship in a species with extreme adaptations, the king penguin (*Aptenodytes patagonicus*). These birds have a peculiar growth, interrupted 3 months after hatching by the austral winter. Before this interruption, chicks undergo extremely rapid statural and ponderal growth. We recorded experimentally (by means of ßuorescent labelling) the growth rate of bone tissue in four long bones (humerus, radius, femur and tibiotarsus) of four king penguin chicks during their fastest phase of growth (3Ð5 weeks after hatching) and identiÞed the associated bone tissue types (ÔlaminarÕ, ÔlongitudinalÕ, ÔreticularÕ or ÔradialÕ Þbro-lamellar bone tissue). We found the highest bone tissue growth rate known to date, up to 171 μm day^{Ð1} (mean 55 μm day^{Ð1}). There was a highly signiÞcant relationship between bone tissue type and growth rate ($P<10^{Ð6}$). Highest rates were obtained with the radial microarchitecture of Þbro-lamellar bone, where cavities in the woven network are aligned radially. This result supports the heuristic value of a relationship between growth rate and bone primary microstructure. However, we also found that growth rates of bone tissue types vary according to the long bone considered ($P<10^{Ð5}$) (e.g. growth rates were 38% lower in the radius than in the other long bones), a result that puts some restriction on the applicability of absolute growth rate values (e.g. to fossil species). The biomechanical disadvantages of accelerated bone growth are discussed in relation to the locomotor behaviour of the chicks during their Þrst month of life.

Key words: Þbro-lamellar bone tissue, biomechanical properties, bone microstructure, growth rate, long bone, structureÐfunction.

Introduction

The relationships between structure and function in compact bone tissues of vertebrates have been more thoroughly studied in secondary remodelled bone (e.g. human Haversian bone) than in primary bone (see review in Currey, 2002). A Þrst and essential contribution came from Amprino (1947), who predicted that the structure of primary bone tissue can be understood as the expression of growth rates. Experimental ßuorescent labelling has documented this functional relationship in some species (e.g. de BuffrŽnil and Pascal, 1984; Castanet et al., 1996, 2000). Accordingly, the current structural classiÞcation of primary bone tissues (de Ricql•s, 1975; de Ricql•s et al., 1991) relies on distinctions of tissue types mainly underlain by differing growth rates (e.g. slow-growing Ôlamellar-zonalÕ tissues*vs* fast-growing ÔÞbro-lamellarÕ tissues). However, some recent results challenge AmprinoÕs theory; de Margerie et al. (2002) found that different types of Þbro-lamellar bone tissue can grow at similar rates during the skeletal ontogeny of the mallard (*Anas platyrhynchos*), for instance. Conversely, Starck and Chinsamy (2002) reported a wide range of growth rate in the Japanese quail (*Coturnix japonica*), without any correlative shift of bone tissue type. These Þndings raise doubts about the validity of the relationship between growth rate and bone tissue type during skeletal ontogeny, especially within Þbro-lamellar (i.e. fast-growing) bone tissues. Believing that some clariÞcation of this controversy could arise from the study of a model species with extreme adaptations, we have investigated the periosteal (i.e. diametric) long bone growth of the king penguin (*Aptenodytes patagonicus* Miller).

King penguins are ßightless seabirds with a unique growth strategy (Stonehouse, 1960; Barrat, 1976): between hatching and ßedging (i.e. departure to sea), chicks spend almost one year on land in the breeding colony. They experience a Þrst phase of rapid growth (approximately 3 months between February and early May). During this phase, chicks are intensively fed by their parents; they achieve a 40Ð60-fold increase in body mass (from 0.2 kg to 9Ð12 kg) and almost reach the adult size, which is among the largest in extant

neognathe birds (1 m tall, ~12 kg; PrŽvost and Mougin, 1970; Barrat, 1976). A period of very low feeding rate follows, during the austral winter (4.5 months, May to mid-September). Chicks lose half of their body mass, and only chicks that have large initial body energy reserves have a high survival rate (Barrat, 1976; Cherel et al., 1987). The last 3.5 months in the colony (mid-September to late December) start with the recovery of an intensive parental feeding rate, see ponderal recovery of chicks and end with chick moulting, immediately followed by their departure to open sea.

Our study focused on the Þrst Ð very active Ð growth phase. Chicks were studied between their third and Þfth week, i.e. when the growth rate of their ßipper and leg bones was the highest (Stonehouse, 1960). Bone tissue growth rates were measured in the appendicular skeleton, coupled with the investigation of associated bone tissue types, which yielded appropriate data to test experimentally AmprinoÕs theory (Amprino, 1947).

Materials and methods

Animals and injections

The Þeld study was performed in the colony of the Baie du Marin, Possession Island, Crozet Archipelago (46¡26′ S, 51¡52′ E; Indian Ocean), where ~40 000 pairs of king penguins breed (Weimerskirch et al., 1992). Four wild chicks, hatched in the Þrst half of February, were triple-labelled with vital ßuorescent dyes at the ages of 2, 3 and 4 weeks to measure bone growth rate. Fluorescein and alizarin solutions (1 g per 100 ml of sterile saline) were injected intraperitoneally with sterile single-use material at a dose of 30 mg $kg^{Ð1}$ body mass and 80 mg $kg^{Ð1}$ body mass, respectively. Prior to the injections, solutions were sterilised using 0.2 µm Minisart high-ßow Þlters (Sartorius, Goettingen, Germany). Fluorescein was injected at the end of weeks 2 and 4 and alizarin was injected at the end of week 3.

During these Þrst weeks of life, chicks were either always with a parent or, during the time (~5Ð10 min) a chick was

