Histological examination of the antlerogenic region of red deer (*Cervus elaphus*) hummels

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Abstract

Hummels are antlerless red deer stags. This abnormality has been attributed to poor feeding conditions which result in failure by the deer to grow complete pedicles. Incomplete pedicles lack the potential to develop normal antlers. The histological examinations in this study showed that the rudimentary pedicles (5 mm and 15 mm in height) grown by the two hummels respectively were developed by intramembranous ossification. This is the way that the frontal lateral crests of normal deer are formed, while, in contrast, antlers grow by endochondral ossification. The transition between the ossification types is most important, and occurs during pedicle growth. Therefore, we suggest that the antlerless condition of hummels is due to their failure to initiate true pedicle growth (which has the potential to develop into an antler) because the change from one ossification type to another does not occur. In view of the fact that both hummels were very small in terms of weight (33.7 kg and 51.8 kg, respectively) for their age, it is possible that nutritional cues are necessary for complete pedicle formation. Presumably these cues act synergistically with androgens for the development of the pedicle/antler as a secondary sexual character. It is possible that nutrition controls pedicle initiation through the IGF1 pathway. (New Zealand Veterinary Journal 44, 126–130, 1996.)

Introduction

Hummels are antlerless red deer stags and have been frequently reported in the literature[1][2][3][4]. As hummels mainly occur in nutritionally impoverished habitats, and as their offspring all grow antlers, this abnormality has been attributed to the poor feeding conditions early in their life and may not be controlled by a simple genetic relationship[5][6].

Red deer are not born with pedicles, but these develop from the antlerogenic periosteum[7] overlying the frontal lateral crests (≥5 mm in height) when their live weights reach about 56 kg (4–6 months old)[8]. Normally, male red deer calves start to grow their antlers from the permanent pedicles when they are about 8 months old[9]. Prepubertal castration of stags will completely prevent pedicle and antler formation[10]. Hummels have either very poorly developed pedicle rudiments (<20 mm in height) or no development at all from the frontal lateral crests[3]. In order to illustrate the role of wound healing in the regeneration of antlers in normal stags[3][5], the frontal lateral crests/rudimentary pedicles of hummels were experimentally amputated at the apex. After wound healing, antlers were formed. Consequently Lincoln *et al.*[3] concluded that the primary abnormality of the hummel lay not in its inability to grow antlers, but in its failure to produce complete pedicles from which normal antler tissue could differentiate. The ability of the pedicle to produce antler tissue is acquired during its formation in the deer's early life, but this ability can be readily gained by an incomplete pedicle in response to wounding in the deer's adult life.

Histological examination of the incomplete pedicles grown by hummels have not yet been reported. Li and Suttie[9] studied the histological structure of antlerogenic periosteum and histogenesis of the frontal lateral crest, pedicle and first antler in red deer. They found that antlerogenic periosteum consists of two layers, an outer fibrous layer and an inner cellular layer. The thickness and cell density of these layers are related to nutritional status, and a change in ossification type from intramembranous through a transition to modified endochondral must be accomplished before a pedicle could proceed to an antler. Therefore, incomplete pedicle formation in hummels could reflect a failure in the ossification type to change either from intramembranous to transitional, or from transitional to endochondral.

The aim of the study was to use light microscopic techniques to examine the histological structure of the frontal lateral crests/rudimentary pedicles grown by hummels and to compare the results of the present examination with those of the pedicles grown by normal red deer for further elucidating underlying mechanisms of pedicle and antler formation.

Materials and Methods

Animals

Two hummels were identified from a group of deer slaughtered at a Dunedin deer slaughtering premises. The first animal (Hummel 1), which had unpalpable pedicles about 5 mm in height (frontal lateral crests), was killed on 1 June 1994, and weighed 33.7 kg. The other

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(Hummel 2), which had 15 mm high pedicles, was killed on 24 June and weighed 51.8 kg. Both animals were 18 months old. Two 6-month-old normal red deer stags with similarly sized frontal lateral crests or incipient pedicles were selected from a deer herd grazing on the pasture of Invermay Flat Deer Yard for comparison. One (Normal 1) weighed 43 kg and had unpalpable pedicles (crests), and the other (Normal 2) weighed 56 kg and had 15 mm high pedicles. Biopsies of the frontal lateral crest and the pedicle, respectively, of the normal deer were carried out on 30 June.

