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Light Microscopic Studies of Pedicle and Early First Antler Development in Red Deer (*Cervus elaphus*)

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ABSTRACT ***Background:*** **Although it is known that deer antlerogenic potential resides in the periosteum of an antlerogenic region and antler forms through modified endochondral ossification, how a deciduous antler forms histologically through a permanent pedicle from the periosteum has not been reported.**

Methods: **Histogenesis of the pedicle and the early first antler in red deer was systematically examined using light microscopy techniques.**

Results and Conclusions: **At the pre-pedicle stage, the frontal lateral crest (under 5 mm in height) consisted horizontally of antlerogenic periosteum and underlying cancellous bone. Both the cellular layer (3.74 times, $P<0.01$) and the fibrous layer of the antlerogenic periosteum were much thicker than those of the margin of the antlerogenic region or the facial periosteum. The crest was formed through intramembranous ossification. When the pedicle began to develop (5–15 mm in height), some discrete clusters of mature chondrocytes appeared in the bony trabeculae, which signified the beginning of the transition of the ossification pattern from the intramembranous to the endochondral. The pedicle consisted of three portions from distal to proximal, periosteum/perichondrium, osseocartilaginous tissue, and osseous tissue. When the pedicle became visible (about 20 mm in height), it consisted of the same three portions as the pedicle initiation stage, but the osseocartilaginous portion was expanded compared to the initiation stage and the cartilaginous proportion increased distally. When the pedicle grew to 25–40 mm in height, continous cartilaginous trabeculae appeared under the apical perichondrium. The pedicle consisted of four portions from distal to proximal: perichondrium, cartilaginous tissue, osseocartilaginous tissue, osseous tissue. It was formed through endochondral ossification. All these ossification pattern changes could not be seen externally as the overlying integument was characterised by typical scalp skin. When the pedicle grew to about 60 mm in height, antler tissue was visually apparent at the apex as the hair type changed from scalp hair to the velvet-like hair of growing antler. However, this transformation could not be distinguished internally as the inside tissues were all continuous between pedicle and antler. Therefore, the histogenesis of the deer pedicle and the first antler originated from the antlerogenic cells and covered two phases: an internal phase through which pedicle was formed and an external phase which signalled the beginning of antlerogenesis.**

Key words: Deer, Pedicle, Antler, Intramembranous ossification, Endochondral ossification, Antlerogenic periosteum, Perichondrium, Transitional ossification

Deer are the only mammals which annually grow and cast osseous appendages, the antlers. The antlers develop from pedicles. The pedicles are permanent bony protuberances, which become apparent around the onset of puberty, having developed from the cells with antlerogenic potential on the frontal bone of the deer skull (Chapman, 1975; Goss, 1985). It has been convincingly demonstrated by a combination of dele-

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TABLE 1. Allocation of the experimental animals

Group	Sex	Number	Age (mths)	Liveweight range (kg)	Pedicle length (cm)	Comments
I	Male	6	4	42	Unpalpable	
		10	6	45	Unpalpable	Malnourished[1]
		3	6	51	Unpalpable	Castrated[2]
	Female	10	8	48	0	Malnourished[3]
		10	14	81	0	
		10	>24	96	0	
II	Male	5	6	53	Palpable (0.5–1.5)	
III	Male	8		59	Visible (about 2.0)	
IV	Male	8	8.5	65	2.5–4.0	
V	Male	3	8–9	69	5.5–7.0 (1 cm antler)	

[1]Typical weight for age: about 50 kg (Suttie et al., 1987).
[2]Castrated 3 weeks prior to the biopsy.
[3]Typical weight for age: about 57 kg (Suttie et al., 1987).

tion and transplantation experiments that the antlerogenic potential resides exclusively in the periosteum of the frontal lateral crest of the deer skull (Hartwig and Schrudde, 1974; Goss 1987; Goss and Powel, 1985; Goss et al., 1964). The frontal lateral crest periosteum of female deer also has antlerogenic potential if it is sufficiently stimulated by exogenous androgen hormones (Wislocki et al., 1947; Jaczewski, 1982), although the only genus in which the females normally develop antlers is *Rangifer* (Lincoln, 1992). The antler develops through a modified form of mammalian endochondral ossification (ECO) (Banks and Newbrey, 1982a,b), although this was controversial for a long time, as the cartilage-like tissue formed during antler growth is well vascularized. In non-antlerogenic somatic cartilage formation vascularisation is limited. However, the vascularized tissue has been confirmed as true cartilage by ultrastructural (Banks and Neal, 1970; Newbrey and Banks, 1975) and histochemical studies (Frazier and Banks, 1973; Frazier et al., 1975).

The histological features of the antlerogenic periosteum have not been described despite the fact that the discovery that the histogenesis of the pedicle and the first antler is dependent on the existence of the antlerogenic periosteum has been considered as one of the most important landmarks in the history of deer antler research (Goss and Powel, 1985). Ham and Harris (1971) reported that somatic periosteum consists of two layers, an outer fibrous layer and an inner cellular layer. The latter comprises mainly a population of committed osteogenic cells, of a special lineage, which promotes bone formation through intramembranous ossification (IMO). Murakami and Emory (1967) found that there were some elastic fibres between the two layers in developing periosteum and speculated that these fibres were related closely to subperiosteal bone formation. However, the ossification pattern whereby the pedicle is initiated from the antlerogenic periosteum is unknown. Macewen (1920) thought that pedicle was formed by ECO, while Wislocki (1942) believed that the pedicle formed by IMO, as the pedicle was covered by a cap of osteogenic tissue. In red deer after the pedicle has reached a length of around 5 cm (Fennessy and Suttie, 1985), the first velvet antler begins to grow. This is an important change because although the pedicle is permanent, the antler is deciduous; that is, the antler but not the pedicle is the part which is cast and regenerated annually. However, the first antler which is grown is not regenerated; rather, it develops from the pedicle spontaneously. This transition from a permanent to a pre-programmed deciduous tissue is a unique zoological event in mammals (Goss, 1983). Goss (1983) presented an hypothesis that the onset of chondrogenesis in the pedicle might signal the beginning of true antler growth but this hypothesis has neither been tested nor further explored.

The aim of this study was to investigate systematically the progression of events during pedicle and first antler histogenesis using light microscopy techniques.

MATERIALS AND METHODS

Tissues

Tissues, from red deer (*Cervus elaphus*), were taken from biopsy and slaughter samples. Tissues were allocated to one of five groups based on different pedicle and antler developmental stages. The details for each group allocation are shown in Table 1. Because female and pre-pubertal castrated male deer do not grow pedicles and antlers, they were classed as Group I (unpalpable pedicles). Antlerogenic and facial periostea were obtained from all Group I deer.

Tissue Collection Techniques

i. Biopsy: the deer were sedated with intravenous Xylazine (0.75 mg/kg live weight) (Rompun, Bayer NZ Ltd) after a 24 hr fast; anaesthesia was induced with a mixture of halothane, nitrous oxide, and oxygen following intubation and the surgery performed under aseptic conditions. All biopsy deer were allocated to a group prior to pedicle initiation so that general body development and pedicle development would not be confounded with time.

For group I, the antlerogenic periosteum was sampled as follows. A crescent shaped incision was made on the scalp 2 cm medial to the left frontal lateral crest, from which the pedicle would grow (in all deer including females there is an alteration in skin thickness above the antlerogenic periosteum which can be felt and seen and which aids the placement of the incisions). The flap of skin, which was separated from the frontal bone by blunt dissection, was reflected laterally. A full thickness piece of antlerogenic periosteum about 3 × 5 mm in size was removed with a scalpel from the dorsal surface of the crest. The piece was deep enough to include a sliver of bone. After removal of the biopsy specimen, a circle (22 mm diameter) was marked over the presumptive antlerogenic region with the biopsy site at the centre. From a site medial to the boundary of the antlerogenic region and outside it, a piece of margin periosteum (about 3 × 5 mm) with a layer of subperiosteal bone was taken using the same method as for antlerogenic periosteum. The wound was closed with a silk suture. Next, a 4 cm long skin incision was made along the midline of the nasal bone. This incision was continued laterally from both ends of the first incision on the skin to the left side. Taking care not to injure major blood vessels, a flap of skin was separated by blunt dissection and reflected laterally to expose the left nasal bone. A piece of facial periosteum (3 × 5 mm) was taken following the same method as above. The wound was closed with silk suture.

For all subsequent groups, an incision was made for 270° around the base of the pedicle with the uncut edge laterally and a second was made from the top of the pedicle to the base until it met the first incision. The skin was separated from the pedicle/antler by blunt dissection. The mid part of the pedicle/antler (about 3 mm in thickness) was removed and the remaining stump was detached using embryotomy wire. The skin of the pedicle was trimmed to fit the wound and then was closed with silk suture.

ii. The heads from slaughtered deer were taken immediately to the laboratory from the abattoir and tissue samples taken. The procedures for the biopsies in Group I were used to take antlerogenic and facial periostea from the slaughtered deer.

Techniques for Histology

All tissue samples were fixed in 10% buffered formalin immediately after removal. After a minimum of 24 hr in the fixative, the samples were then decalcified in Raymond Lamb "R.D.C." commercial decalcification solution (BDH Chemical NZ Ltd) for 4–15 hr and washed in tap water for 2–4 hr. The samples were embedded in paraffin wax and sectioned at 5 μm. Three different stains were employed: Gill's haematoxylin and alcoholic phloxine/eosin for general histological interpretation; Verhoeff's elastin haematoxylin and alcoholic phloxine/eosin for identifying elastic fibres; and alcian blue and haematoxylin/eosin for confirming the presence of cartilage tissue. The tissue sections were observed and photographed using a Zeiss Axioplan Microscope.

Techniques for Measuring the Periostea of Group I

The two-dimensional quantitation method (Gundersen et al., 1988) was employed using a modified Olympus BH-2 microscope. The microscope was connected via a camera to a television monitor on which a counting frame with 64 regularly spaced points was attached. The magnification of the image was verified by using a micrometer with 10 μm intervals.

The cell density of both the cellular and fibrous layers was counted and the thickness of the cellular layer was measured (the thickness of the fibrous layer was not measured as we could not get a perfectly intact fibrous layer after processing). The procedure for selecting counting or measuring areas was as follows. The image of a periosteal section was moved into the monitor screen, and one end of the image was adjusted to occupy the whole screen horizontally. The area positioned in the central part of the screen was selected and the counting frame was attached to this part. The thickness of the cellular layer was measured using a ruler and the cell numbers in the frame of both the cellular and fibrous layers were counted. Thereafter, the periosteal image was moved horizontally towards the other end until a length approximating one and a half screens was passed. The central part was again selected as the measuring or counting area. If the area chosen for measuring or counting had artifacts, such as broken layers (especially in the fibrous layer, fibrous bundles being very easily separated from one another), or tissue defects such as a subperiosteal bone surface with tunnel absorptions, this area was ignored. The image was kept moving toward the opposite end until an area which had no artifacts or defects for measuring or counting reached the central part of the screen. Using the same procedure as above, the cellular layer thicknesses were measured at five different sites and cell density was counted at three different areas for each section. Tissue thickness and density among the periostea were analysed using ANOVA, with individual deer as the blocking stratum where appropriate.