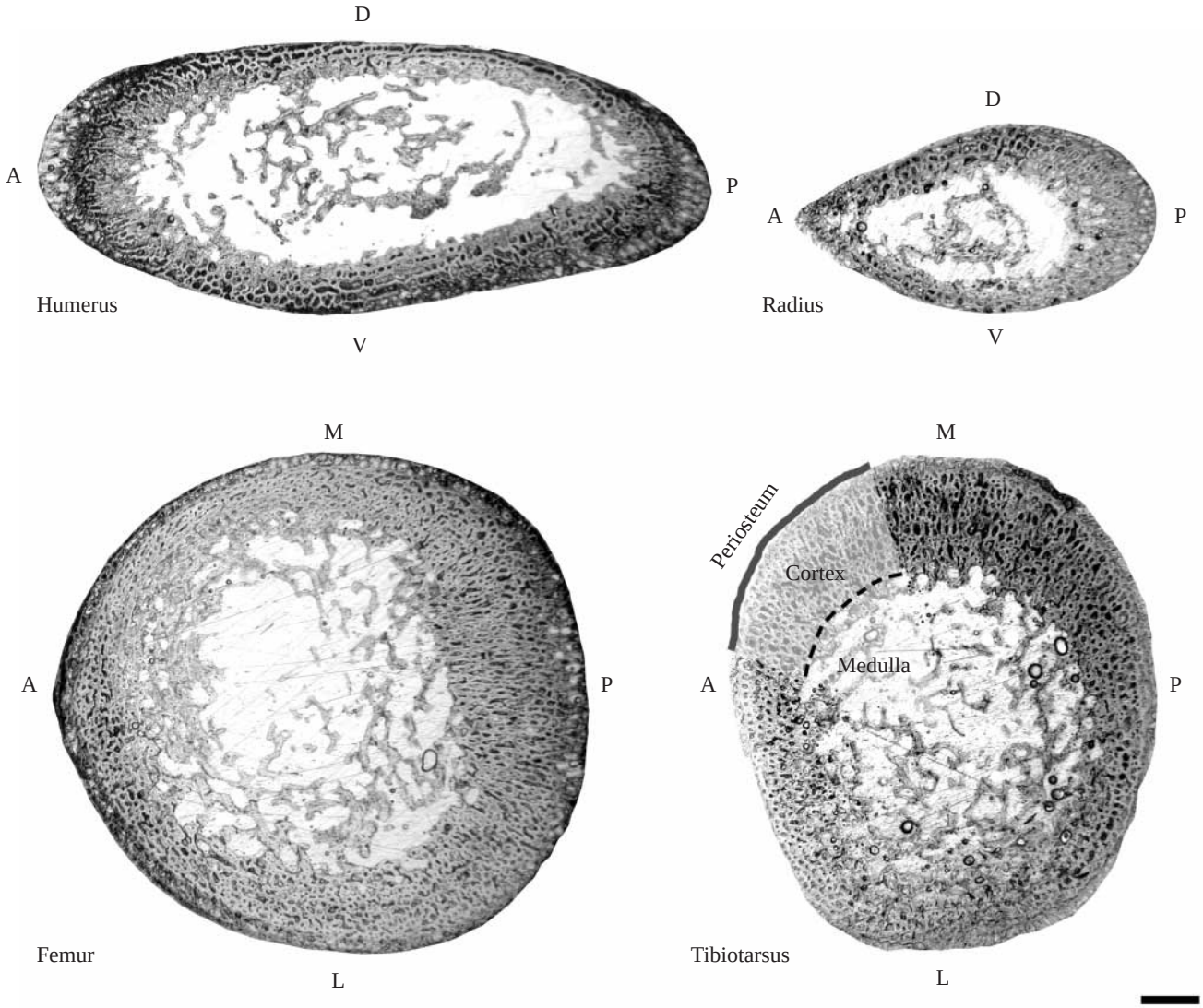

Fig. 1. Undemineralized diaphyseal thin-sections of the four bones studied (a single chick shown). Ordinary transmitted light. Anterior (A), posterior (P), dorsal (D), ventral (V), medial (M) and lateral (L) sides of bone sections are indicated. Scale bar, 1 mm.

manipulated in a nearby shelter for injections and body measurements, were exchanged with a dummy egg, a procedure well accepted by the parents. Until the chick was returned, the ÔincubatingÕ parent was continuously observed. Each time they were manipulated, birds were weighed (±10 g) and their bill, ßipper and foot lengths measured (±0.5 mm). Between each weekly manipulation, chicks were left undisturbed in the breeding colony with their parents and were observed daily. They were identiÞed with coloured Þsh-tags implanted dorsally in the skin under local anaesthesia (1 ml xylocaine 2%) at the Þrst manipulation. Each chickÕs parents were identiÞed through dye marks on the chest. The growth curve (body mass and body measurements) of the four labelled chicks was compared with that of 30 unlabelled chicks born in the same area of the colony at similar dates (identiÞed in the same manner as labelled chicks).

The labelled chicks were sacriÞced by cervical vertebrae dislocation after being emancipated (i.e. left alone in the colony by their parents between two feedings) when they were 5 weeks old (except for one chick, who died accidentally during a storm 4 days after the third labelling and suffered partly from predation). They were kept frozen (Ð20¡C) until further analysis.

The authorisation to perform these experiments in the breeding colony and to sacriÞce chicks was obtained from the Ethics Committee of the Institut Fran•ais pour la Recherche et la Technologie Polaire (present name Institut Polaire Paul-Emile Victor). The study followed the ÔAgreed Measures for the Conservation of Antarctic and Subantarctic FaunaÕ.

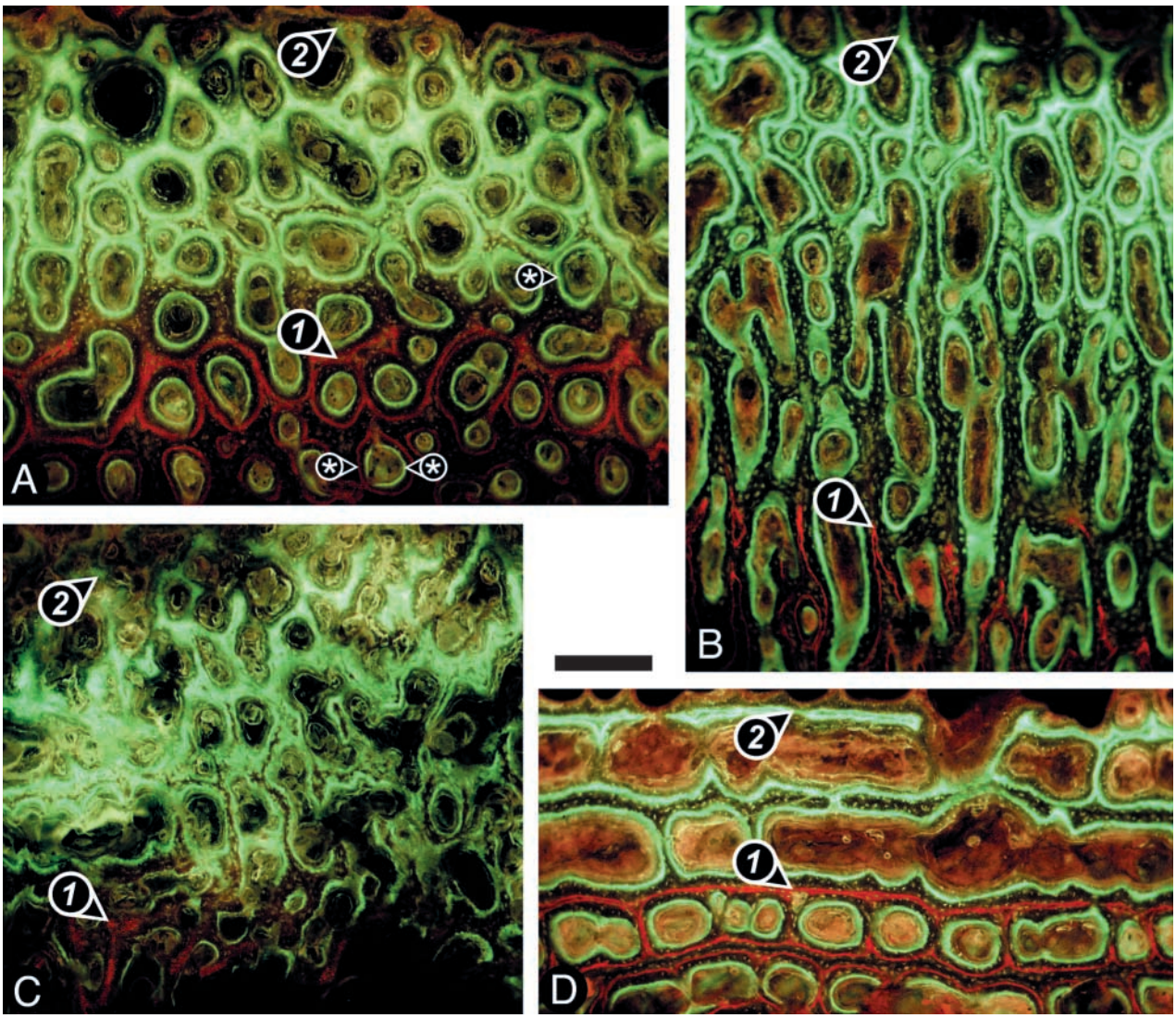

Fig. 2. The four Þbro-lamellar bone types observed in the long bones of the king penguin chick. Undemineralized diaphyseal thin-sections, observed under ultraviolet light, bone periphery at the top. Two periosteal labels are shown: (1) alizarin injection 3 weeks after hatching; (2) ßuorescein injection at 4 weeks. The outer (top) border of the two labels and the sides of the Þeld delineate a tissue ÔpatchÕ, the thickness of which was measured to yield growth rates. (A) longitudinal bone; the asterisks indicate labelling of the primary osteon Þlling; (B) radial bone; (C) reticular bone; (D) laminar bone. Scale bar, 200 μm.