Tissue samples

The sampling procedures, either from slaughtered deer heads or biopsy, follow those described by Li and Suttie[9], and are summarised below.

Hummels

A crescent-shaped incision was made on the scalp of the slaughtered hummel head medially to the crest/pedicle. After reflecting the overlying skin, the upper part (3 mm in height) of each crest was taken from Hummel 1, and the upper parts of the rudimentary pedicles (10 mm in height) were taken from Hummel 2.

Normal red deer stags

The two normal deer were sedated with xylazine (Rompun, Bayer NZ Ltd; 0.75 mg/kg live weight) after a 24 h fast, and anaesthetised with a mixture of halothane, nitrous oxide, and oxygen following intubation. The surgery was carried out under aseptic conditions. Similar sampling procedures were used as those for hummels. Thus the upper parts of the crests (3 mm in height) and the incipient pedicle buds (10 mm in height) were taken from Normal 1 and Normal 2, respectively. After the surgery, the incisions were sutured with silk.

Histology

All of the sampled tissues were fixed in 10% buffered formalin immediately after removal. After decalcification with commercial de-calcification solution (Raymond Lamb "RDC", BDH Chemical NZ Ltd), tissue blocks were embedded in paraffin wax and sectioned at 5 μm. Gill's haematoxylin and alcoholic phloxine/eosin (H&E) stains, with or without alcian blue counter staining, were used for staining. The sections were examined and photographed using an dissecting microscope (Olympus SZH10) and a microscope (Zeiss Axioplan).

The quantification of the antlerogenic periosteum was made following the same method used by Li and Suttie[9]. The thicknesses of the cellular layers were measured at five different sites, except for that of Normal 2, as the boundary between the cellular layer and the underlying bone no longer existed in that deer. The cell density of the cellular layers was counted in three different areas. This replication was used to analyse differences between individual animals by least squares using Student's *t* test. The ossification type by which the crests/rudimentary pedicles were growing was evaluated by comparing them with the pattern those occurring in normal pedicle formation[9].

Results

Normal 1

The crest could be divided longitudinally into two portions, antlerogenic periosteum and underlying bone. The periosteum consisted of two layers, an outer fibrous layer and an inner cellular layer (Figure 1a). The cellular layer was composed of osteogenic (antlerogenic) cells and fine fibres. The cells