The ossification patterns by which the pedicle was formed were evaluated by comparison with somatic bone (Ham, 1969) and antler bone (Banks and Newbrey, 1982a,b) formation.

RESULTS

Group I (Unpalpable Group)

In this group all tissue samples, including the frontal lateral crest, the margin, and the facial tissue, consisted of two horizontal portions, periosteum, and underlying osseous tissue.

Male

Margin and facial periosteum and underlying bone. The margin periosteum and facial periosteum of the normally nourished deer consisted of two layers, an outer fibrous layer and an inner cellular layer (Fig. 1). The fibrous layer was made up of collagenous fibres, fibroblasts, and fibrocytes. These cells were spindle or fusiform shaped with elongated or oval nuclei and were sparsely distributed among the fibres. The fibres were arranged in thick bundles which ran in more or less straight lines parallel to the periosteum.

The cellular layer was composed of osteogenic cells and fine fibres. The osteogenic cells were oval and their long axis ran parallel to the periosteum. The fine fibres were distributed in a much more reduced intercellular space than that of the fibrous layer but they were also

Fig. 1. Facial periosteum and underlying bone from a 4-month-old male calf. F, fibrous layer; C, cellular layer; and B, bone. A discrete row of resting osteoblasts (arrows) was distributed along the interface of the C and B. The underlying bone was compact (hematoxylin and eosin = H and E). Bar = 0.1 mm.

arranged parallel to the periosteum. The nuclei of the osteogenic cells were larger and the cytoplasm more abundant than those of the fibrous cells.

A discrete row of resting osteoblasts was arranged along the interface of the innermost region of the cellular layer and subperiosteal osseous portion. The osseous tissue was composed of compact bone and its surface appeared smooth.

All facial periostea of the malnourished and the castrated deer were very similar to those of the normally nourished deer. There was no significant difference in terms of the mean thickness of the cellular layer, or mean cell density in the fibrous layer. The cell density of the cellular layers of the malnourished deer was greater than that of the normally nourished and the castrates ($P<0.05$) (Table 2).

Frontal lateral crest periosteum and underlying bone. The gross structure of the antlerogenic periosteum of the normally nourished deer at biopsy was similar to the margin or the facial periosteum (Fig. 2), but both the cellular layer and the fibrous layer were much thicker than those of the margin or the facial periosteum.

The osseous part consisted of cancellous trabeculae. Active osteoblasts covered the surfaces of the trabeculae and the spicules. The subperiosteal cancellous bone was formed by means of typical mammalian IMO. The innermost boundary of the cellular layer was still distinguishable from the cancellous bone. The cells of the lower region were heterogenous, but were mainly preosteoblasts. The intercellular spaces were greater in the lower region compared with those of the mid and upper regions. Osteoclasts were rare in the cancellous bone. The cellular layers of the three regions for both the castrated (Fig. 3) and malnourished (Fig. 4) deer were similar to, but on average thinner than those of the normally nourished deer ($P<0.05$) (Table 2).

The mean cell density of the cellular layer was significantly greater ($P<0.05$) and the fibrous layer significantly lower ($P<0.01$) in the malnourished deer compared to both the normally nourished and the castrated deer (Table 2). There was no significant difference in mean cell density between the normal and the castrated deer (Table 2). Both the cellular layers ($P<0.01$) and the fibrous layers of the antlerogenic periostea of the malnourished and the castrated deer were much larger than their own facial periostea. However, there was no significant difference in mean cell density between the antlerogenic periosteum and the facial periosteum in both the malnourished and castrated deer.

The overall mean cellular layer thickness of the antlerogenic periostea of the three male sub-groups was greater than that of the facial periostea (difference = 83.3; SED 6.9 μm). The mean overall cell density of the antlerogenic periostea was significantly more dense than that of the facial periostea for the cellular and fibrous layers, with differences of 1.47, 1.65 (SEDs 0.30, 0.28) μm, respectively. The subperiosteal bone of the castrated deer was cancellous. However, the trabeculae were thicker and the vascular spaces peripheral to these trabeculae were, therefore, narrower than those of the intact normally nourished deer. New lamellae were apparently being added to both sides of the trabeculae and isolated spicules over the cancellous network were rare. There were more osteoclasts in the castrated deer than in the intact normally nourished deer distributed along the surface of the trabeculae. The most obvious feature of the osseous part of the malnourished deer was that the bone was compact and no isolated spicules were found. Active osteoclasts, laid in Howship's lacunae, were scattered along the compact bone surface and no bone formation was observed.

Female

Facial periosteum and underlying bone.

The facial periostea of all female deer were histologically similar to those of the males above. The subperiosteal bone surface was smooth and nearly devoid of osteoblasts and the bone was compact (Fig. 5). There were no significant differences among the facial periostea of these three sub-groups in cellular layer thickness or cell density (Table 2).

Frontal lateral crest periosteum and underlying bone. The antlerogenic periostea of the female deer was histologically similar to their facial periostea (Fig. 6). But the

TABLE 2. Mean thickness and density of the periostea in Group 1

Sex	Age (mths)	n	Mean thickness of cellular layer (μm)		Mean density (cell/mm^2)			
					Antlerogenic		Facial	
			Antlerogenic	Facial	Cellular layer	Fibrous layer	Cellular layer	Fibrous layer
Male	4	6	160	33.5	6.73	3.80	5.97	1.30
	6	5	104	34.5	8.25	2.57	6.83	1.60
	6	3	123	35.4	6.82	3.58	5.46	1.26
		sed	12.3–14.8*	4.0–4.7^{NS}	0.51*	0.22**	0.35*	0.14^{NS}
Female	14	6	116	44.8	5.53	2.42	5.79	1.78
	>24	5	114	42.8	5.75	2.12	5.87	1.52
	8	5	107	47.1	6.12	2.76	6.58	1.82
		sed	10.5–10.9^{NS}	4.9^{NS}	0.50^{NS}	0.19*	0.67^{NS}	0.20^{NS}

NS = not significant; * = $P<0.05$; ** = $P<0.01$.

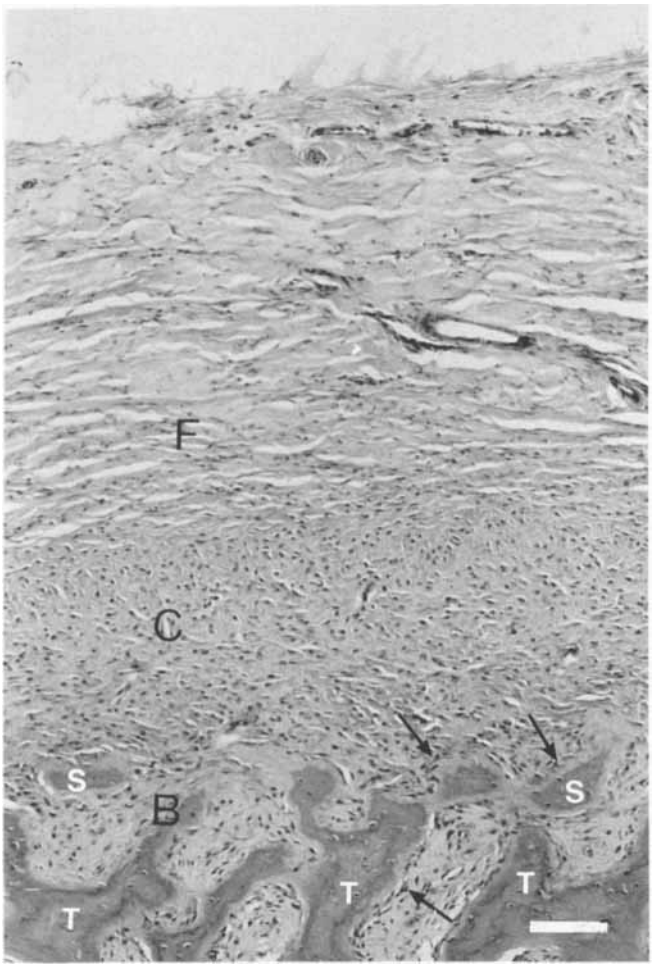

Fig. 2. Antlerogenic periosteum and underlying bone from a 4-month-old male calf. The underlying bone was cancellous. The surfaces of the trabeculae (T) and the spicules (S) were covered with active osteoblasts (arrows). F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

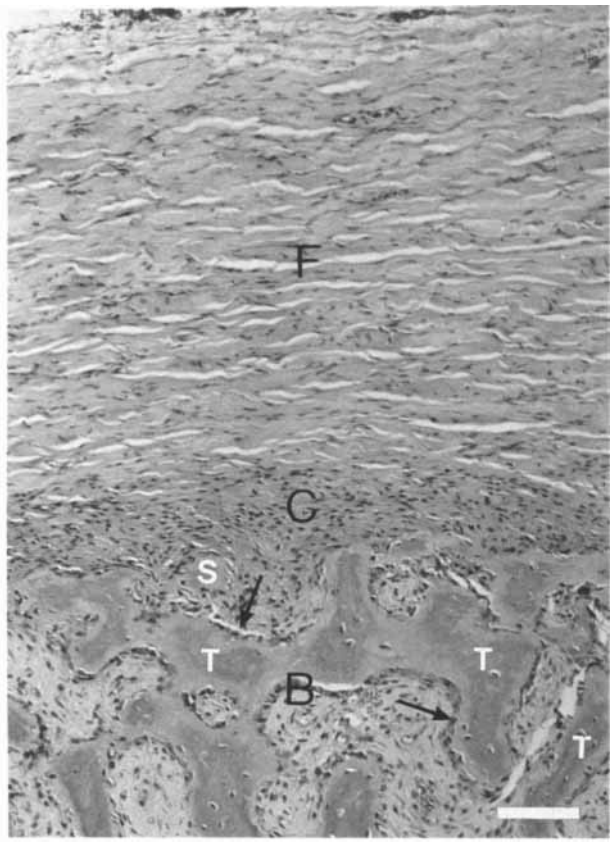

Fig. 3. Antlerogenic periosteum and underlying bone from a 6-month-old castrated calf. The underlying bone was cancellous. The surfaces of the trabeculae (T) and the spicules (S) were covered with active osteoblasts (arrows). F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

cellular and fibrous layers of the antlerogenic periostea were much larger in thickness than their facial periostea, (difference = 70.3; SED 5.9; $P<0.01$) (Table 2). The cell density of the cellular layer did not differ significantly between the antlerogenic periosteum and the facial periosteum, over or within sub-groups (Table 2). The mean fibrous layer cell density of the antlerogenic periosteum of 8-month-old malnourished deer was greater ($P<0.05$) than those of the two other female sub-groups (Figs. 7, 8). The overall mean cell density of the antlerogenic periostea was significantly