Table 1. *Periosteal bone tissue types and growth rates (µm day^{D}) observed in the four chicks*

Long bone anatomical direction	Humerus				Radius				Femur				Tibiotarsus			
	A	P	D	V	A	P	D	V	A	P	L	M	A	P	L	M
Chick 1																
Week 3			Lam 14	Ret 39				Lon 14	Ret 70			Lon 65	Lon 89	Lam 60		Rad 135
Week 4	Rad 63	Lon 41	Lam 10	Lam 17	Rad 34	Rad 27	Lon 10	Lam 10	Ret 34	Rad 77	Lon 34	Lon 31	Ret 29	Lam 22	Rad 34	Rad 70
Week 5	Rad 130	Lon 34				Rad 58			Ret 58	Rad 130	Lon 92	Lon 34	Ret 48	Lam 29	Rad 77	Rad 68
Chick 2																
Week 3			Lam 14					Lon 10				Lon 63	Lon 104	Lon 75		
Week 4	Rad 125	Ret 109	Lam 17	Lam 46	Ret 82	Rad 92	Lam 39	Lon 27	Lam 46	Rad 123	Lon 75	Lon 51	Lon 39	Lon 53	Rad 41	Rad 130
Week 5	Ret 97	Rad 84		Lam 22		Ret 46			Ret 22	Rad 82	Ret 27	Lon 31	Lam 17	Lam 29	Lon 24	Lon 31
Chick 3																
Week 3			Lam 27	Lon 53			Lon 46	Ret 27	Lam 72	Rad 171		Lon 43	Lon 77	Lon 65	Lon 80	Rad 133
Week 4		Rad 116	Lam 12	Lon 41			Lon 17	Lam 14	Lam 29	Lon 70	Lon 77	Lam 27	Lon 14	Lon 36	Lon 34	Rad 84
Week 5	Rad 165	Rad 121		Rad 47	Ret 72	Rad 94			Lam 39	Rad 99	Lon 91	Lon 39	Lon 25	Lon 47	Lon 66	Lon 41
Chick 4																
Week 3			Lon 60	Lam 17			Ret 19	Lam 22	Ret 65	Rad 135	Ret 101	Lon 63	Lon 43	Lon 51	Lon 99	Lon 65
Week 4			Lam 22	Lam 24		Rad 101	Lon 7	Lam 14	Lam 39	Lon 77	Lam 36	Lam 34	Lon 27	Lon 75	Lon 68	Lon 55
Week 5	Rad 125	Rad 70			Ret 53	Ret 48	Lon 12	Lon 14	Lam 22	Rad 84	Lam 22	Lam 22				

A, anterior; P, posterior; D, dorsal; V, ventral; M, medial; L, lateral. Associated bone tissue type is given (lon, longitudinal bone; rad, radial bone; ret, reticular bone; lam, laminar bone).

Bone sections

Four long bones (humerus, radius, femur and tibiotarsus; Fig. 1) were removed per individual and embedded in polyester resin, after dehydration in graded ethanol and defatting in acetone and trichloroethylene. A 500 µm-thick mid-diaphyseal cross section was sawed out of each bone using a Presi P-100 diamond saw (Grenoble, France). Sections were glued to a glass object-holder with epoxy glue (Devcon, Riviera Beach, FL, USA) and ground to 80 µm using graded abrasive material.

Measurements of growth rates

Periosteal growth rates were measured optically under ultraviolet light on a ßuorescence microscope (Zeiss Axiovert 35; Jena, Germany; magniÞcation 50×), using the following method. Each section was observed in four orthogonal anatomical directions (e.g. anterior, posterior, dorsal and ventral). In each Þeld, three successive ßuorescent labels and the periosteum delimited three bone tissue ÔpatchesÕ (examples in Fig. 2), corresponding to week 3, week 4 or week 5 in the chickÕs growth. Each patch was characterised by a bone tissue type, identiÞed under natural light, following de Ricql•sÕ classiÞcation of bone tissues (de Ricql•s, 1975; de Ricql•s et al., 1991). Using an optical micrometer, the thickness of each patch was measured once (±10 µm) as the distance between the outer borders of labels (Fig. 2) and then divided by the time elapsed between two labels to yield a local value of growth rate. Out of a theoretical sum of 192 (three patches per direction, four directions per section, four sections per chick, four chicks), we were unable to analyse 52 patches because, occasionally, the Þrst (and even second) label was locally destroyed by perimedullar resorption. Moreover, the periosteum was sometimes difficult to locate precisely, invalidating some measures of week 5. The data from the 140 analysed patches are presented in Table 1.

Statistical analysis

We analysed our data using the following analysis of variance (ANOVA) design: growth rate (the quantitative variable) was considered as the dependent variable. Growth rates were log-transformed to satisfy the Ôhomogeneity of varianceÕ assumption of parametric ANOVA. This transformation also conferred a normal distribution to the 140 growth rate values. In agreement with the histological level of integration of our study, bone tissue patches were the statistical elements of the design (and not the four chicks, which constitute a grouping variable here). Four Þxed factors (categorical grouping variables) were assumed to have a possible effect on growth rate:

(1) chick (four levels: chick 1, chick 2, chick 3, chick 4);

Table 2. *Results of statistical tests*

ANOVA	Factor	d.f.	MS	*F*	*P*	
One-way	Chick	3	0.425	0.838	0.475	
	Error	136	0.507			
One-way	Week of growth	2	1.200	2.421	0.093	
	Error	137	0.496			
Two-way	Long bone	3	2.267	10.754	$<10^{-5}$	***
	Bone tissue type	3	8.533	40.483	$<10^{-6}$	***
	Long bone × bone tissue type	9	0.748	3.548	$<10^{-3}$	***
	Error	124	0.211			
Multiple comparisons	Long bone	HumerusÐradius			$<10^{-4}$	***
		HumerusÐfemur			0.808	
		HumerusÐtibiotarsus			0.845	
		RadiusÐfemur			$<10^{-5}$	***
		RadiusÐtibiotarsus			0.002	**
		FemurÐtibiotarsus			0.186	
		Grouping	(radius) (humerus, femur, tibiotarsus)			
	Bone tissue type	LaminarÐlongitudinal			$<10^{-3}$	***
		LaminarÐreticular			$<10^{-4}$	***
		LaminarÐradial			$<10^{-5}$	***
		LongitudinalÐreticular			0.203	
		LongitudinalÐradial			$<10^{-5}$	***
		ReticularÐradial			0.002	**
		Grouping	(laminar) (longitudinal, reticular) (radial)			

Multiple comparisons: HSD test for unequal replication Ð 5% experiment-wise error rate.
d.f., degrees of freedom; MS, mean squares; **$P<0.01$; ***$P<0.001$.

(2) week of growth (three levels: week 3, week 4, week 5);
(3) long bone (four levels: humerus, radius, femur, tibiotarsus);
(4) bone tissue type (four levels: longitudinal, reticular, radial, laminar; see below).