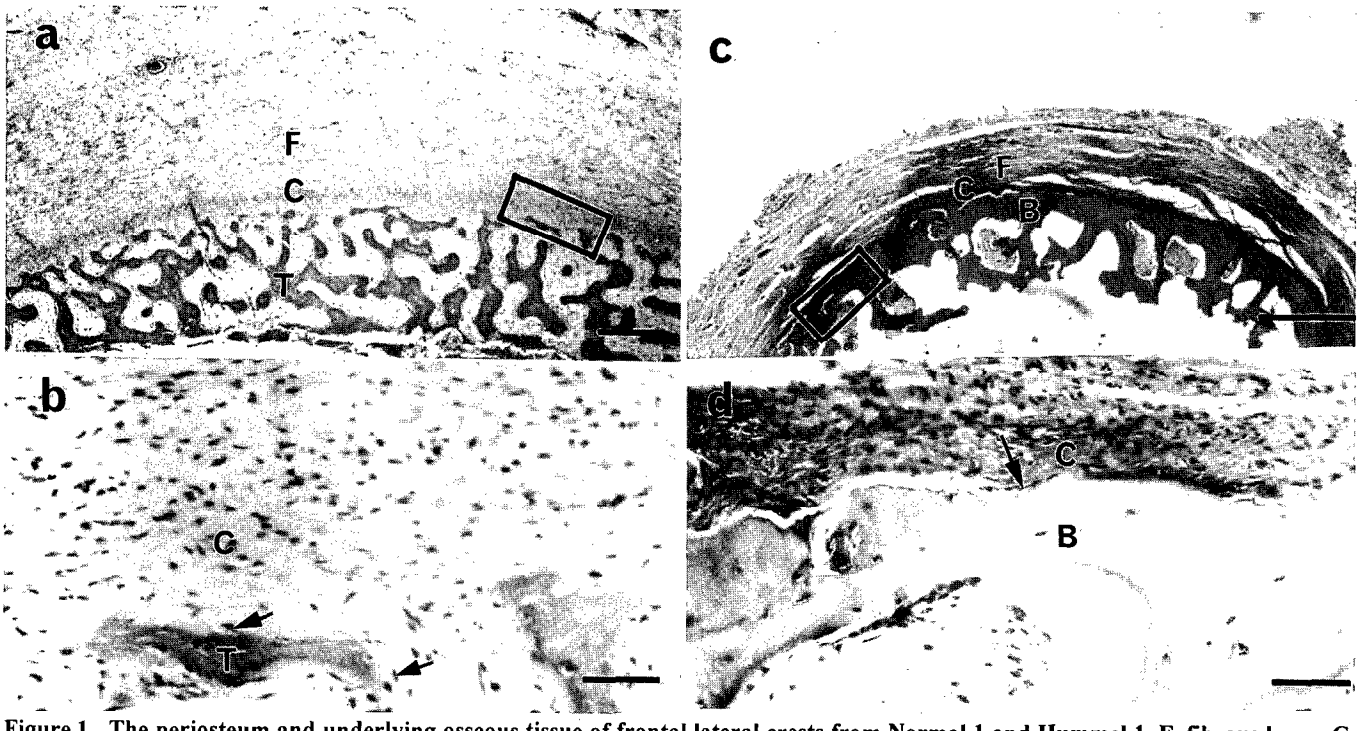

Figure 1. The periosteum and underlying osseous tissue of frontal lateral crests from Normal 1 and Hummel 1. F, fibrous layer; C, cellular layer; T, trabecular bone; and B, mature bone. H&E. a. Normal 1 (6-month-old male calf with frontal lateral crests). The bone was cancellous and immature. Bar = 0.3 mm. b. Enlargement of the rectangle in 1a. Note the active osteoblasts covering the trabeculae (arrows). Bar = 0.05 mm. c. Hummel 1 (18-month-old male stag with frontal lateral crests). Although the bone was not totally compact it was more mature compared with Normal 1. Bar = 1 mm. d. Enlargement of the rectangle in 1c. A discrete row of resting osteoblasts (arrow) was arranged along the interface of the cellular layer and underlying bone. Bar = 0.05 mm.

were oval and the fibres were evenly distributed in the intercellular space (Figure 1b). The subperiosteal osseous tissue consisted of cancellous trabeculae. The surfaces of these trabeculae were lined with active osteoblasts (Figure 1b). Therefore the crest of this deer was being formed through typical mammalian intramembranous ossification.

Hummel 1

The gross structure (Figure 1c) of the vertical section of the crest of Hummel 1 was similar to that of Normal 1, but both cellular ($p<0.01$) and fibrous layers (not quantified) were much thinner than those of Normal 1 (Table I). The cells in the cellular layer were spindle-shaped with elongated nuclei. The mean cell density of the cellular layer was not significantly different from that of Normal 1 (Table I). The surfaces of the bony trabeculae were lined with discrete rows of resting osteoblasts. The trabecular bone was more mature than that of Normal 1 (Figure 1d). The ossification type occurring in the crest was the same as that in Normal 1 intramembranous ossification.

Normal 2

The incipient pedicle could be classified longitudinally into three portions, apical hyperplastic periosteum/perichondrium, osseocartilaginous tissue and osseous tissue from distal to proximal. However, the boundary between the cellular layer and underlying tissue no longer existed (Figure 2a). In the periosteum/perichondrium, juxtaposed to the overlying fibrous layer, large sized, randomly oriented cellular layer cells were dispersed evenly and fine fibres filled in the intracellular space. The deep region of the layer contained mainly spindle-shaped cells, which were oriented perpendicular to the long axis of the pedicle (Figure 2b). The cellular layer cell density was not significantly different from those of Normal 1 and Hummel 1 (Table I). The osseocartilaginous portion constituted a large proportion of the pedicle at this stage. It could be classified into two zones, the osseocartilage forming zone and the osseocartilage remodelling zone, from distal to proximal, with a smooth transition between the zones. The structure of the osseous portion was similar to that of Normal 1, but osteoblasts were less active (Figure 2a). The pedicle was forming through an ossification type transitional between intramembranous and endochrondral.