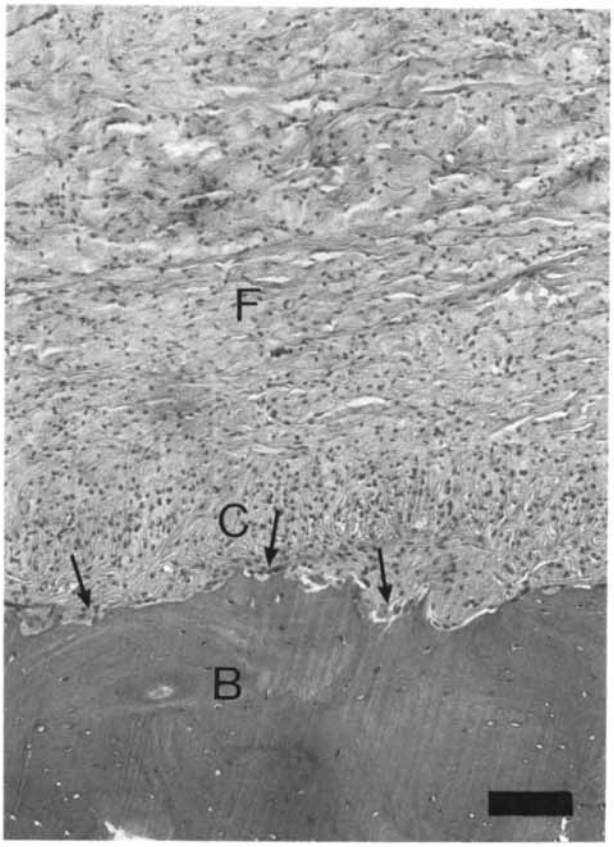

Fig. 4. Antlerogenic periosteum and underlying bone from a 6-month-old malnourished male calf. The distribution and the orientation of the cellular layer cells were disorganized. The underlying bone was compact and its surface was irregular due to the activity of the osteoclasts (arrows). F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

Fig. 5. Facial periosteum and underlying bone from a 14-month-old female calf. The underlying bone was compact and its surface was smooth and nearly devoid of osteoblasts. F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

greater than that of facial periostea for the fibrous layer, with differences of 0.70 (SED 0.11) μm, while there was no difference in the cellular layer (mean = −0.55 (SED 0.40) μm).

The subperiosteal bone of the 14-month-old deer was cancellous and was formed through the same ossification pattern as occurred in the frontal lateral crest of the male deer. However, the trabeculae and spicules constituting the cancellous network ran parallel to the hyperplastic periosteum instead of oblique or perpendicular, and the surfaces of these trabeculae were covered with fewer osteoblasts. Osteoclasts were infrequent in the cancellous bone.

The subperiosteal bones of both older than 24-month normally nourished and 8-month-old malnourished deer were compact and the surfaces were smooth and virtually devoid of osteoblasts. The subperiosteal compact bone had regular Haversian systems. The antlerogenic periostea of both sub-groups of deer were at a resting stage.

Comparison between male and female

The overall mean density of the antlerogenic periostea was greater ($P<0.001$) for males than for females, by 1.66 (SED 0.30) μm for the cellular layer and by 0.88 (SED 0.12) μm for the fibrous layer. The pattern of overall mean cell density for the facial periosteum was quite different. There were no significant differences for the cellular layer (male—female = 0.04 (SED 0.33) μm, but the females had greater fibrous layer cell density than the males by 0.30 (SED 0.11) μm. For both males and females and for both antlerogenic and facial periostea, the cell density of the cellular layer was greater than that of the fibrous layer, by an overall average of 4.12 (SED 0.18) μm.

No elastic fibre or cartilage was found in any tissue sample from male, castrate, or female deer.

Group II (Palpable Group)

The major change in the histological structure of the incipient pedicle at the palpable stage compared with the unpalpable stage was that discrete clusters or colonies of mature chondrocytes were visible and the innermost, previously clear, boundary between the cellular layer and the underlying bone no longer existed. The appearance of cartilage indicated that an ossification pattern change (OPC) from typical IMO had taken place.

The pedicle tissue at this stage could be visually separated into three portions from distal to proximal, namely, the hyperplastic antlerogenic periosteum/per-

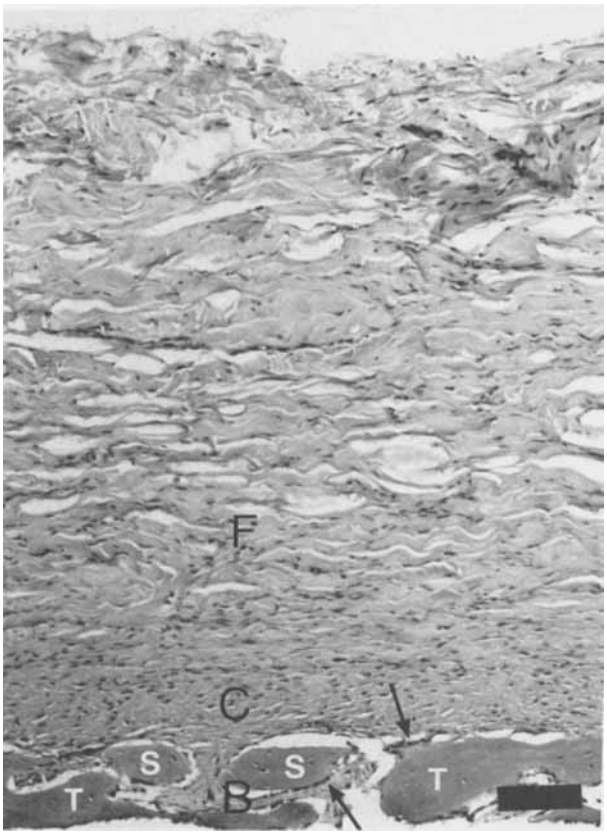

Fig. 6. Antlerogenic periosteum and underlying bone from a 14-month-old female calf. The bone was cancellous and the surfaces of the trabeculae (T) and the spicules (S) were covered with osteoblasts (arrows). F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

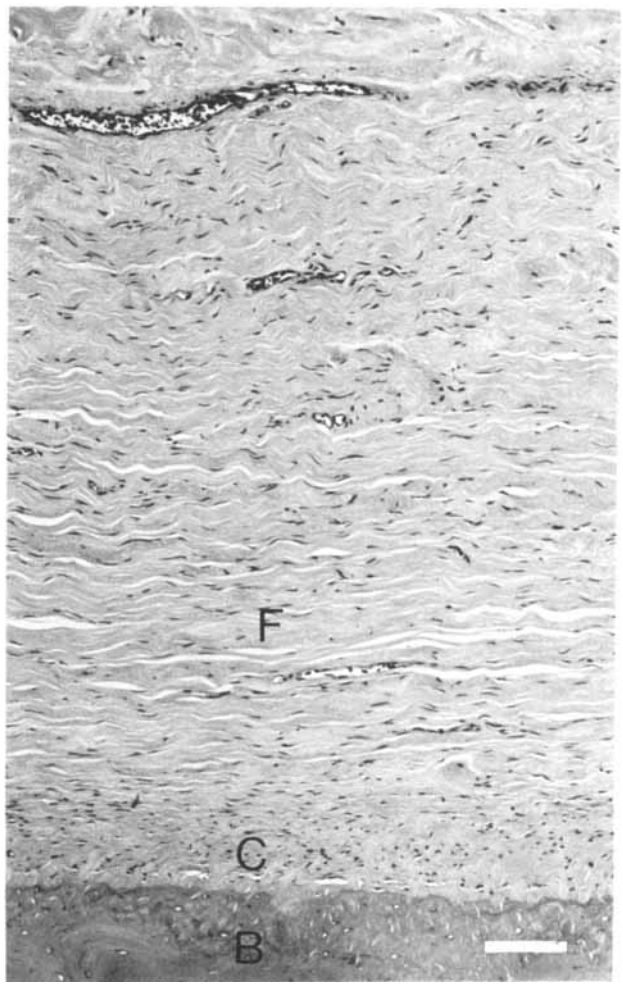

Fig. 7. Antlerogenic periosteum and underlying bone from a 24-month-old hind. The bone was compact and its surface was smooth and devoid of osteoblasts. F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

ichondrium, the osseocartilaginous tissue, and the osseous tissue (Fig. 9).

In the hyperplastic antlerogenic periosteum/perichondrium, the fibrous bundles of the fibrous layer were arranged in regular waves (Fig. 10), unlike the straight bundles of Group I. The long axis of the fibroblasts or fibrocytes which were located in this layer followed the same pattern as the fibrous bundles. In the superficial region of the hyperplastic cellular layer, juxtaposed to the fibrous layer, large sized, randomly oriented cells with round to oval nuclei were dispersed evenly. Fine fibres filled in the intercellular spaces which were reduced compared with the fibrous layer and were oriented perpendicular to the fibrous layer. Adjacent to this region, there was about a 4–5 cell wide region which was characterised by even finer and more randomly oriented fibres compared with the previous regions. However, the cell properties (size, shape, and orientation) were identical to that of the distal region. In both these regions numerous mitotic figures were noted and the matrix was slightly alcianophilic. These regions corresponded to the antlerogenic periosteum cellular layer of Group I. The deep region of the cellular layer contained mainly spindle-shaped cells which were oriented perpendicular to the long axis of the palpable pedicle and had eccentrically positioned nuclei. The cytoplasm of these cells was basophilic and had numerous fine cellular processes extending into scant intercellular spaces. Mitotic figures were a characteristic feature of this region and were found in preosteoblasts/prechondroblasts. The matrix of the region was not alcianophilic. Longitudinal vascular channels which were not previously visible were observed in this portion. However, these channels may not have completely formed since they appeared discrete in appearance and had very narrow or no compartments and no blood cells were found within these channels.