Unfortunately, as a consequence of working on limited and precious material, our data were insufficient to test the effects of all factors and interactions simultaneously in a complete controlled four-way ANOVA design. Indeed, our data set contains too few replications of measurements per cell in the table and a substantial amount of missing data. To circumvent this restriction, we Þrst tested the difference between chicks and between weeks of growth using one-way ANOVAs. Since these tests returned no signiÞcant effects of the two factors (see Table 2), we pooled growth rate data across chicks and weeks of growth. This reduced the number of cells in the data set and therefore increased replication of measurements within each cell. This enabled us to test the effects of the two remaining factors (long bone and bone tissue type, which are of main interest here) in a complete controlled two-way ANOVA design. *Post-hoc* multiple comparisons were carried out using HSD Tukey test for unequal replication (5% experiment-wise error rate). They allowed groupings of non-signiÞcantly different levels within each factor.

Body mass and measurements at the beginning and end of the experiment were compared using paired *t*-tests. Ages of emancipation between labelled and non-labelled chicks were compared using *t*-test. Growth curves of labelled and non-labelled chicks (Fig. 3) were compared using the 95% conÞdence intervals of their linear slopes.

STATISTICA and STATVIEW software were used to perform the analysis.

Results

Body mass and body measurements

In the labelled chicks, body mass increased from 680±160 g (mean ± S.D.) at the Þrst injection to 2370±165 g at sacriÞce, a 3.6±0.6-fold increase (paired *t*-test, d.f.=2, *t*=Ð29.721, *P*=0.001, Þnal body mass missing for the predated chick). Bill, ßipper and foot lengths increased from 39±2 mm, 86±4 mm and 75±6 mm, respectively, to 52±3 mm (d.f.=3, *t*=Ð8.385, *P*=0.004, no missing data), 151±20 mm (d.f.=3, *t*=Ð6.901, *P*=0.006) and 123±10 mm (d.f.=3, *t*=Ð12.325, *P*=0.001). Ponderal and statural growth curves of the four labelled chicks were very close to those of 30 other chicks that were not labelled (Fig. 3; only bill length daily increase differed between labelled and non-labelled chicks). Moreover, labelled chicks were emancipated at the same age (32.3±2.1 days, *N*=3; emancipation date missing for the predated chick) as non-labelled chicks (32.2±3.2 days, *N*=30) (d.f.=31, *t*=0.089, *P*=0.930). These results indicated that the labelling procedure

we used had no strong adverse effect on the chicksÕ development.

Bone microstructure

Each bone section comprised two concentric regions: a peripheral crown, or ÔcortexÕ, encircling a central area of lesser compactness, the ÔmedullaÕ (Fig. 1).

Cortex

Five-week-old chicks had bone cortices made of Þne cancellous bone (Fig. 1). This structure results from periosteal growth of ÔÞbro-lamellar boneÕ, the rapidly growing type of bone tissue (see de Ricqlès et al., 1991). Fluorescent labels (Fig. 2) demonstrated rapid periosteal bone growth, i.e. apposition of a highly lacunar woven network at the bone periphery, completed by a progressive Þlling by primary osteons in the depth of the cortex. Such Þne porosity of the whole cortex is due to fast centrifugal progression of the periosteum, exceeding the primary osteon Þlling process, which thus remains temporarily incomplete. Primary osteon orientation was quite variable, even within a single cross section. Accordingly, four Þbro-lamellar bone tissue types could be distinguished:

(1) Ôlongitudinal boneÕ (Fig. 2A): longitudinally oriented primary osteons (i.e. Ò*issu Þbro-lamellaire ^ ostŽones primaires longitudinaux dispersŽs*Ó, according to de Ricqlès, 1975; p. 89);

(2) Ôradial boneÕ (Fig. 2B): radially and longitudinally oriented primary osteons (i.e. Ò*issu rayonnant*Ó, according to de Ricqlès, 1975; p. 91);

(3) Ôreticular boneÕ (Fig. 2C): obliquely and irregularly oriented primary osteons (i.e. Ò*issu rŽticulaire*Ó, according to de Ricqlès, 1975; p. 92);

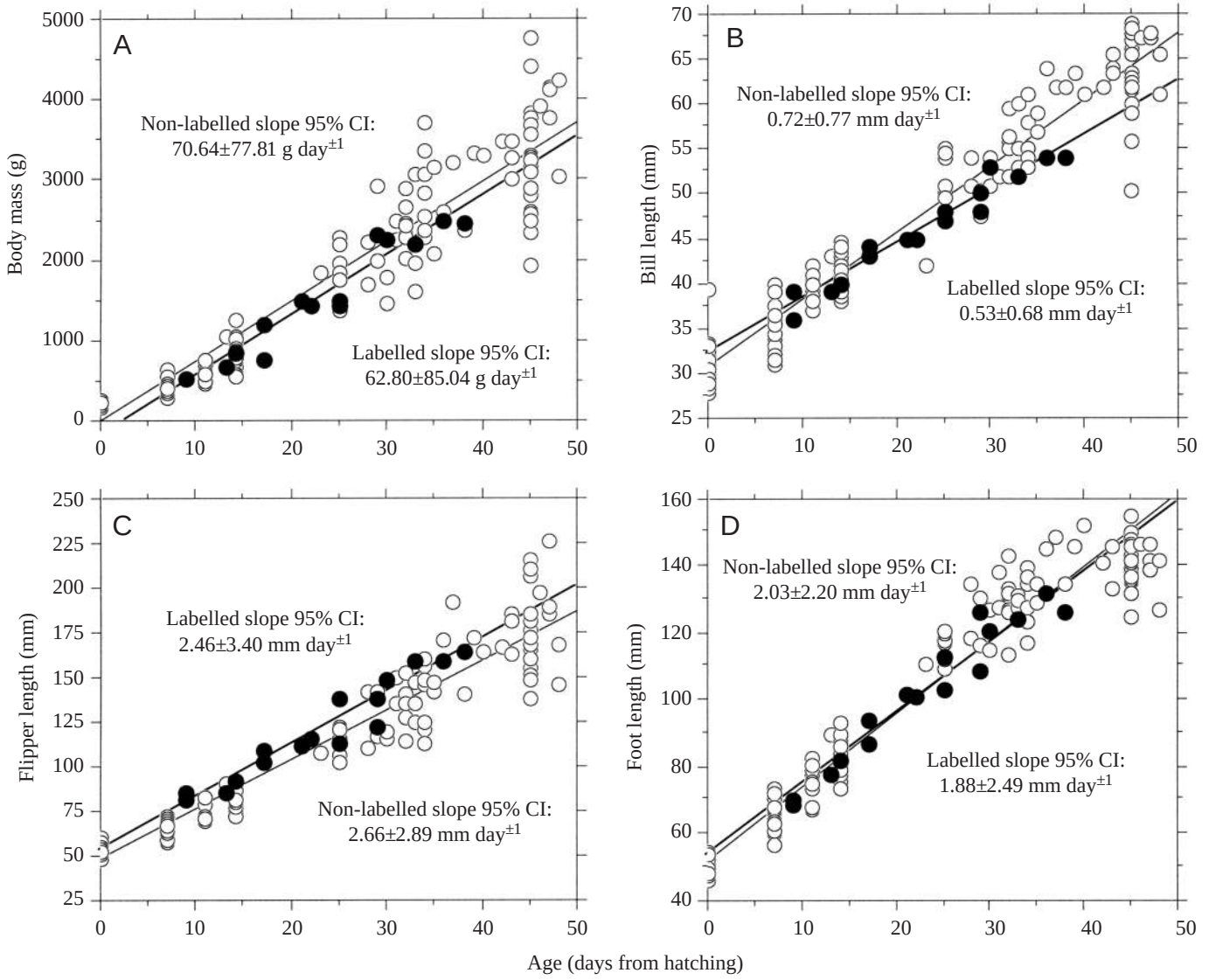

Fig. 3. Regressions of body mass (A), bill length (B), ßipper length (C) and foot length (D) on time from hatching in 30 non-labelled chicks (open circles) and in the four labelled chicks studied in the present paper (Þlled circles). Overlapping conÞdence intervals (CI) of regression slopes (except for bill length) indicate a normal growth of labelled chicks.

