

Histological Studies of Pedicle Skin Formation and Its Transformation to Antler Velvet in Red Deer (*Cervus elaphus*)

CHUNYI LI* AND JAMES M. SUTTIE

AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand

ABSTRACT

Deer antlers and their antecedent pedicles are made up of two components, interior osseocartilage and exterior integument. In a previous study, we described that histogenesis of the interior osseocartilage proceeds through four ossification stages. These are intramembranous (IMO), transition (OPC), pedicle endochondral (pECO), and antler endochondral (aECO). In the present study, we used histological techniques to examine pedicle skin formation and its transformation to antler velvet. The results showed that pedicle skin initiated from the apex of a frontal lateral crest and was formed through three distinctive stages. These stages are 1) compression of the subcutaneous loose connective tissue at the OPC stage, 2) stretching of the undulated epidermis at the early pECO stage, and 3) neogenesis of the skin and its associated appendages at the mid pECO stage. Transformation into antler velvet, which occurs at the late pECO stage, is mainly associated with alteration in the skin appendages. This alteration includes the loss of arrector pili muscle and sweat glands, and the gain of the large bi- or multi-lobed sebaceous glands. These results suggest that pedicle skin expansion occurs to release the mechanical tension created by underlying forming antlerogenic tissue, initially in response to it by mechanical stretch, and then by neogenesis of skin. In turn, the stretched pedicle skin may exert mechanical pressure on the underlying antlerogenic tissue causing it to change in ossification type. Antler velvet generation may be accomplished by both mechanical stimulation and chemical induction from the underlying pECO stage antlerogenic tissue. If this hypothesis is correct it is likely that mechanical stimulation would drive skin formation and chemical induction then determine skin type. Furthermore, asynchronous transformation of the interior and exterior components during pedicle formation and antler generation may result from the delayed chemical induction and the way antler velvet initially generates. The results from both mitotic cell labelling of the basal layer and ultrastructure of the basement membrane of the apical skin in the study support these hypotheses. Anat Rec 260:62–71, 2000.

© 2000 Wiley-Liss, Inc.

Key words: pedicle; antler; antler velvet; antlerogenic tissue; skin

Deer antlers are male cephalic appendages that are cast and regenerate each year from the apices of pedicles. Pedicles are permanent protuberances that start to develop from the lateral crests of deer frontal bone during puberty which occurs in the first year of a deer's life (Fennessy and Suttie, 1985). First antlers are generated spontaneously after pedicles grow to a species-specific height (50–60 mm high in red deer). Thereafter, antler formation is a regenerative response to annual androgen

hormone fluctuation following casting of the previous hard antlers. So far, no reasonable hypothesis has been ad-

*Correspondence to: Chunyi Li, AgResearch, Invermay Agricultural Centre, Private Bag 50034, Mosgiel, New Zealand. E-mail: lic@agresearch.cri.nz

Received 29 March 2000; Accepted 15 May 2000

vanced to explain the mechanism underlying the transformation from a permanent pedicle to a deciduous antler.

Pedicles and growing antlers are made up of exterior and interior components. The exterior component consists of pedicle skin and antler velvet (velvet-like skin). Pedicle skin is typical deer scalp skin (Goss, 1983), whereas antler velvet is specialised skin. In contrast to deer body and pedicle skin, antler velvet lacks subcutaneous loose connective tissue but has the ability to form new hair follicles. These newly formed follicles lack associated arrector pili muscle and sweat glands, but possess well-developed sebaceous glands (Billingham et al., 1959; Bubenik, 1993; Goss, 1964). However, histological description of the transformation from pedicle skin to antler velvet has not been reported.

The interior component is composed of osseocartilaginous tissue. The histogenesis of this component in red deer has previously been systematically studied (Li and Suttie, 1994). The results have shown that it proceeds through four distinct development stages. Intramembranous ossification (IMO, pedicle under 5 mm), transitional ossification or ossification pattern change (OPC, pedicle under 25–30 mm), pedicle endochondral ossification (pECO, pedicle over 25–30 mm), and antler ECO (aECO, entire antler). Both pECO and aECO are modified endochondral ossification and essentially the same as they are histologically indistinguishable. Thus a change in ossification from pedicle type (IMO and OPC) to antler type (modified ECO) is complete when a pedicle grows to only half (25–30 mm) of its full length (50–60 mm). Hence, the anatomical definition of antler as opposed to pedicle is when a change in the exterior component from pedicle skin to antler velvet takes place and not due to an internal change. It was not understood why transformations between the interior component and the exterior component take place asynchronously. However, histological examination of the exterior component may hold the key to answering this question.