The narrow osseocartilaginous portion comprised the spicules and free tips of the trabeculae juxtaposed to the hypertrophied antlerogenic periosteum/perichondrium. Discrete clusters of very large cells with round nuclei were located in lacunae. The lacunae were found in the centre of the spicules or mid trabecular regions; these were the proliferating and differentiating areas in the central apex of the growing pedicle. The matrix between these cells was intensely alcianophilic, especially the capsular margins of the lacunae, indicating

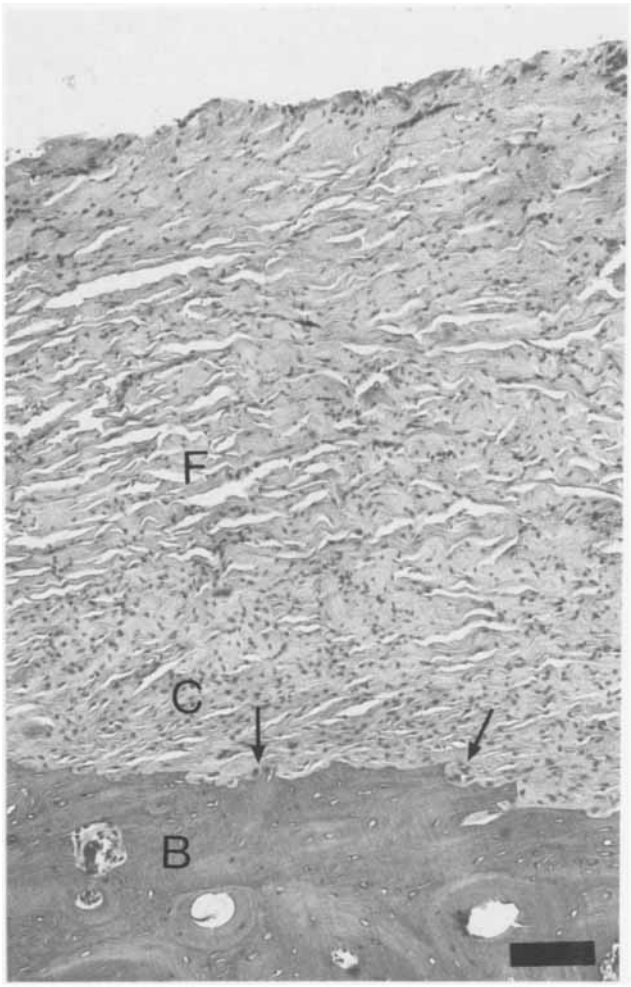

Fig. 8. Antlerogenic periosteum and underlying bone from an 8-month-old malnourished female calf. The bone was compact and its surface was irregular to the activity of the osteoclasts (arrows). F, C, B are the same as shown for Figure 1 (H and E). Bar = 0.1 mm.

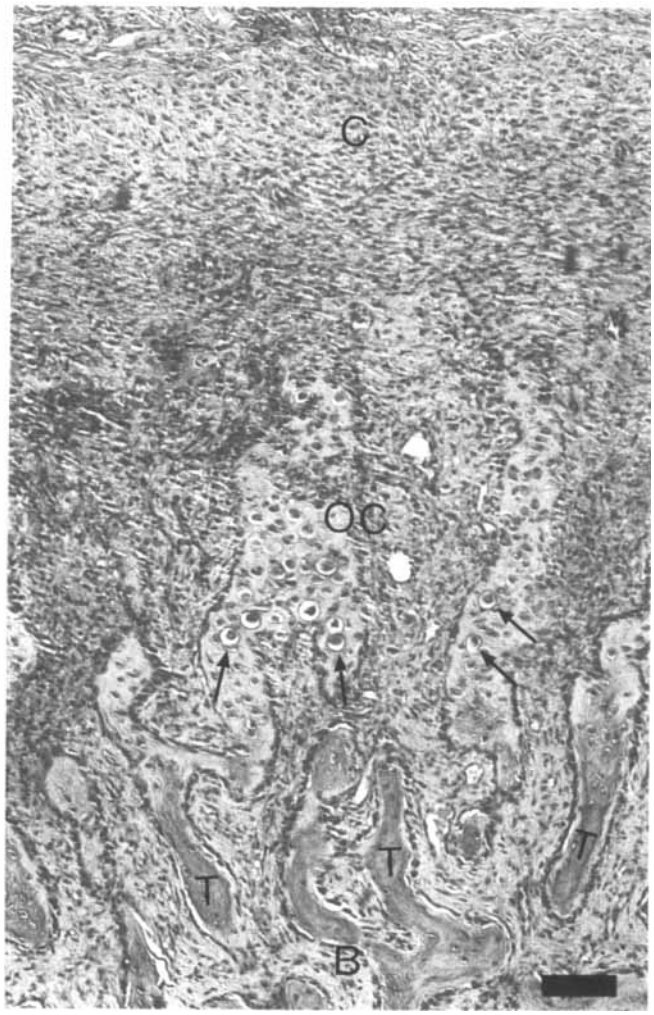

Fig. 9. A vertical section of a palpable pedicle through the cellular layer (C), the osseocartilaginous tissue (OC), and cancellous bone (B) from a 6-month-old male calf. It shows that some discrete clusters of mature chondrocytes (arrows) appeared in the fast forming bony trabeculae (T). (Alcian Blue/H and E). Bar = 0.1 mm.

that these cells were probably mature chondrocytes. Therefore, a unique configuration was present with the bone trabeculae having discrete cartilaginous cores (Fig. 13). Chondroblasts were found between the discrete clusters of mature chondrocytes and the prechondroblasts positioned distally. These cells were round and polarised and the cellular margin was clearly defined from the matrix. There was a longitudinal gradient of differentiation in these chondroblasts as follows: less differentiated cells with a weaker alcianophilic matrix occupied the distal region, whereas more differentiated cells with a stronger alcianophilic matrix were located in the proximal region. In the proximal part, the discrete channels and perivascular compartments were becoming enlarged. However, the perivascular compartments peripheral to the trabeculae, which had discrete cartilaginous cores, were very narrow or did not have vascular channels. The trabeculae in this part were more or less parallel to the long axis of the pedicle.

In the osseous portion, the bone trabeculae were anastomosed with one another; this constituted a scaffolding of cancellous bone. Most of the surfaces of these trabeculae were rimmed with osteoblasts. Osteoclasts were rare.

Group III (Visible Group)

The princple distinguishing feature of the incipient pedicle at this stage was that chondroclasia was observed in the proximal region of the osseocartilaginous portion and was associated with the earliest formed chondrocytes. The distally positioned cap of the hyperplastic antlerogenic periosteum/perichondrium was continuous with the peripheral periosteum, which had an inverted "U" shape appearance.

The pedicle could also be longitudinally separated into three portions: the hyperplastic antlerogenic periosteum/perichondrium, the osseocartilaginous tissue, and the osseous tissue from distal to proximal.

In the hyperplastic antlerogenic periosteum/peri-

Fig. 10. A part of the fibrous layer of the hyperplastic antlerogenic periosteum/perichondrium from a 6-month-old male calf. The fibrous bundles had regular waves. (H and E). Bar = 0.05 mm.

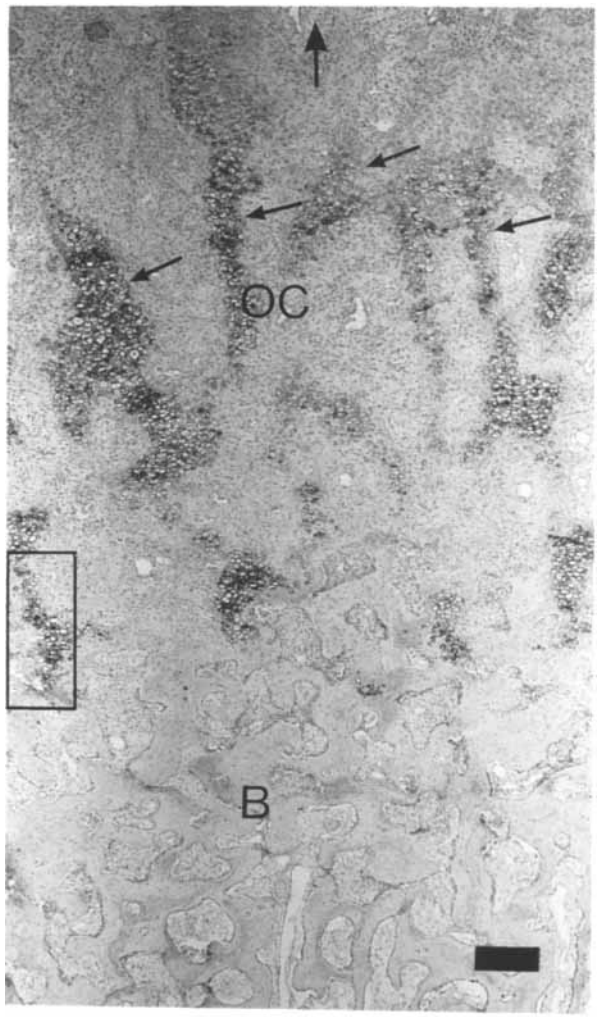

Fig. 11. A vertical section through the osseocartilaginous tissue (OC) and cancellous bone (B) of an incipient pedicle from an 8-month-old male calf. It shows that in the osseocartilaginous portion, the proportion of the cartilaginous tissue (arrows) increased towards the distal side (large arrow). (Alcian blue/H and E). Bar = 0.3 mm.

chondrium portion, the amplitude of the fibrous bundle waves in the fibrous layer was smaller than, but the cellular properties were identical to those of Group II. The three regions of the cellular layer were similar to, but more pronounced than those of Group II.

The osseocartilaginous portion (Figs. 11–13) formed a large proportion of the pedicle at this stage. It could be divided into two zones from distal to proximal: the osseocartilage forming zone and the osseocartilage remodelling zone (Fig. 13), with a gradual transition from the distal portion to the forming zone.

The gross appearance of the osseous portion (Fig. 12) was the same as that of Group II. Typical lamellar bone was being laid down on the trabecular surfaces.

Group IV (2.5–4 cm Long)

The most obvious histological characteristic of the pedicle at this stage was that the continuous cartilaginous trabeculae over the bony trabeculae with discrete cartilaginous cluster cores within the central pedicle (this was a special structure which resembled the configuration of an adrenal gland cross section) was enclosed within an inverted "U" formed by the distally positioned hyperplastic perichondrium and the peripheral periosteum. In the structure, the fibrous layer of the perichondrium could be compared to the capsule of the adrenal gland; the cellular layer to the zona glomerulosa; the parallel cartilaginous trabeculae to the zona fasciculata; the remodelling osseocartilaginous trabecular zone to the zona reticularis; and the anastomosed bony trabeculae to the medulla. The pedicle could be longitudinally divided into four portions: the hyperplastic perichondrium; the cartilaginous tissue; the osseocartilaginous tissue; and the osseous tissue.

In the hyperplastic perichondrium (Fig. 14), the amplitude of the fibrous wave bundles in the fibrous layer diminished further and the layer became more tightly packed, although the size, shape, and orientation of the cells in these layers were similar to those of Group III. The descriptions for the three regions of cellular layer of group III were suitable for those of this group. The perichondrium was more cellular than Group III and merged with a smooth transition into cartilage.

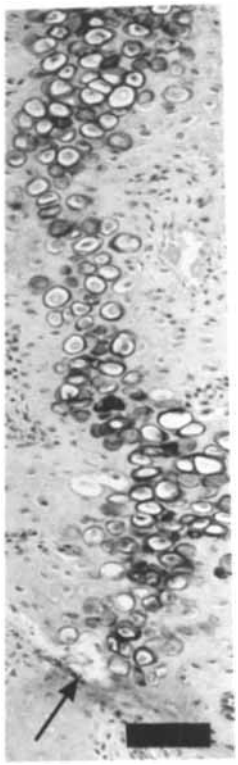

Fig. 12. A high power micrograph from a part of Figure 11. It shows an area of chondroclasia (arrow). (Alcian blue/H and E). Bar = 0.05 mm.