(4) ÔlaminarboneÕ (Fig. 2D): circularly and longitudinally oriented primary osteons (i.e. Ò*tissu laminaire* sensu strictoÓ, according to de Ricql•s, 1975; p. 90).

All four tissue types were common, exhibited by every bone of our study, with various frequencies (Fig. 4).

Finally, the chicksÕ cortices were thick, occupying the outer third of the shaftÕs radius, on average. This high cortical thickness is the result of limited perimedullar resorption (osteoclasts progressing centrifugally at the inner surface of the cortex), which proceeds slowly and/or starts late compared with periosteal apposition.

Medulla

A rather loose medullary spongiosa occupied the central area of the sections (Fig. 1). It was made of remnants of cortical primary bone, spared by perimedullar resorption. These trabeculae had been remodelled and consolidated by some endosteal bone apposition, as shown by ßuorescent labels (Fig. 5).

Periosteal bone growth rate

The 140 growth rate values ranged from 7 µm day^{-1} to 171 µm day^{-1} (mean 55 µm day^{-1}). There was no signiÞcant heterogeneity of growth rate among chicks or weeks of growth (P=0.475 and 0.093, respectively; Table 2). However, the Ôlong boneÕ factor had a highly signiÞcant effect on growth rate (P<10^{-5}; Table 2), mainly because of growth rates 38% lower in the radius than in other bones (P<0.002; multiple comparisons; Table 2; Fig. 4).

The Ôbone tissue typeÕ factor had the strongest effect on growth rate (P<10^{-6}; Table 2). Despite the four types having overlapping ranges (Fig. 4), laminar bone had signiÞcantly lower growth rates (P<10^{-3}; multiple comparisons; Table 2), while radial bone had signiÞcantly higher growth rates (P<0.002; multiple comparisons; Table 2). Radial bone was the only tissue apposited at a rate above 109 µm day^{-1}. Longitudinal and reticular bone had similar intermediate growth rates. It is noteworthy that the effect of Ôbone tissue typeÕ was measured while controlling for the Ôlong boneÕ effect (and *vice versa*; principle of a two-way ANOVA). Moreover, a signiÞcant interaction between Ôlong boneÕ and Ôbone tissue typeÕ was detected (P<10^{-3}; Table 2), which means that the two effects were not simply linearly additive. Graphical representation (Fig. 4) illustrates the results of the ANOVA; while the radius indeed has lower growth rates than other long bones, a shared pattern of increasing growth rate from laminar to radial bone is perceptible in all four long bones.

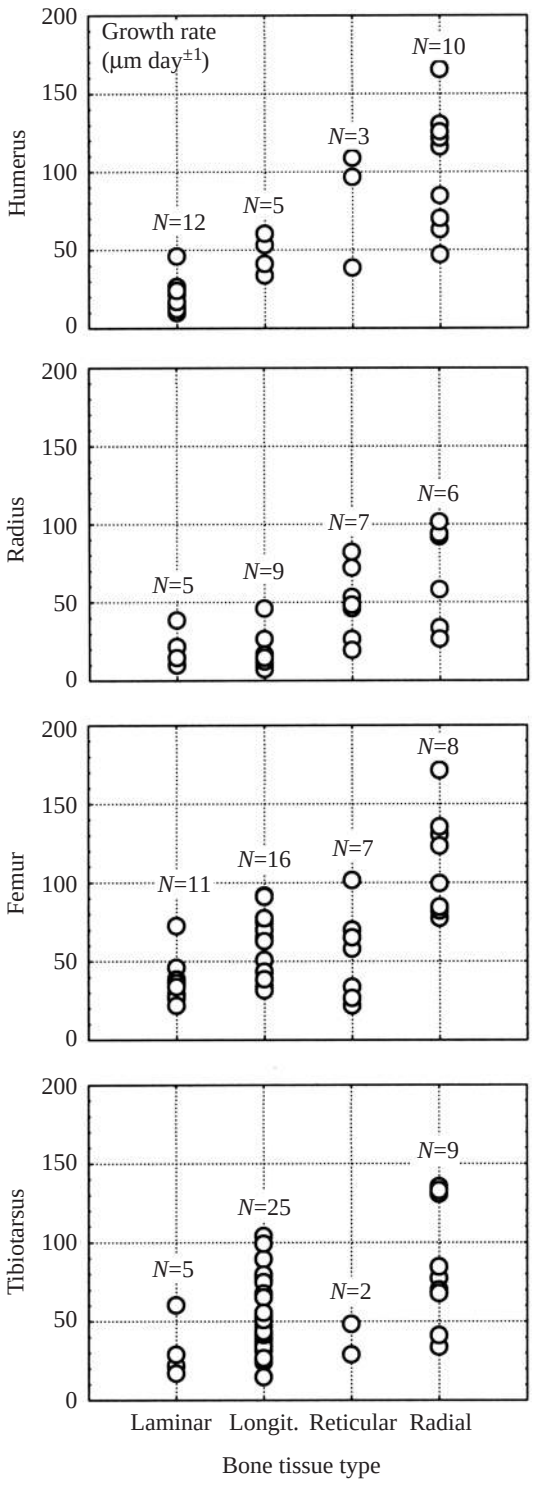

Fig. 4. Scatterplot of growth rates within tissue types and long bones. Associated detailed statistics are given in Table 2.

Discussion

High-speed osteogenesis

Fibro-lamellar bone is typical of early growth in endothermic vertebrates (review in de Ricql•s et al., 1991). Previous studies using ßuorescent labelling have measured its growth rate (e.g. de BuffrŽnil and Pascal, 1984; Castanet et al., 1996, 2000; de Margerie et al., 2002; Starck and Chinsamy, 2002) and all found high rates of periosteal bone growth (>5Ð10 µm day^{-1}) compared with other bone tissues (i.e. lamellar-zonal tissues).

The range we report in king penguin chicks (7Ð171 µm day^{-1}) is congruent with those previous Þndings.

Moreover, it extends the known range of periosteal growth rates up to previously unreported values; the highest rate was previously 112 μm day^{-1}, observed locally in the Mallard chick (de Margerie et al., 2002). High-speed osteogenesis in early growth of the king penguin might be required by peculiar life history traits in this species (i.e. large body size and only 3 months available to reach that size before winter starvation). Even the Emperor Penguin (*Aptenodytes forsteri*), which is larger and hatches further south on sea ice during austral winter, has a more evenly spread growth, and thus may not grow as fast as the king penguin (Stonehouse, 1960; p. 60).

Test of AmprinoÕs theory

According to our results, a relationship between growth rate and bone tissue type does exist in king penguin chicks, even after controlling for other factors (e.g. long bone); a special type of Þbro-lamellar bone tissue (i.e. radial bone) grows faster, while another type (i.e. laminar bone) has comparatively moderate rates of growth. Nevertheless, aside from this central result supporting AmprinoÕs prediction, some other Þndings indicate that AmprinoÕs theory must be applied carefully.