Hummel 2

The gross structure (Figure 3a) of the vertical section of the rudimentary pedicle was similar to those of Normal 1 and Hummel 1, and consisted of a fibrous layer, a cellular layer and underlying trabecular bone from distal to proximal. The cellular layer was significantly thicker ($p<0.01$) than that of

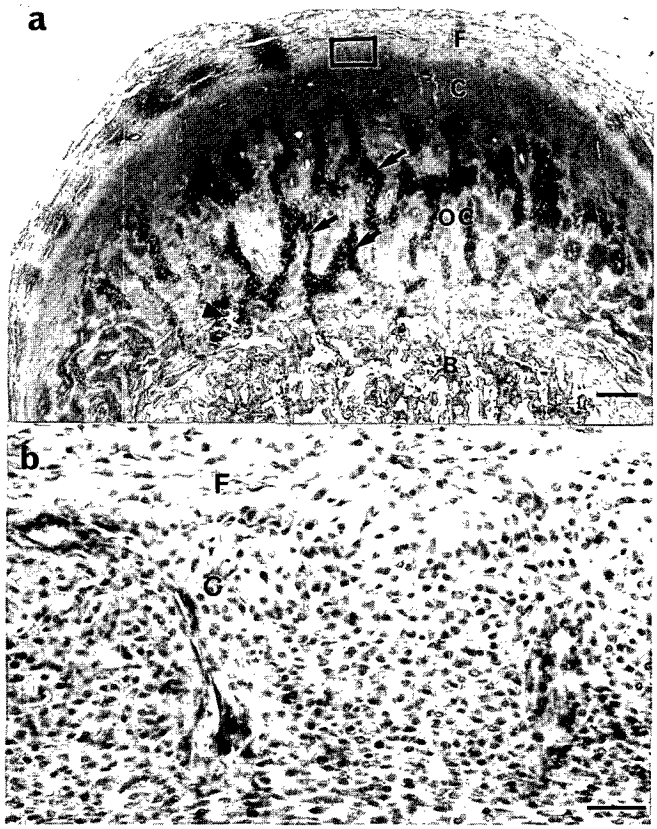

Figure 2. An incipient pedicle (15 mm in height) tissue from Normal 2. a. A vertical section of the pedicle through the fibrous (F) and cellular (C) layers, osseocartilaginous tissue (OC) and bone (B). Note the cartilaginous columns (arrows) were still discrete. Toward the proximal end of the osseocartilaginous portion, chondroclasia had already started (arrow head). Alcian blue/H&E. Bar = 1 mm. b. Enlargement of the rectangle in 2a. It shows the detail of the region between the cellular layer (C) and the fibrous layer (F). H&E. Bar = 0.05 mm.

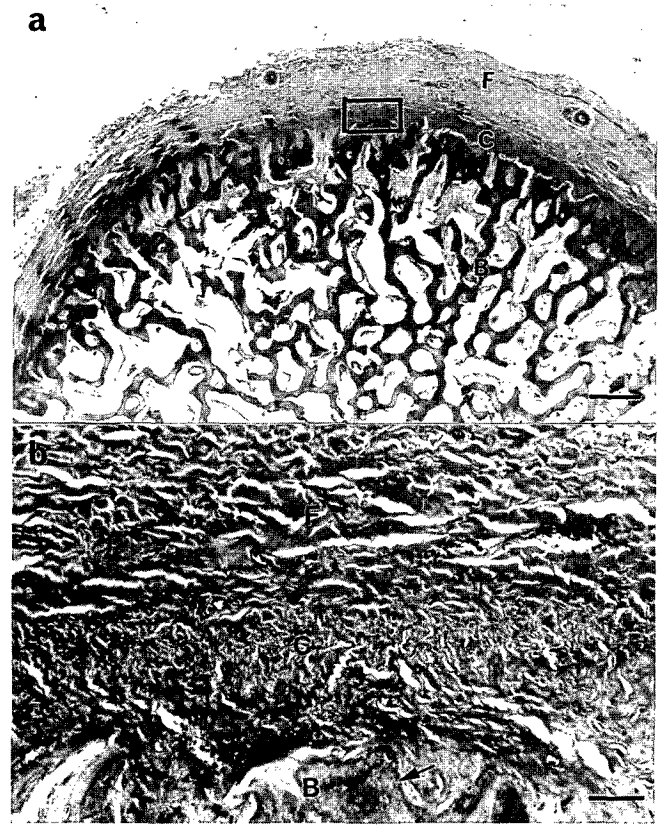

Figure 3. Rudimentary pedicle (15 mm in height) tissue from Hummel 2. a. A vertical section of the pedicle through the fibrous (F) and cellular (C) layers and underlying bone (B). Note that the cellular layer was very narrow and underlying tissue was pure, mature trabecular bone. Bar = 1 mm. b. Enlargement of the rectangle in 3a. F, C and B are the same as shown above. Note the distribution and orientation of the cellular layer cells were disorganised, and the surfaces (arrow) of the bone trabeculae were nearly devoid of osteoblasts. Bar = 0.05 mm.