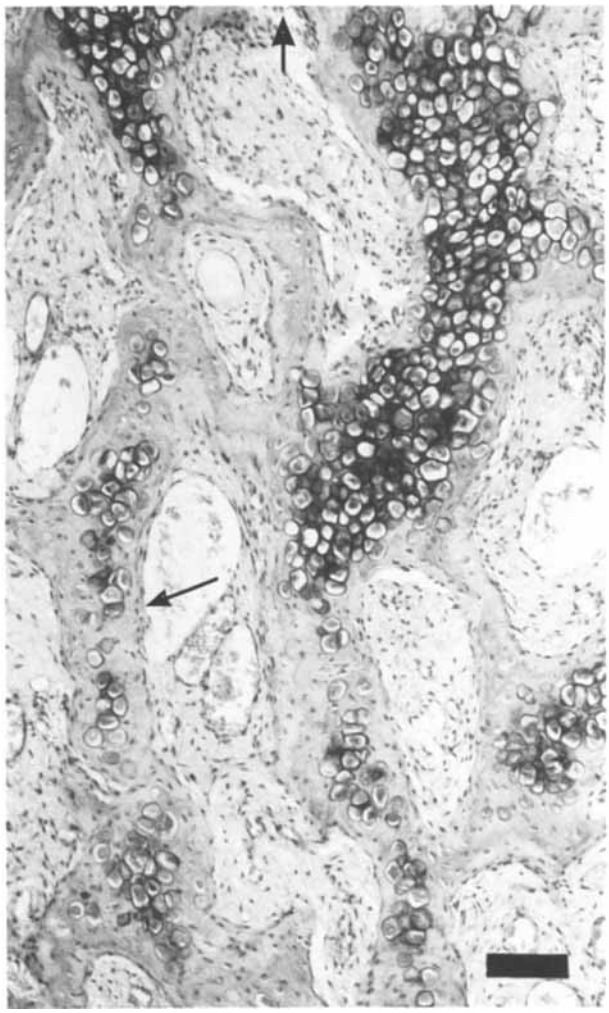

Fig. 13. A vertical section through the osseocartilaginous tissue of an incipient pedicle from an 8-month-old male calf. It shows a bony trabecula (arrow) with discrete cartilaginous cores. Note that all the cartilaginous cells from the cores were alive. The cartilaginous proportion increased towards the distal side (large arrow). (Alcian blue/H and E). Bar = 0.05 mm.

Nearly all of the cartilaginous trabeculae in the cartilaginous portion (Fig. 15) were continuous and parallel to the long axis of the pedicle. The cartilaginous cells varied in their state of maturity and cells of different maturity were located in a particular pattern in the tissue. Longitudinally, less differentiated cells occupied distal parts, whereas more matured cells were located in proximal parts; latitudinally, matured or hypertrophied chondrocytes occupied mid trabecular regions and young chondrocytes and chondroblasts were positioned peripherally. Alcianophilia occurred along these trabeculae, and was especially apparent on the capsular margins of lacunae.

In the osseocartilaginous portion, the osseocartilage forming zone was reduced compared with the corresponding portion of Group III, whereas the remodelling zone nearly occupied the whole portion, although the process was more pronounced in the proximal than in the distal region (Fig. 16). The remodelling in this unique structure consisted mainly of chondroclasia which was complemented by osteoclasia. At the proximal region, some discrete chondrocytic clusters in the mid trabecular sites were almost totally dissolved away, whereas others were being dissolved to a varying extent by chondroclast activity. The spaces left by removing chondrocytic clusters and the perivascular compartments became continuous as osteoclasts destroyed the peripheral bony wall. The activities of chondroclasts and osteoclasts converted the smooth-surfaced osseocartilaginous trabeculae into irregular and broken columns. Osteogenesis could be seen at the sites where chondrocytes had almost totally disappeared.

In the osseous portion, although osteoclasts were occasionally seen, active osteoblasts dominated the trabecular surfaces. Therefore, the whole portion was probably in transition from cancellous to compact bone.

The peripheral periosteum through the whole pedicle formation kept its original properties. Subperiosteal cancellous bone was formed through IMO, which was responsible for the latitudinal growth of the pedicle.

Group V (1 cm Antler Bud)

The gross structure of the whole tissue in this group was similar to that of Group IV, except for the integument. The histological feature of the tissue was that all cartilaginous trabeculae, peripheral periosteum, and underlying bone were continuous at the pedicle and newly formed antler bud junction. Externally this junction was characterised by a skin transformation

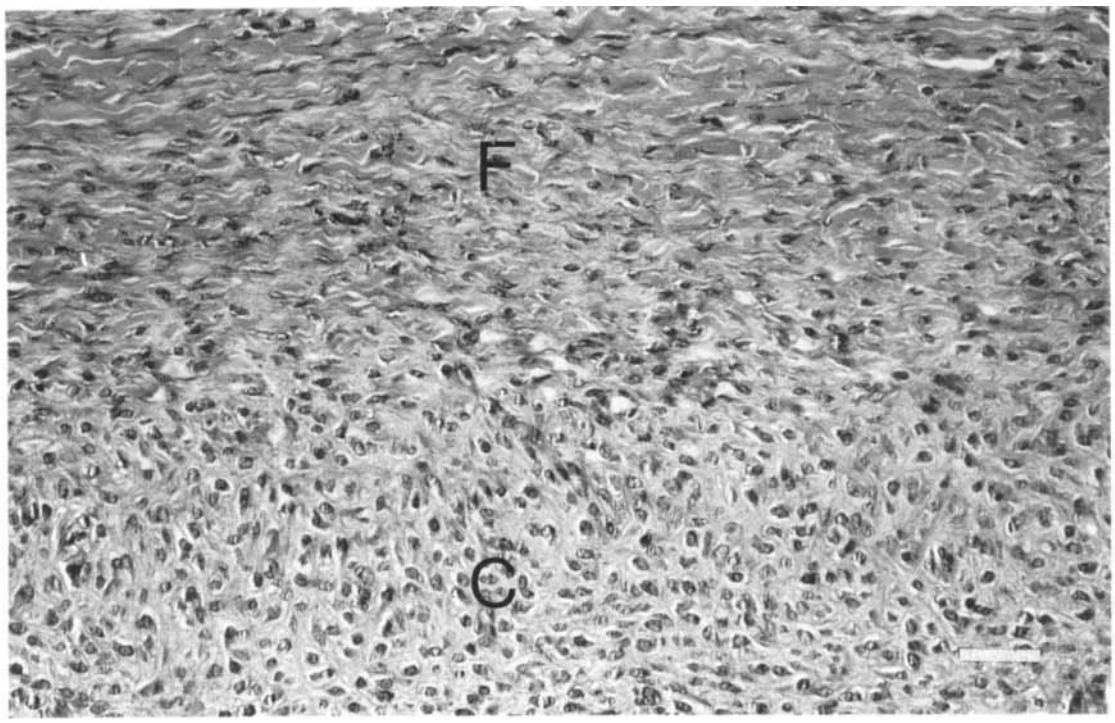

Fig. 14. A junction of the fibrous layer (F) and the cellular layer (C) of a pedicle apical perichondrium from an 8.5-month-old male calf. It shows that the cells in the superfacial region of the cellular layer, juxtaposed to the fibrous layer are large sized, randomly oriented with round nuclei. The fibres which filled in the intercellular spaces were oriented perpendicular to the fibrous layer (H and E). Bar = 0.05 mm.

from typical scalp to velvet. Therefore, at this stage antler development was simply the continuation of pedicle growth. The whole structure of the tissue could be also longitudinally divided into 4 portions: the hyperplastic perichondrium; the cartilaginous tissue; the osseocartilaginous tissue; and the osseous tissue.

In the hyperplastic perichondrium (Fig. 17), the fibrous layer was similar to that of Group IV. However, in the superficial region of the cellular layer juxtaposed to the fibrous layer, large sized, randomly oriented cells no longer existed but instead spindle-shaped cells oriented perpendicular to the long axis of antler predominated in the region. Adjacent to this region, large sized randomly oriented cells with round to oval nuclei were found. The deep region of the layer was similar to, but more pronounced than that of Group IV.

The cartilaginous portion (Fig. 18) consisted of two zones, from distal to proximal, namely a cartilage forming zone and a cartilage remodelling zone. The progression of cartilage formation occurred distally, whereas cartilage remodelling took place from the proximal region upward. The remodelling frontier from the proximal part advanced in parallel with the progression of cartilage formation from the distal part. The cartilage forming zone was similar to the cartilaginous portion of Group IV. In the cartilage remodelling zone, the activity of chondroclasts had changed the smooth-surfaced trabeculae into irregular, broken, and attenuated columns.

The osseocartilaginous portion was made up of two zones, namely a distal zone and a proximal zone. The distal zone was derived from the proximal region of the cartilaginous part of Group IV. The characteristic of the zone was predominant chondroclasia complemented by osteogenesis. The activity of the chondroclasts continued attenuating the cartilaginous trabeculae, whereas cancellous bone filled in the space created by chondroclasia. Therefore the osseous trabeculae with cartilaginous cores appeared in the remodelling zone. However, all of the nuclei of the cartilage cells in the cores were pyknotic (Fig. 19). The proximal zone corresponded to the osseocartilaginous portion of Group IV. However, the unique bone trabeculae with discrete clusters of chondrocytes had almost totally disappeared. In this zone, the remodelling pattern was apparent: at the distal region, chondroclasia was predominant (Fig. 20) and complemented by osteoclasia and osteogenesis, whereas at the proximal region, osteogenesis was predominant and accompanied by osteoclasia and chondroclasia (Fig. 21).

The osseous portion developed from both the corresponding portion and the deep region of the osseocartilaginous portion of Group IV. Cells lining the surfaces of bone trabeculae consisted mainly of osteoblasts. The laying down of typical lamellar bone continued, although it was cancellous, rather than compact.