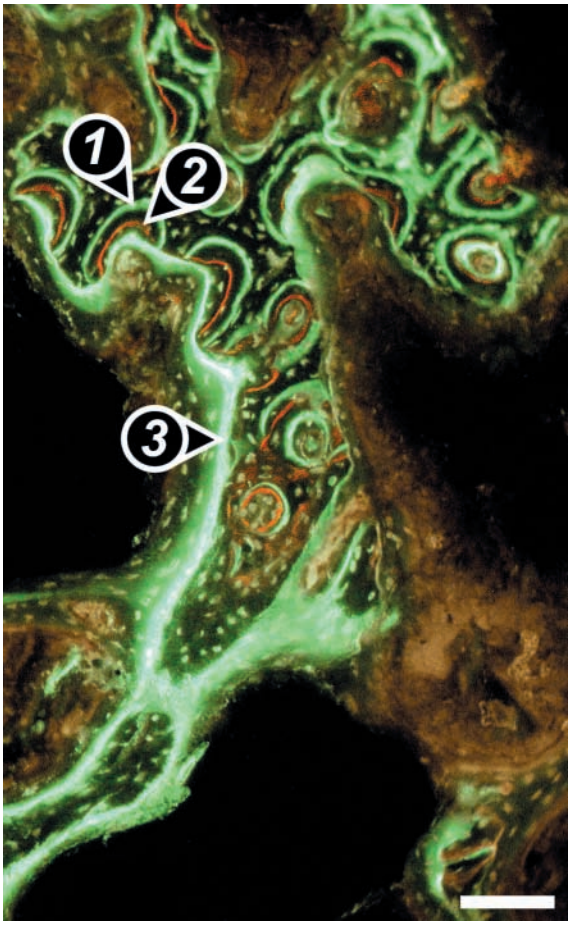

Fig. 5. Spongiosa (femur). The core of the trabeculae is constituted by remnants of primary periosteal bone, labelled at the time of its original apposition: label 1 (ßuorescein injection at 2 weeks) and label 2 (alizarin injection at 3 weeks) can be seen in partly resorbed primary osteons. Locally, resorption has stopped and some endosteal bone has been apposited (label 3; ßuorescein injection at 4 weeks). Scale bar, 100 μm.

First, we found a signiÞcant effect of Ôlong boneÕ on growth rate, even after controlling for the effect of Ôbone tissue typeÕ. This means that a given bone tissue type can have different absolute growth rates in different parts of the skeleton. Starck and Chinsamy (2002) also reported a Ôlong boneÕ effect on growth rate of longitudinal bone tissue in the Japanese quail. Moreover, the signiÞcant interaction we observed between factors attests once more that the relationship between bone tissue type and growth rate can vary somewhat across parts of the skeleton.

Second, growth rate within each tissue type is highly variable, and ranges extend widely. This point had been raised in other species (Castanet et al., 2000; de Margerie et al., 2002; Starck and Chinsamy, 2002). For instance, Starck and Chinsamy (2002) observed a range of 10Ð50 μm day^{-1} for longitudinal bone in the Japanese quail. Consequently, when several tissue types are observed (as in the present study), there is a consequential overlapping of growth rate ranges between tissue types (Fig. 4), and some differences fall below the signiÞcance threshold (e.g. comparison between longitudinal and reticular bone; Table 2). Variations in porosity might explain such variation of growth rates within tissue types (de Margerie et al., 2002; Starck and Chinsamy, 2002).

Far from nullifying AmprinoÕs theory, which becomes more widely documented after the present work, the preceding points still have restrictive outcomes. A single and precise growth rate value can hardly be extracted from bone type. Moreover, extrapolation of extant growth rates to fossils on the basis of a shared bone tissue type (e.g. Curry, 1999; Horner et al., 2000; Padian et al., 2001) should be conducted very carefully, as already emphasised (de Margerie et al., 2002; Starck and Chinsamy, 2002).

Role of radial bone

Radial bone has the fastest growth and is the only tissue type found at rates of 109Ð171 μm day^{-1} (Fig. 4). Radial bone has already been observed in the bones of some wild or domesticated tetrapods (Table 3), especially in immature specimens, but its growth rate had never been measured. Nevertheless, in the light of comparative paleohistological observations, de Ricql•s (1977; p. 139) already hypothesised that radial primary osteonal orientation could be the signature of very rapid bone growth. Moreover, in aviculture, radial bone is observed in the limb bones of birds artiÞcially selected for rapid growth (DŠmmrich and Rodenhoff, 1970; Itakura and Yamagiwa, 1970; Leblanc et al., 1986; Leterrier and Nys, 1992; Wyers et al., 1993). Finally, radial bone has not been observed in growth series of mallard (Castanet et al., 1996; de Margerie et al., 2002), ostrich (*Struthio camelus*) or emu (*Dromaius novaehollandiae*; Castanet et al., 2000), where growth rates did not attain the highest values measured here in the king penguin. These results suggest that radial bone could be a microstructural adaptation permitting higher rates of

Table 3. *Previous reports of radial Þbro-lamellar bone in wild or domesticated tetrapods*

Species	Taxon	Reference	Material
Placodus sp.	Diapsida, Sauropterygia, Placodontia	de BuffrŽnil and Mazin (1992; plate I)	Adult humerus, fossil
Notosollasia sp.	Synapsida, Therapsida, Therocephalia	de Ricql•s (1969; plate IV)	Juvenile radius, fossil
Macropus sp.	Synapsida, Mammalia, Metatheria	Amprino and Godina (1947; plate XIV)	Juvenile metatarsus, extant
Canis familiaris	Synapsida, Mammalia, Eutheria	Torzilli et al. (1982; Þg. 4a)	Juvenile femur, extant
Bos taurus	Synapsida, Mammalia, Eutheria	Smith (1960; plate II)	Juvenile metacarpus, extant
Homo sapiens	Synapsida, Mammalia, Eutheria	Foote (1916; plate XXI)	Foetal femur, extant
		Amprino and Bairati (1936; p. 442)	Foetal femur, extant

Additional data from the aviculture literature are discussed in the text.

diametric bone growth, within an already fast-growing tissue type (i.e. Þbro-lamellar bone). Functionally, the way through which the radial microarchitecture yields faster growth remains unclear. This would require Þne comparative ultrastructural analysis of bone production by the periosteum, which was not conducted here. Nevertheless, we do notice that radial struts of woven bone can be produced continuously by the periosteum, contrary to a discontinuous ÔsaltatoryÕ pattern in other bone tissue types (as illustrated in Fig. 6).

Biomechanical considerations

It is well-known that Ò*mature function and growth are antagonistic... features*Ó (Ricklefs et al., 1994). A Ò*trade-off between growth rate and functional maturity*Ó is a general constraint in various tissues of vertebrate organisms (review in Ricklefs et al., 1998). For example, Carrier and Leon (1990) addressed a Ò*conßict between development and skeletal function*Ó. Therefore, the question of the biomechanical outcome of very rapid bone growth in the king penguin chick arises. Although this point would deserve experimental investigations (i.e. mechanical testing), two facts already support the idea that king penguin chick bone tissue has indeed poor mechanical attributes: (1) high cortical thickness in chicks, as observed in our material, is known to partly compensate for low resistance of the bone tissue, as Carrier and Leon (1990) pointed out in the California gull; (2) during their Þrst month (i.e. before emancipation), king penguin chicks have a parsimonious locomotor behaviour: a chick always remains in the immediate vicinity of its parents (curled up in the brood patch or just standing close to it). The needs for active locomotion are very low, as they do not have to escape from predators, for example. As in other altricial birds, growth itself, rather than body support and transmission of forces, becomes the primary function of the birdÕs skeleton (Starck, 1998). Moreover, research in aviculture has shown that birds artiÞcially selected for high growth rates tend to have