Table I. Comparison of the crest/pedicle between hummels and normal stags

Animal	Age (months)	Live weight (kg)	Crest/pedicle				
			Tissue	Height (mm)	Ossification type[a]	Cellular layer of PO/PC[b] Thickness (μm)	Cell density (cell/mm²)
Normal 1	6	43.0	Crest	<5	IM	158.3	5.76
Hummel 1	18	33.7	Crest	<5	IM	48.7	6.16
Normal 2	6	56.0	Pedicle	15	TS	ND[c]	5.53
Hummel 2	18	51.8	Pedicle	15	IM	120.0	5.70
SED[d]						10.9**	0.47ns

a IM = intramembranous ossification; TS = transitional ossification.
b PO/PC = periosteum/perichondrium.
c ND = not measured.
d SED = standard error of the difference; ns = not significant; ** = $p<0.01$.

Hummel 1, but significantly thinner ($p<0.05$) than that of Normal 1 (Table I). The distribution and orientation of the cellular layer cells were disorganised (Figure 3b). The cell density of the cellular layer was not significantly different from those of Normal 1, Hummel 2 and Normal 2. The underlying trabecular bone was similar to that of Hummel 1 and more mature than that of Normal 1. Few resting osteoblasts were found along the surface of these trabeculae (Figures 3a and b). The crest was forming through mammalian intramembranous ossification.

Discussion

The results in this study, that in the normal red deer stag calves the 5 mm high frontal lateral crest was forming through intramembranous ossification and the 15 mm high incipient pedicle by transitional ossification, are consistent with those reported by Li and Suttie[9]. Therefore, the crest or pedicle formation from Normal 1 or Normal 2 represent normal crest and incipient pedicle formation, respectively, in red deer stags.

That hummels cannot grow antlers is attributed to the incomplete formation of their pedicles[3][5]. Li and Suttie[9] reported that complete histogenesis of a frontal lateral crest and a pedicle in red deer covers three different ossification stages: intramembranous, transitional and modified endochondral. The ossification type change, from intramembranous to transitional, signals the end of crest formation and the start of pedicle growth. The results from the present study show that the frontal lateral crests/rudimentary pedicles from both hummels were forming through intramembranous ossification. That means histologically the hummels were still constructing their frontal lateral crests, although the height of the bony protuberance achieved by Hummel 2 had already reached 15 mm and this height is typically associated with the transitional ossification stage seen in Normal 2. However, chronologically these hummels should have already finished one antler cycle, i.e. they were more than 1 year old. Therefore, we suggest that the antlerless state of hummels is caused by their failure to initiate true pedicle growth rather than incomplete pedicle formation.

It has been well accepted that incomplete pedicle formation, or more precisely the lack of true pedicle initiation, in hummels results from poor nutrition rather than androgen hormone status. The results from the present study support this notion, as the body weights of these 18-month-old hummels (33.7 kg and 51.8 kg) were lower than those from 6-month-old stag calves (43 kg and 56 kg). That the cellular layer thickness of the apical periosteum from both hummels was significantly less than that of a normal stag, while cell density did not differ significantly, was consistent with comparisons between normal and malnourished deer[9]. This provides further evidence that hummels result from malnutrition.

It is very well established that deer pedicle initiation is dependent on androgen hormones[10]. Li and Suttie[9] thought that the ossification type change from intramembranous to transitional, that is pedicle initiation, is stimulated by an increase in androgen hormone secretion at that particular period[9]. There are two phenomena which cannot be explained solely by androgen hormone stimulation. One is that deer need a threshold body weight to initiate their pedicles[11], and the other is that hummels should have normal androgen hormone status as they have normal testes, and apart from lacking antlers their other secondary sexual characters are fully expressed.