DISCUSSION

The results of this examination show that the histogenesis of the pedicle and the early first antler proceeds in two distinct phases, namely a phase of internal

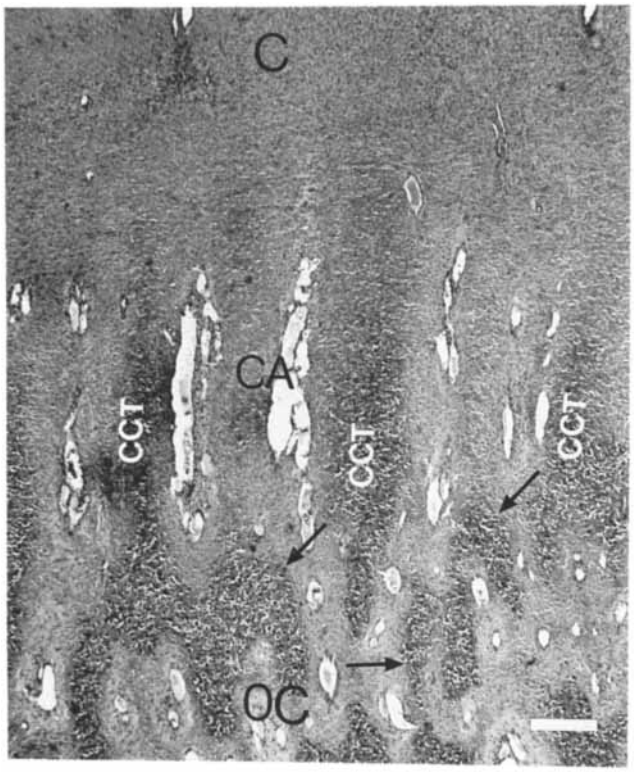

Fig. 15. A vertical section through the hypertrophied cellular layer (C), cartilaginous tissue (CA), and osseocartilaginous tissue (OC) of a pedicle from an 8.5-month-old male calf. It shows that the continuous cartilaginous trabeculae (CCT) were forming on the bony trabeculae with discrete cartilaginous cores (arrows). (Alcian blue/H and E). Bar = 0.3 mm.

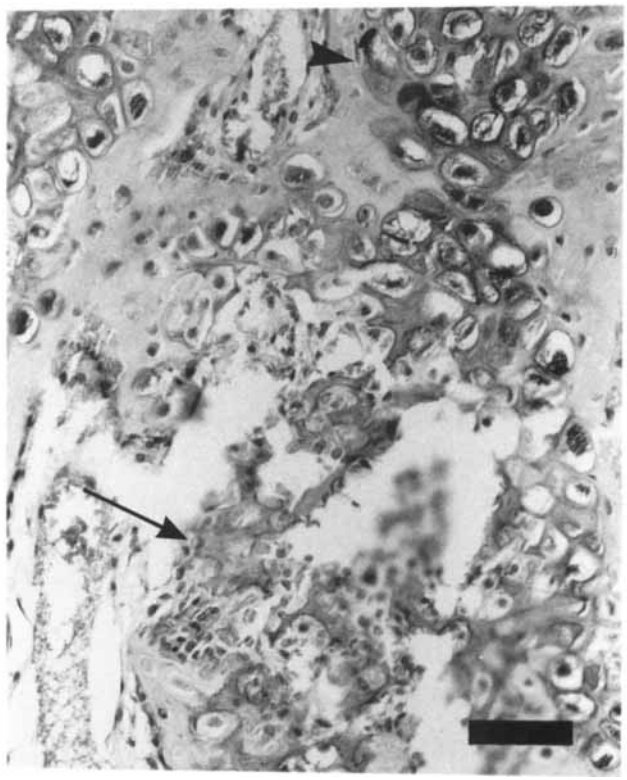

Fig. 16. A part of the osseocartilaginous portion of a pedicle from an 8.5-month-old male calf. The arrow head points to the forming zone and the arrow points to the remodelling zone. (Alcian blue/H and E). Bar = 0.03 mm.

change through which the pedicle is formed and an external change phase, which signals the beginning of antlerogenesis. The internal phase which is associated with cellular and matrical changes is divisible into three distinct, yet continuous stages, namely IMO, OPC, and modified ECO. The changes are initiated from the antlerogenic periosteum of the frontal lateral crest (Fig. 22).

Antlerogenic Periosteum and Antlerogenic Cells

It is well known that the periosteum of the frontal lateral crest, the antlerogenic periosteum of pre-pubertal deer, can induce ectopic pedicle and antler formation (Hartwig and Schrudde, 1974; Goss and Powel, 1985). The histological results of this study showed that both the cellular layer and the fibrous layer of the antlerogenic periosteum of both male deer before transforming to antlerogenic perichondrium, and of female deer was much thicker than the facial periosteum but there were no significant differences in cell density between the periostea. Therefore, the antlerogenic periosteum contains more cells than the facial periosteum in a piece of tissue of the same area. Inasmuch as the cellular layer of the periosteum accounts for bone formation (Ham and Harris, 1971), antlerogenic periosteum possesses a greater potential than facial periosteum in terms of its capacity to form bone. The fibrous layer of the antlerogenic periosteum becomes hyperplastic during pedicle growth unlike the somatic periosteum fibrous layer, which maintains its thickness unchanged when the osteogenic layer reaches its maximum thickness during bone formation (Sissons, 1971). It is worth noting that at the OPC stage the fibrous bundles in the fibrous layers have characteristic regular waves, which may imply that at this stage the fibrous layer grows even faster than the underlying tissue, although the amplitude of the waves decreases gradually as the pedicle formation speeds up. Another specific feature of the antlerogenic periosteum is that elastic fibres, which are present between the cellular and fibrous layer in developing somatic periosteum of the guinea pig (Murakami and Emery, 1967), were not observed in the present study during the pedicle and antler development period. Murakami and Emery (1967) thought the elastic fibres acted as a limiting membrane for a proliferating osteogenic layer. Therefore, without the restriction of a limiting membrane, the antlerogenic periosteum cellular layer might become more hypertrophied and consequently promote pedicle and antler formation.

The finding in this study that the female deer antlerogenic periosteum was histologically identical to that of the male deer before transforming to perichondrium may provide further evidence to explain why female deer antlerogenic periosteum can also be induced to grow pedicles and antlers with the appropriate stimulation (Jaczewski, 1982).

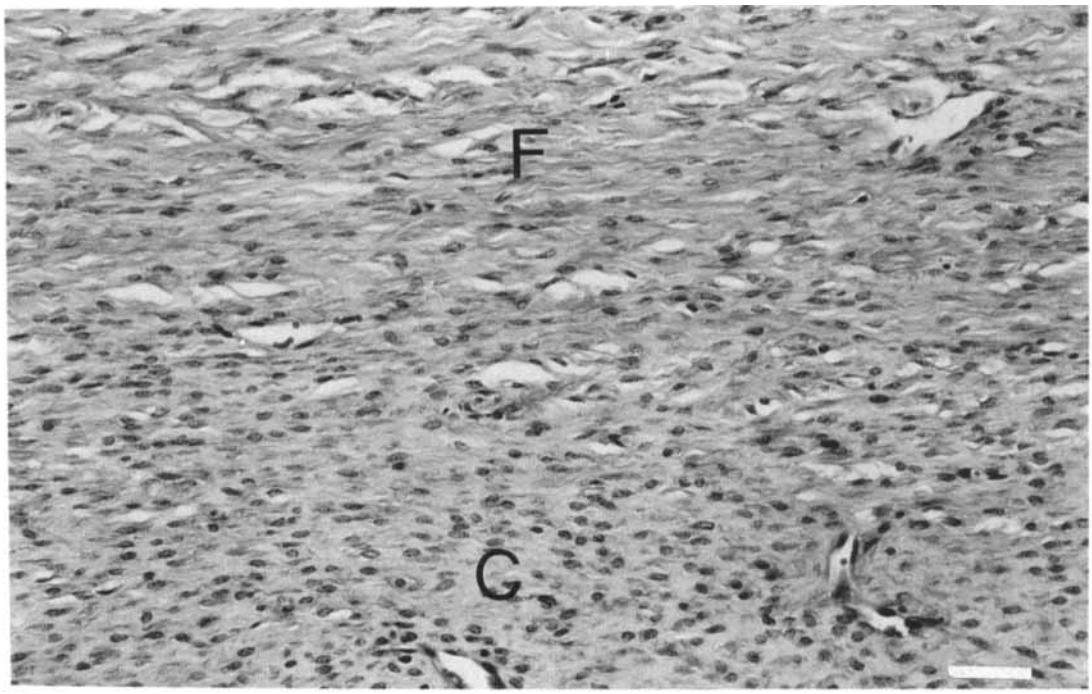

Fig. 17. A junction of the fibrous layer (F) and the cellular layer (C) of an antler apical perichondrium from a 9-month-old male calf. It shows that the cells in the superfacial region of the cellular layer, juxtaposed to the fibrous layer were spindle-shaped and oriented parallel to the fibrous layer (H and E). Bar = 0.05 mm.

The fact that mitotic figures were only found in the cellular layer of the hyperplastic antlerogenic periosteum/perichondrium during pedicle formation is consistent with the finding of Banks and Newbrey (1982a) in antler development. Unlike somatic cartilage development in which appositional growth as well as interstitial growth are responsible for cartilage growth as the chondrocytes undergo both division and hypertrophy, pedicle and antler cartilage formation is primarily achieved by appositional growth as the chondrocytes are not able to divide and can only become hypertrophied. This was considered as one of the characteristic features distinguishing antler cartilage formation from somatic counterparts by Banks and Newbrey (1982b). Consequently, cell division in the apical antlerogenic periosteum/perichondrium region accounts for pedicle and antler elongation, whereas cell proliferation in the peripheral periosteum is responsible for the latitudinal growth of the pedicle and antler, but at the onset of growth, both types of cells are derived from the osteogenic tissues of the antlerogenic periosteum cellular layer. Consequently, the periosteum/perichondrium contains types of bone cells which may only represent different functional states of the same osteogenic cells. Since the frontal lateral crest periosteum which has the capacity to form pedicle and antler is termed antlerogenic periosteum, the cellular layer osteogenic cells can be defined as antlerogenic cells.

Intramembranous Ossification Stage

Under normal nutritional conditions, 4-month-old males and 14-month-old females had externally unpalpable pedicles. However, the subperiosteal cancellous bone is being formed by means of typical mammalian IMO in the frontal protrusion, whereas the subperiosteal bone of the facial periosteum is compact and at resting stage. It is well established that osteogenic cells of periosteum differentiate into osteoblasts and then bone is formed in the presence of capillaries (Ham, 1969). Since the periosteum is richly supplied with blood vessels (Ham, 1969), when it is stimulated, the osteogenic cells form bone directly; this occurs in the early stages of bone fracture repair (Ham and Harris, 1971). Deer antlerogenic periosteum is also richly supplied with capillaries derived from temporal and supraorbital blood vessels (Waldo et al., 1949; Adams, 1979). Therefore, it is conceivable that the antlerogenic cells of the antlerogenic periosteum differentiate into osteoblasts, and cancellous bone is directly formed in the growing apex of the frontal protrusion.