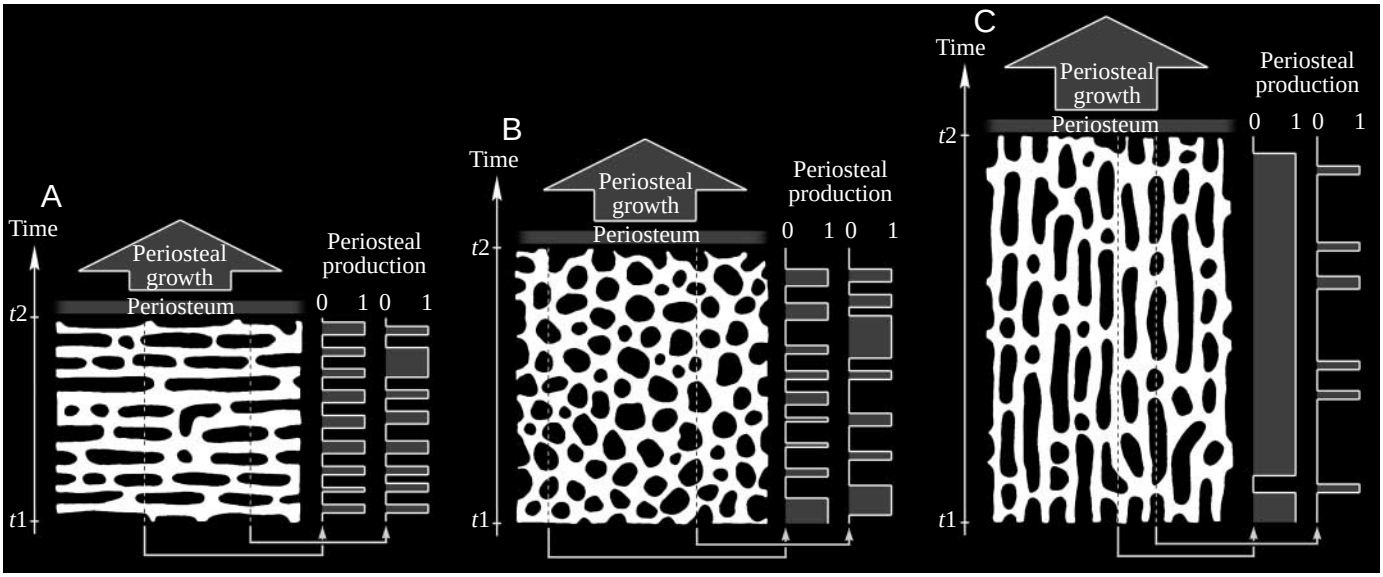

Fig. 6. A putative explanation of faster growth in radial bone. Schematic cross sections of tissues. (A) Laminar bone. Circumferential alignment of woven bone struts results from a saltatory and discontinuous activity of the periosteum (proÞles expressed on the right of each section). Repeated onset and arrest of production are suspected to be time-consuming. (B) Longitudinal bone. Same phenomenon as in laminar bone (although less pronounced). (C) Radial bone. Radially aligned bone struts are produced continuously by the periosteum. Efficiency of growth is increased. This holds independent of bone porosity.

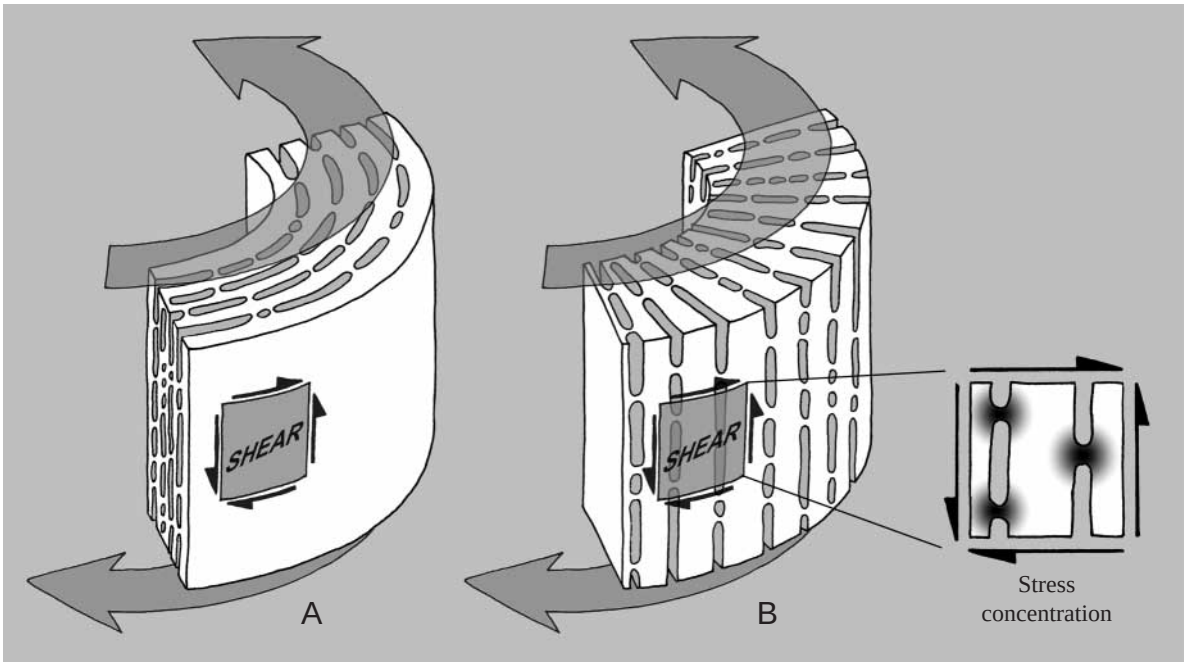

Fig. 7. Interaction between osteonal orientation and shear ßow, under torsional load. Schematic diagrams of tissues. (A) In laminar bone, shear ßow is continuous in the circumferencial ÔsheetsÕ of bone. (B) In radial bone, radial cavities interrupt the shear ßow, because they go through circumferential planes. Bone tissue near cavities undergoes highly concentrated stresses and will yield prematurely. Longitudinal bone would have intermediate characteristics.

weaker long bones (e.g. Leterrier and Nys, 1992), sometimes resulting in pathological conditions (DŠmmrich and Rodenhoff, 1970; Itakura and Yamagiwa, 1970).

It is likely that radial bone has the most detrimental effect on mechanical resistance, because radial cavities between bone struts interrupt the shear ßow around bone (Fig. 7) and concentrate stresses at their corners. These Ôopen section effectsÕ are known to dramatically reduce stiffness and strength, as has been modelled for torsional loads (Elias et al., 2000). Conversely, it has been proposed that laminar bone, which incidentally grows more slowly, would have better mechanical properties (de Margerie, 2002).

Adult king penguins exhibit strong (Currey, 2002; p. 130), matured bone (completed primary osteons, peripheral layer of lamellar-zonal bone, intracortical Haversian bone, perimedullar layer of endosteal bone; Meister, 1962). The timing of the bone maturation process in juveniles with regard to winter fast and to the onset of full locomotor activity (i.e. departure to sea after the Þrst year on land) remains to be studied.

The authors would like to thank N. Lambert and J.-C. Fournier for their expert advice in the tagging and manipulating of young chicks in the breeding colony and M.-M. Loth for technical help during the histological preparations. We are grateful to V. de BuffrŽnil, M. Laurin and A. de Ricql•s for comments on earlier versions of the manuscript and to A. Chagnon and A. Camproux for advice on statistical methods. Financial support was provided by the Institut Fran•ais pour la Recherche et la Technologie Polaires (present name Institut Polaire Paul-Emile Victor; Programme 119) and logistical support by the Terres Australes et Antarctiques Fran•aises.

References

Amprino, R. (1947). La structure du tissu osseux envisagŽe comme expression de diffŽrences dans la vitesse de lÕaccroissement*Arch. Biol.* **58**, 315-330.