Why don't these androgen hormones stimulate pedicle initiation? We can speculate that deer pedicle initiation or the ossification type change, from intramembranous to transitional, depends on both appropriate androgen hormone and nutrition. How androgen hormone functions on pedicle initiation has been a controversial issue. Bubenik[12] hypothesised an indirect mechanism whereby a nervous connection between the antlerogenic periosteum and the antler growth centre (a putative growth centre which was thought to be in the central nervous system) was essential for pedicle development with androgen hormone acting on the establishment of the nervous connection. However, Fennessy and Suttie[8] considered that pedicle formation resulted from the direct stimulation of androgen hormone on the antlerogenic periosteum. Li *et al.* found that antlerogenic periosteum had specific binding sites for testosterone using a nuclear binding assay[13] and autoradiographic localisation[14]. These latter two papers are in favour of the hypothesis advanced by Fennessy and Suttie. However, the results from an experiment which was recently conducted in our laboratory[15] showed that the osteogenic cells of the antlerogenic periosteum taken from the pedicle initiation period only responded to androgen hormone when sufficient insulin-like growth factor 1 (IGF1) was present *in vitro*, and reacted to IGF1 in a dose-dependent manner.

Through which pathway could nutrition control pedicle initiation? Bubenik[12] thought that hummels formed because of poor nutritional conditions, such that the antler growth centre or connections between the pedicle and the antler growth centre did not develop sufficiently[12]. Suttie and Kay[11] postulated that poor nutrition would cause hummels after the age at which the presumptive pedicle region is responsive to the changes in luteinising hormone (LH) and testosterone associated with puberty[11]. Suttie *et al.*[16] reported that IGF1 level correlated significantly and positively with live weight gain during the period of pedicle and first antler formation. Therefore, it is conceivable that nutrition controls pedicle growth through the IGF1 pathway.

Acknowledgments

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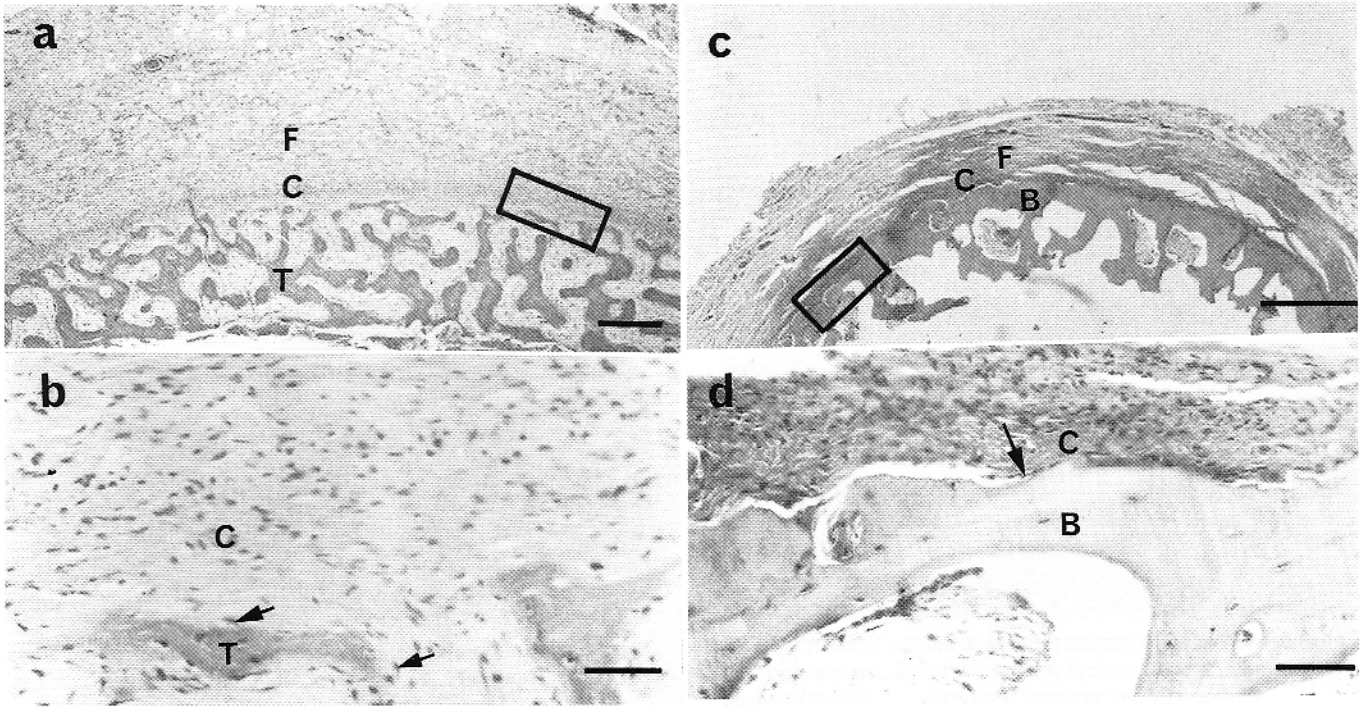