One of the astonishing features of pedicle skin and antler velvet is their capacity to expand rapidly. This expansion rate is commensurate with an elongation of the interior component (Billingham et al., 1959; Goss, 1964). However, the question of whether there is a driving force for pedicle skin and antler velvet expansion, or whether they themselves have the ability to do that, has not been previously asked.

First antler generation is considered unique, as this process is not an example of regeneration since there has been nothing lost which could be replaced (Goss, 1983). In his transplantation experiments, Goss (1987) noted that ectopic antlers can form only if the tissue derived from the grafted antlerogenic periosteum becomes closely associated with the overlying skin. Goss (1990) thought that this indispensable tissue association is a prerequisite for the inductive molecules from the antlerogenic tissue to reach and effect the overlying skin. However, it was not known whether this was also the case for normal first antler generation.

The aim of the present study was to use histological techniques to examine pedicle skin formation and its transformation to antler velvet, to explore the following questions: 1) What factors cause the rapid expansion of the pedicle/antler skin? 2) Why transformations from a pedicle to an antler between the interior and the exterior components are asynchronous? 3) Does normal first antler

generation also require the close association between antlerogenic tissue and the overlying skin?

MATERIALS AND METHODS

Tissue Sampling

The tissues were taken either from slaughtered animals (frontal lateral crests [FLC] and early pedicles), or from biopsy (late pedicles and early antlers) of red deer (*Cervus elaphus*) during the period of pedicle initiation and first antler generation (17 August–11 November, Southern Hemisphere).

The number of deer sampled and the heights of the FLCs, pedicles or antlers are shown in Table 1.

FLC and Early Pedicle Sampling

In order to ensure that the sampled FLCs or OPC stage pedicles were suitable for measurement of the apical skin (defined as the thickness of epidermis and subcutaneous loose connective tissue [SLCT]), two heads from each stage were fixed by perfusion. This is because it had been previously observed that a layer of SLCT exists between the skin and the underlying FLCs at the intramembranous ossification (IMO) or the early pedicle at transitional ossification (OPC) stages. Difficulties were encountered in our previous studies when handling samples with SLCT and the perfusion technique was developed so as not to dislodge the overlying skin from the FLC or the OPC stage pedicle during sampling.

The perfusion was carried out immediately after slaughter and prior to sampling as follows. Fifteen min after an injection of heparin (50,000 IU), the deer were euthanised by lethal injection of pentobarb 500 (Sodium pentobarbitone solution 500 mg/ml, Chemstock Animal Health Ltd., New Zealand). The deer heads were removed immediately after death and perfused by pumping fixative (6 litres 10% buffered Formalin/head) through the carotid arteries. After perfusion, the hair around the FLC region was shaved and an oval incision was made around the FLC on the scalp using a scalpel. The incision was deep enough to cut through the skin and the incision line was approximately 5 mm from the crest boundary. Then the FLC was removed by using a bone saw to cut through the skull following the skin incision line.

For the OPC stage pedicle, after shaving the hair around the region, an incision was made circumferentially along the joint line of the scalp and the pedicle skin using a scalpel. The bony bump was then sawn off through the skin incision line. After trimming, all the tissue blocks were fixed in 10% buffered Formalin for histology.

The rest of the FLCs and the OPC stage pedicles were taken from slaughtered animals without perfusion, following the same dissection procedures described above. Extreme care was taken at each sampling and trimming step to avoid separating the skin from the underlying tissue.