Two questions arise: Firstly, should the IMO stage be defined as frontal lateral crest formation or pedicle initiation? Although the protrusion was not investigated in the present study between birth and 4 months it is highly likely that the IMO is integral to frontal lateral crest formation. There are two reasons: pedicle initiation depends on androgen hormones (Bubenik, 1982; Jaczewski, 1982), and at the IMO stage, endogenous testosterone is still undetectable in male calves (Suttie and Fennessy, 1990), while the more convincing argument is that the frontal lateral crest in both female deer and castrated male deer develops to the same stage. Secondly, how is the IMO stage commenced or sustained? Sissons (1971) reported that the proliferat-

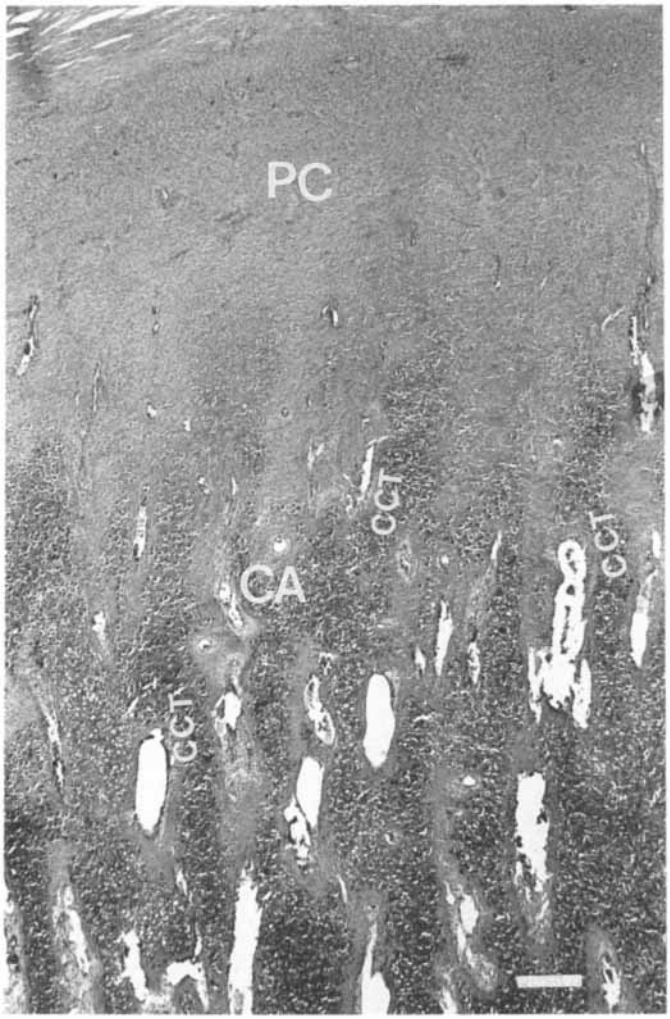

Fig. 18. A vertical section through the hypertrophied perichondrium (PC) and cartilaginous tissue (CA) of an incipient antler from a 9-month-old male calf. The continuous cartilaginous trabeculae (CCT) were being exclusively formed underlying the perichondrium. (Alcian blue/H and E). Bar = 0.3 mm.

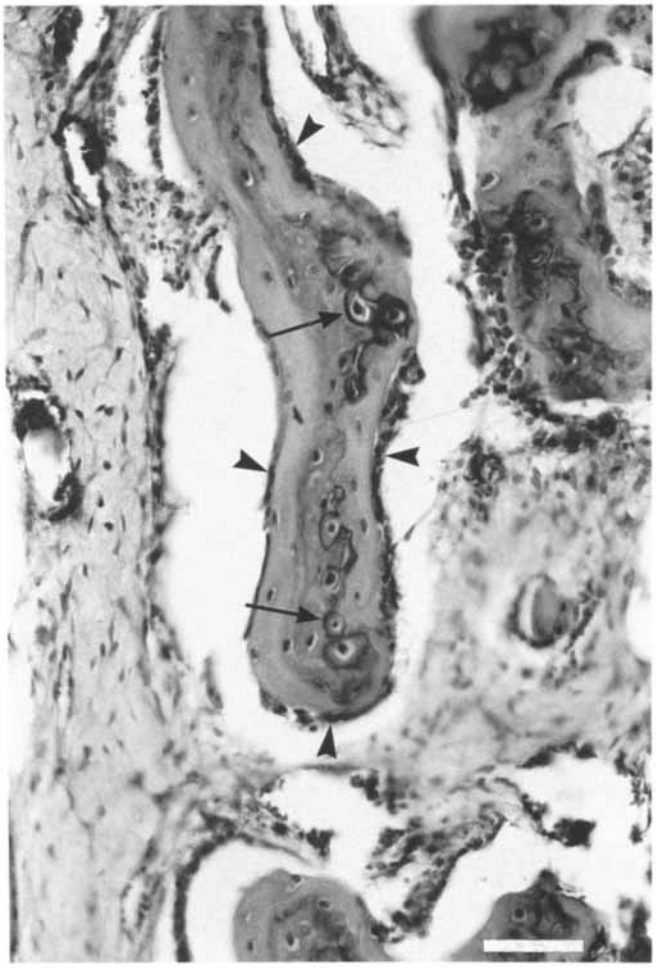

Fig. 19. A part of the osseocartilaginous portion of a pedicle with an incipient antler from a 9-month-old male calf. It shows that a bony trabecula covered by osteoblasts (arrowheads) had cartilaginous cores (arrows). Notice that the nuclei of the cartilaginous cells were pyknotic. (Alcian blue/H and E). Bar = 0.03 mm.

ing tissue of bone has considerable autonomous powers of growth. In the course of normal development, this undergoes important modifications under the influence of local and general factors. These include nutritional, genetic, and other less precisely specified factors which all play their part in sustaining or modifying bone growth. It seems quite possible that the IMO stage is related to dietary factors since the IMO stage does not occur in malnourished deer of either sex.

Ossification Pattern Change (OPC) Stage

When the incipient pedicle of a male red deer calf becomes externally palpable, the tissue has begun to undergo OPC from IMO to modified ECO in the apex of this protrusion. The antlerogenic cells of the antlerogenic periosteum covering the free ends of the trabeculae and the surfaces of the spicules of some areas differentiate into chondroblasts rather than osteoblasts. A similar event occurs in secondary type cartilaginous tissue formation (such as mandibular condyle) (Hall, 1978), in solitary osteochondroma formation (the most common benign tumour of the skeleton) (Dahlin and Unni, 1986), and bone fracture repair (Ham and Harris, 1971). Ham and Harris (1971) thought that differentiating osteogenic cells of the periosteum lost their vascular environment and that this was the reason for the OPC in bone fracture repair. However, unlike ordinary somatic cartilage which is virtually devoid of a capillary system, both pedicle and antler cartilage are well vascularised. Nevertheless the present results and those of a previous study (Banks and Newbrey, 1982a) provide evidence for the hypothesis that the OPC occuring in pedicle formation is caused by the same mechanism as that of bone fracture repair. This is so because when the OPC begins, cartilage cells only appear at the mid trabecular region of some fast growing central trabeculae in the forming apex of the pedicle. The distal part of these trabeculae are separated by discrete and narrow spaced channels within which no blood cells are found only connective fibre strands. By

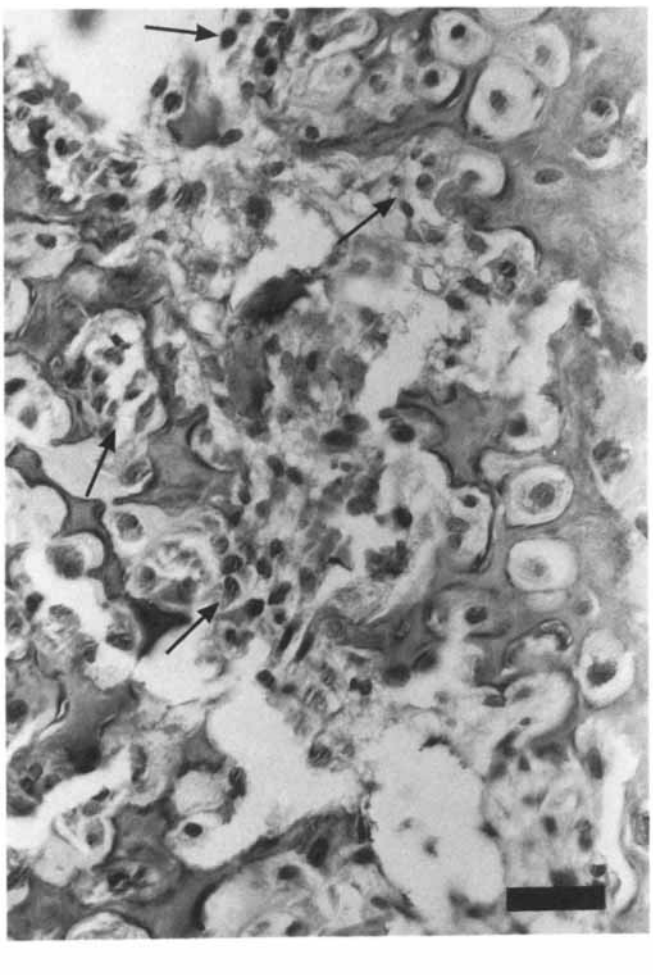

Fig. 20. An active area of chondroclasia of the distal region of an osseocartilaginous portion from an 8.5-month-old male calf. Chondroclasts (arrows) are destroying the cartilaginous part of an osseocartilaginous trabecula. (Alcian blue/H and E). Bar = 0.03 mm.

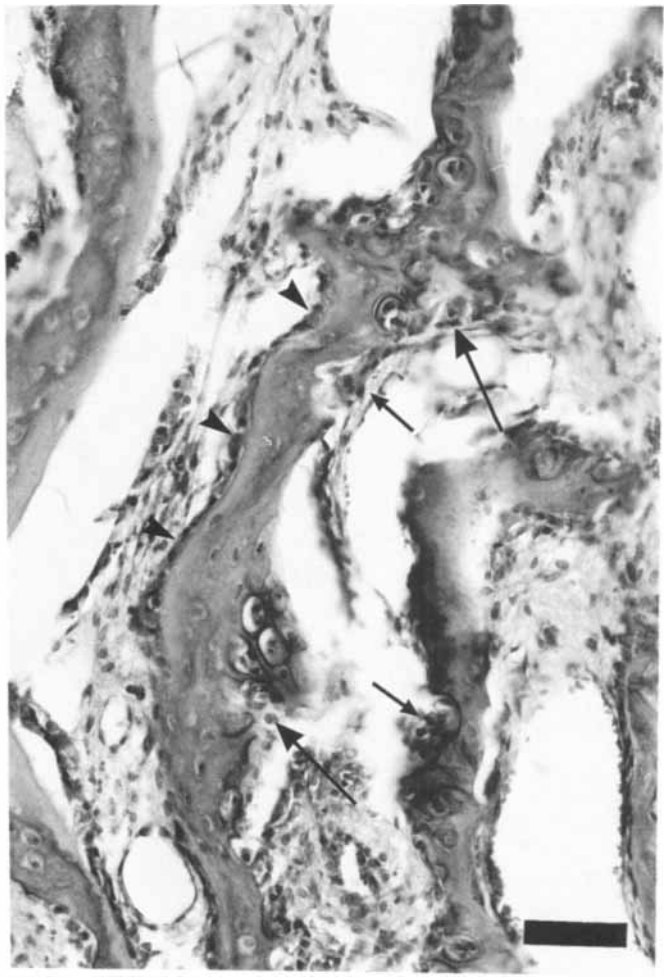

Fig. 21. A remodelling area of the proximal region of an osseocartilaginous portion from an 8.5-month-old male calf. The osseocartilaginous trabecula was partially covered with active osteoblasts (arrowheads). Osteoclasia (arrows) and chondroclasia (large arrows) were also presented in the same trabecula. (Alcian blue/H and E). Bar = 0.03 mm.

the time the ossification pattern changes to modified ECO during the pedicle formation, no blood cells are found in the channels of the region where antlerogenic cells were differentiating into cartilaginous cells, as occurred during antler development (Banks and Newbrey, 1982a). In addition, most cartilaginous cells or clusters are not continuous when they first appear. Therefore there is strong reason to believe that where the antlerogenic cells of the antlerogenic periosteum proliferate and differentiate vigorously, functional capillary formation is unable to keep up with their rapid growth, and cartilage tissue will be formed. But, if for some reason the speed of the antlerogenic cell proliferation and differentiation slows down, a functional capillary formation can catch up with the growth apex, antlerogenic cells can differentiate in the vascular environment, and bone tissue will be formed again directly.