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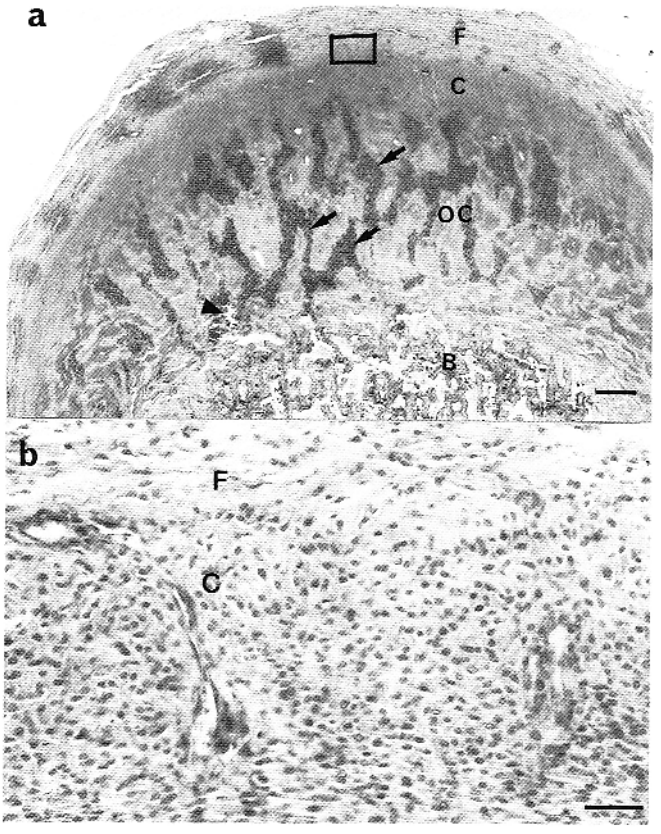

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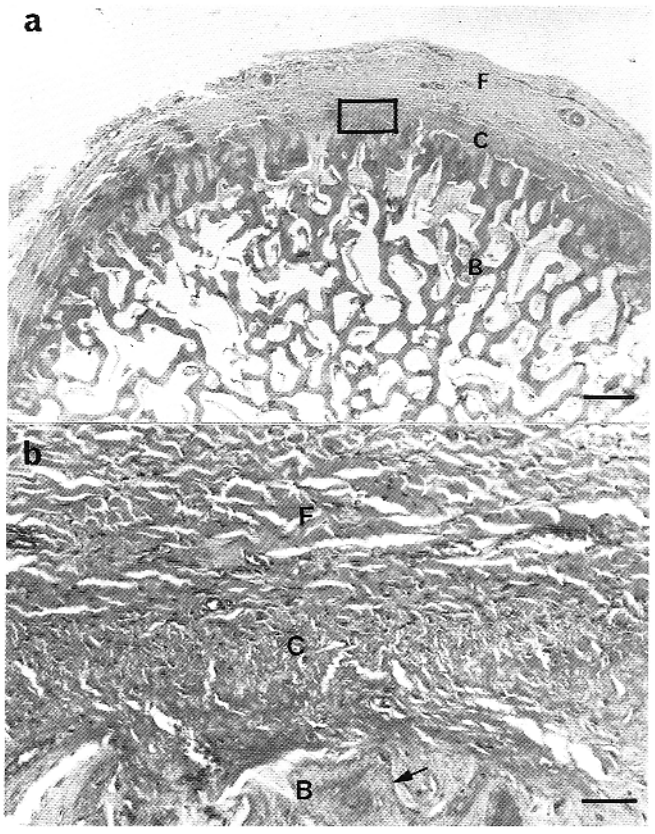

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