Amprino, R. and Bairati, A. (1936). Processi di ricostruzione e di riassorbimento nella sostanza compatta delle ossa dellÕuomo.*Z. Zellforsch.* **24**, 439-511.

Amprino, R. and Godina, G. (1947). La struttura delle ossa nei vertebrati. *Com. Pont. Acad. Sci.* **9**, 329-463.

Barrat, A. (1976). Quelques aspects de la biologie et de lÕŽcologie du manchot royal (*Aptenodytes patagonicus*) des ”les Crozet. *CNFRA* **40**, 9-52.

Carrier, D. and Leon, R. L. (1990). Skeletal growth and function in the California gull (*Larus californicus*). *J. Zool. Lond.* **222**, 375-389.

Castanet, J., Curry-Rogers, K., Cubo, J. and Boisard, J. J. (2000). Periosteal bone growth rates in extant ratites (ostriche and emu). Implications for assessing growth in Dinosaurs. *C.R. Acad. Sci. III Vie* **323**, 543-550.

Castanet, J., Grandin, A., Abourachid, A. and de Ricql•s, A. (1996). Expression de la dynamique de croissance osseuse dans la structure de lÕos pŽriostique chez *Anas platyrhynchos*. *C. R. Acad. Sci. III Vie* **319**, 301-308.

Cherel, Y., Stahl, J. C. and Le Maho, Y. (1987). Ecology and physiology of fasting in king penguin chicks. *Auk* **104**, 254-262.

Currey, J. D. (2002). *Bones: Structure and Mechanics*. Princeton: Princeton University Press.

Curry, K. A. (1999). Ontogenetic histology of *Apatosaurus* (Dinosauria: sauropoda): new insights on growth rates and longevity. *J. Vert. Pal.* **19**, 654-665.

DŠmmrich, K. and Rodenhoff, G. (1970). SkelettverŠnderungen bei MastkŸken.*Zbl. Vet. Med. B* **17**, 131-146.

de BuffrŽnil, V. and Mazin, J.-M. (1992). Contribution de lÕhistologie

osseuse ˆ lÕinterprŽtation palŽobiologique du genre *Placodus* AGASSIZ, 1833 (Reptilia, Placodontia). *Rev. PalŽobiol.* **11**, 397-407.
de BuffrŽnil, V. and Pascal, M. (1984). Croissance et morphogen•se postnatales de la mandibule du vison (*Mustela vison*, Schreiber): donnŽes sur la dynamique et lÕinterprŽtation fonctionnelle des dŽp™ts osseux mandibulaires. *Can. J. Zool.* **62**, 2026-2037.
de Margerie, E. (2002). Laminar bone as an adaptation to torsional loads in ßapping ßight. *J. Anat.* **201**, 521-526.
de Margerie, E., Cubo, J. and Castanet, J. (2002). Bone typology and growth rate: testing and quantifying ÒAmprinoÕs ruleÓ in the mallard (*Anas platyrhynchos*). *C. R. Biol.* **325**, 221-230.
de Ricql•s, A. (1969). Recherches palŽohistologiques sur les os longs des TŽtrapodes. II. Quelques observations sur la structure des os longs des ThŽriodontes. *Ann. PalŽont. (VertŽbrŽs)* **55**, 1-52.
de Ricql•s, A. (1975). Recherches palŽohistologiques sur les os longs des TŽtrapodes. VII: Sur la classiÞcation, la signiÞcation fonctionnelle et lÕhistoire des tissus osseux des TŽtrapodes. Premi•re partie: structures. *Ann. PalŽont. (VertŽbrŽs)* **61**, 51-129.
de Ricql•s, A. (1977). Recherches palŽohistologiques sur les os longs des TŽtrapodes. VII: Sur la classiÞcation, la signiÞcation fonctionnelle et lÕhistoire des tissus osseux des TŽtrapodes. Deuxi•me partie: fonctions (Þn). *Ann. PalŽont. (VertŽbrŽs)* **63**, 133-160.
de Ricql•s, A., Meunier, F. J., Castanet, J. and Francillon-Vieillot, H. (1991). Comparative microstructure of bone. In *Bone*, vol. 3 (ed. B. K. Hall), pp. 1-78. Boca Raton: CRC Press.
Elias, J. J., Frassica, F. J. and Chao, E. Y. S. (2000). The open section effect in a long bone with a longitudinal defect Ð a theoretical modeling study. *J. Biomech.* **33**, 1517-1522.
Foote, J. S. (1916). *A Contribution to the Comparative Histology of the Femur*. Washington, DC: Smithsonian Institution Publication 2382.
Horner, J. R., de Ricql•s, A. and Padian, K. (2000). Long bone histology of the hadrosaurid dinosaur *Maiasaura peeblesorum*: growth dynamics and physiology based on an ontogenetic series of skeletal elements. *J. Vert. Paleont.* **20**, 115-129.
Itakura, C. and Yamagiwa, S. (1970). Histopathological studies on bone dysplasia of chickens. I. Histopathology of the bone. *Jap. J. Vet. Sci.* **32**, 105-117.
Leblanc, B., Wyers, M., Cohn-Bendit, F., Legall, J.-M. and Thibault, E. (1986). Histology and histomorphometry of the tibia growth in two turkey strains. *Poult. Sci.* **65**, 1787-1795.
Leterrier, C. and Nys, Y. (1992). Composition, cortical structure and mechanical properties of chicken tibiotarsi: effect of growth rate. *Br. Poult. Sci.* **33**, 925-939.
Meister, W. (1962). Histological structure of the long bones of penguins. *Anat. Rec.* **143**, 377-386.
Padian, K., de Ricql•s, A. J. and Horner, J. R. (2001). Dinosaurian growth rates and bird origins. *Nature* **412**, 405-408.
PrŽvost, J. and Mougin, J. L. (1970). *Guide des Oiseaux et Mammif•res des Terres Australes et Antarctiques Fran•aises*. Paris: Delachaux et NiestlŽ.
Ricklefs, R. E., Shea, R. E. and Choi, I. H. (1994). Inverse relationship between functional maturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. *Evolution* **48**, 1080-1088.
Ricklefs, R. E., Starck, J. M. and Konarzewski, M. (1998). Internal constraints on growth in birds. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 266-287. Oxford: Oxford University Press.
Smith, J. W. (1960). Collagen Þbre patterns in mammalian bone. *J. Anat.* **94**, 329-344.
Starck, J. M. (1998). Structural variants and invariants in avian embryonic and postnatal development. In *Avian Growth and Development* (ed. J. M. Starck and R. E. Ricklefs), pp. 59-88. Oxford: Oxford University Press.
Starck, J. M. and Chinsamy, A. (2002). Bone microstructure and developmental plasticity in birds and other dinosaurs. *J. Morphol.* **254**, 232-246.
Stonehouse, B. (1960). The king penguin *Aptenodytes patagonica* of South Georgia. I. Breeding behaviour and development. *Falkland Isl. Depencies Surv. Sci. Rep.* **23**, 1-80.
Torzilli, P. A., Takebe, K., Burstein, A. H., Zika, J. M. and Heiple, K. G. (1982). The material properties of immature bone. *J. Biomech. Eng.* **104**, 12-20.
Weimerskirch, H., Stahl, J. C. and Jouventin, P. (1992). The breeding biology and population dynamics of king penguins *Aptenodytes patagonica* on the Crozet Islands. *Ibis* **134**, 107-117.
Wyers, M., Hamon, J. C., Cherel, Y. and Plassiart, G. (1993). Histology and histomorphometry of the tibial diaphyseal growth in two turkey strains during the Þrst six weeks after hatching. *Anat. Histol. Embryol.* **22**, 48-58.