What stimulates the OPC from IMO to modified ECO during the pedicle formation? It seems undoubtedly that the OPC is induced by androgen hormone as pedicle development depends on androgen hormone stimulation. At the OPC stage, endogenous testosterone begins to increase in male deer calves (Suttie et al., 1991). The growing apex of the pedicle bud is the target tissue of androgen hormone (Li et al., 1990). Moreover, the frontal lateral crest development of female deer and castrated male deer calves stops before the OPC stage begins.

Endochondral Ossification Stage

When the incipient pedicle of a male deer calf develops sufficiently to be externally visible, cartilage tissue is being formed in the growth apex by means of a new ossification pattern. This is identical to the pattern which occurs in antler development, namely, modified mammalian ECO (Banks and Newbrey, 1982a,b). The precise features of the vascularized tissue were uncertain until Banks (1973), Frazier et al. (1975), and Banks and Newbrey (1982a) demonstrated that this unique tissue was indeed cartilage by histological, his-

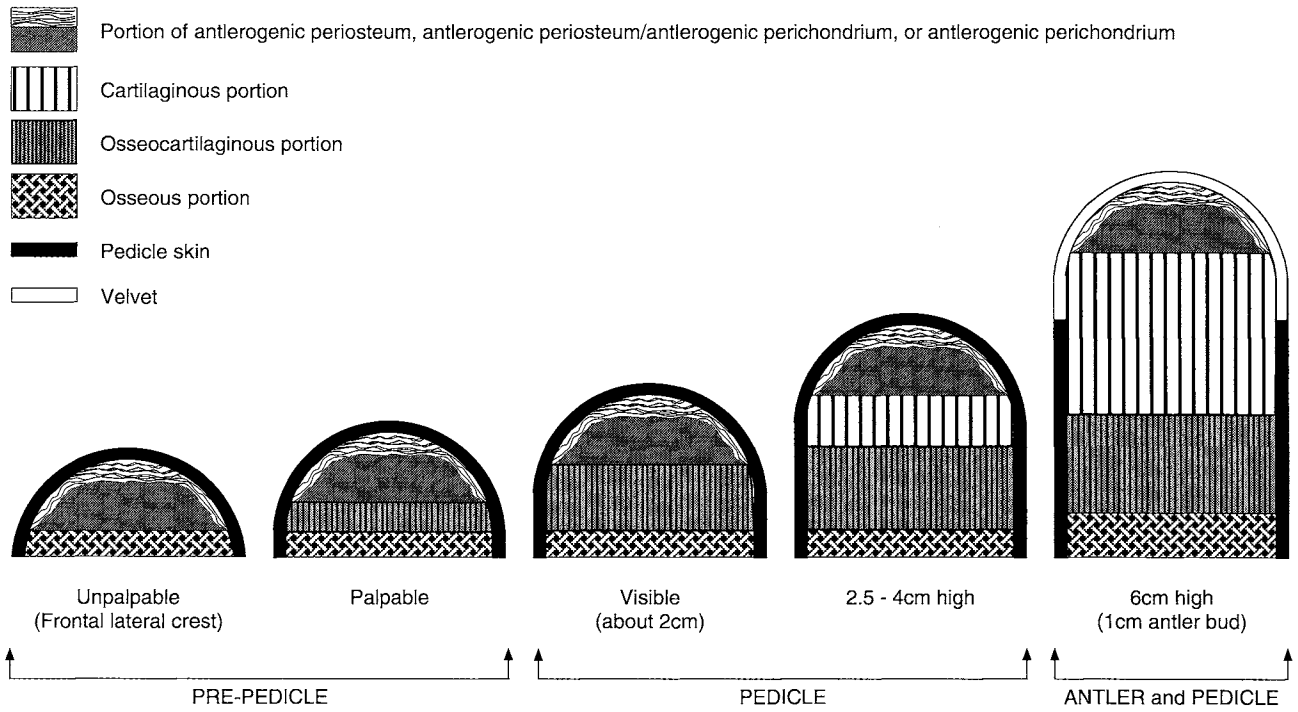

Fig. 22. Diagram of histogenesis of deer pedicle and early first antler.

tochemical, and ultrastructural evidence. The ECO of antler forms vascularised tissue directly in contrast to its somatic counterpart which forms avascular cartilage first followed by vascular bud invasion of the cartilage as soon as it becomes calcified. This normally only occurs in direct bone tissue formation. The ossification pattern of antler development is termed modified mammalian ECO to account for the differences in its vascular structure (Banks and Newbrey, 1982b).

Banks and Newbrey (1982b) considered that the metabolic demands of rapid proliferation, differentiation, and growth of antler might require the additional nutrient supply provided by the unique vascular cartilage. Stockwell (1979) reported that although cartilage matrix was optimal for diffusion (the normal way in which cartilaginous cells obtain their nutrients) there was a limitation over which the chondrocytes could not be adequately nourished by the blood vessels in perichondrium. Therefore if pedicle and antler development adopted typical mammalian ECO, then growth would be limited or cartilage cells in the central part of the tissue block would have died before they became calcified as they could not get adequate nourishment. Conversely if pedicle and antler development proceeded continuously through IMO, that is, no OPC occurred, antlers might not have existed at all because the tissue is formed very slowly through IMO. The growth rate of cortical bone being formed by means of IMO has been measured at 10–15 μm weekly (Sissons, 1971). The antler growth period is about 15 weeks (Fennessy et al., 1992), so the total length of antler would be only about 0.2 mm! The modified mammalian ECO probably evolved for pedicle and antler formation to permit rapid growth over a restricted time period.

The finding that the ossification pattern changes from IMO to modified ECO when the deer pedicle grows over 25 mm long may explain the phenomenon that pedicle size is crucial for antler growth following traumatic induction in castrate males. Antler formation will occur after amputation and wound healing only if the pedicle is over 2 cm high but not if the pedicle is less than that (Jaczewski, 1982).

Consequently, we conclude that under normal nutrition conditions, the frontal lateral crest in both male and female red deer calves is formed through typical mammalian IMO. Gradually the antlerogenic cell proliferation and differentiation of the antlerogenic periosteum slow down. At this stage if no further stimulation or induction occurs, the antlerogenic cell activity will eventually stop. However, if these cells are adequately stimulated by internal (androgen hormones) or external (mechanical or chemical) factors, their proliferation and differentiation will recommence. The antlerogenic cells begin to differentiate into chondroblasts instead of osteoblasts because their growth pace is faster than blood vessel growth, then the OPC from IMO to modified ECO begins and the unique vascularized cartilage is formed. However this whole series of changes cannot be seen externally as the pedicle skin is still characterised by that of typical scalp. Therefore these changes are called the internal changes.

Tissue Transformation From Pedicle to Antler Stage

When the pedicle of a red deer calf has reached about 6 cm in length, shiny velvet skin appears at its distal tip. This externally represents the beginning of the tissue transformation from pedicle to antler. However our results showed that except for the integument covering

the tip, histologically there were no differences found in the periosteum or developing vascularized cartilage tissue between the pedicle and antler. Both pedicle and newly formed antler have the same ossification pattern and all cartilaginous columns and peripheral periosteum are continuous between pedicle and antler. Therefore this change is termed the external change, yet physiologically the two structures vary considerably in their responses to sex hormones (Goss, 1970). Further the cells of the pedicle have the full capacity to regenerate, whereas those of the antler do not (Suttie and Fennessy, 1990). Goss (1985) considered that one cannot precisely distinguish at which point in this process the antler itself first begins to develop, but that there was reason to believe that the onset of chondrification (ECO) might signal the earliest stage of antlerogenesis. At this time, however, there is no overt indication that the pedicle has begun to form an antler, and the overlying skin is still characterised by the typical pelage of the scalp. The present results do not support Goss's hypothesis because chondrification begins when the pedicle bud has just become palpable. However, some questions remain to be answered. Firstly, why does the integument change from typical pelage to velvet only occur when the pedicle reaches about 6 cm in length while the chondrification begins when the pedicle just starts to grow? Secondly, what are the initiating and concluding signals for pedicle formation? Prior to chondrification, the subperiosteal cancellous bone formation occurs as the frontal lateral crest develops and in both female and castrated male deer development also proceeds to this stage, but stops before the chondrification starts. Thirdly, why does the growth of an artificially induced pedicle stop when it reaches a certain length, but the visual appearance of velvet on the distal end of the pedicle needs extra stimulation (Jaczewski, 1982)? Fourthly, why do antlers cast only from the junction of the typical scalp pelage and velvet? We believe that the chondrification must signal pedicle initiation, and the velvet appearance starts antler formation, although the histological differences between pedicle and antler are unknown at this stage.

In conclusion, the histogenesis of deer pedicle and first antler proceeds through a complex series of tissue changes, which originate from the proliferation and differentiation of the antlerogenic cells of the antlerogenic periosteum and covers two distinct phases, namely an internal phase through which pedicle tissue is formed, and an external phase which signals the onset of antlerogenesis. The internal phase starts from IMO, progresses through OPC which signifies the completion of frontal lateral crest formation and the initiation of pedicle development, to modified mammalian ECO in which unique well-vascularized cartilage is formed. However, these changes cannot be seen externally as the overlying skin of the pedicle is still characterised by typical pelage of the scalp. The external phase begins when the shiny skin, with velvet pelage, appears at the distal end of the pedicle and proceeds continuously through the modified ECO until the first antler forms. However, the change cannot be distinguished internally as the peripheral periosteum and cartilaginous trabeculae are all continuous between pedicle and antler, although the antler integument or velvet is considerably different from the pedicle skin.

ACKNOWLEDGMENTS

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