Late Pedicle and Early Antler Sampling

A modified velvet harvesting technique was employed for the biopsy of the late stage pedicles and the early antlers. Briefly, the deer was held in a hydraulic deer crush and local anaesthesia of the pedicles/antlers was achieved by injecting 10 ml/side Lopaine (Lignocaine hydrochloride, U.S.P. 20 mg/ml. Ethical Agents Ltd., Auckland, New Zealand) around the base of a pedicle. After carefully shaving and sterilising the pedicle/antler and

TABLE 1. Histological examination of pedicle skin formation and its transformation to antler velvet*

Stage	n	SLCT (μm)	Height (mm)	Epidermis		Neogenesis of hair follicle	Arrector pili (Y/N)	Sebaceous gland		Sweat gland
				Morphology	Thickness (μm)			Size	Shape	
IMO	4	850 ± 150^a	>5	undulated	18.5 ± 1.85^a	no	Y	small	monolobar	+
OPC	6	20.7 ± 0.67^a	20.7 ± 0.67^a	undulated, but less extent in amplitude and frequency cf. IMO stage	50.0 ± 2.45	no	Y	intermediate	monolobar	++
pECO	10	—	28.3 ± 3.44	flat	68.6 ± 8.96	early, at tip; late, tip to shoulder	Y/N	large	most bilobar	++
aECO	3	—	40.6 ± 5.54	flat	95.4 ± 12.24	at tip (special hair follicle) ^c	N	very large	bi- or multilobar	±
	4	—	74.9 ± 5.61	flat	112.2 ± 11.5	tip to distal shaft (special hair follicle)	N	very large	bi- or multilobar	—

*IMO, intramembranous ossification; OPC, transitional ossification; pECO, pedicle endochondral ossification; aECO, antler endochondral ossification; SLCT, subcutaneous loose connective tissue.

+ common; ++ abundant; ± sparse; — absent.

^aMean ± se.

^bA thin vascular layer.

^cBigger sebaceous glands, arrector-pili-muscle-less and sweat-gland-less.

the adjacent area of the scalp, a pedicle or an antler was biopsied following the same procedure as for sampling OPC stage pedicles. After the biopsy, the wound was closed with a silk suture and the deer was released from the crush. The biopsied samples were fixed in 10% buffered Formalin for histology.

Histology

After a minimum of 3 days in the fixative, the tissue samples were decalcified in a solution containing 40% formaldehyde: 90% formic acid: water (1:2.2:16.8). Decalcification was considered to be complete when no evidence of mineralisation was observed on radiographs. Following decalcification, the tissues were neutralised in saturated aqueous lithium carbonate (1 g or over/78ml water) for 6–8 hr then stored in 70% alcohol before they were embedded in paraplast wax. The embedded tissue blocks were sectioned at 5–10 μm . Haematoxylin (H)/eosin (E) and alcian blue/HE stains were used.

Stage Determination and Thickness Measurement

Developmental stages of the pedicles were determined by histological evaluation of the interior component (ossification types). The height measurement of these pedicles was used only as a reference.

Thickness measurements for the apical epidermis were carried out on the tissue samples from all four ossification stages. Measurement of the SLCT was carried out only on the tissue samples from the IMO stage, as the boundaries between the SLCT and the underlying fibrous layer of the antlerogenic tissue could not be readily distinguished in the subsequent developmental stages. The procedure for thickness measurement has been reported elsewhere (Li and Suttie, 1994). Briefly, a modified Olympus BH-2 microscope that was connected via a camera to a television monitor was used. The magnification of the image was verified by using a micrometer with 10 μm intervals. Ten measurements for epidermis and five measurements for SLCT were taken with an interval of 10 μm . All measurements were made perpendicular to the skin surface at the tip of the FLC, pedicle, or antler. The thickness of the epidermis was analysed using ANOVA. The statistical significance was assessed at the 5% and 1% levels.

BrdU Injection and Mitotic Cell Localisation

BrdU injection was conducted on 3 deer, one from each of the following ossification stages: IMO, OPC, and pECO, for localisation of mitotic cells. Two hr before the deer were killed, BrdU (5'-bromo-2'-deoxyuridine, Sigma Chemical Company, St. Louis, MO) a synthetic thymidine analogue, was injected intraperitoneally to each deer (25 $\mu\text{g/g}$ live weight).

Following the same procedure as for histology, the tissue was sampled, processed, and sectioned at 5 μm . The sections were collected onto gelatin-coated slides. The recommended manufacturer's immunohistochemical technique was used to localise BrdU labelled cells. Anti-BrdU antibody was purchased from Becton-Dickinson and anti-mouse IgG from Amersham. After the localisation, the sections were examined using appropriate fluorescence optics on a Zeiss fluorescence microscope. The mitotic cell distribution in the apical skin from these three ossification stages is reported here.

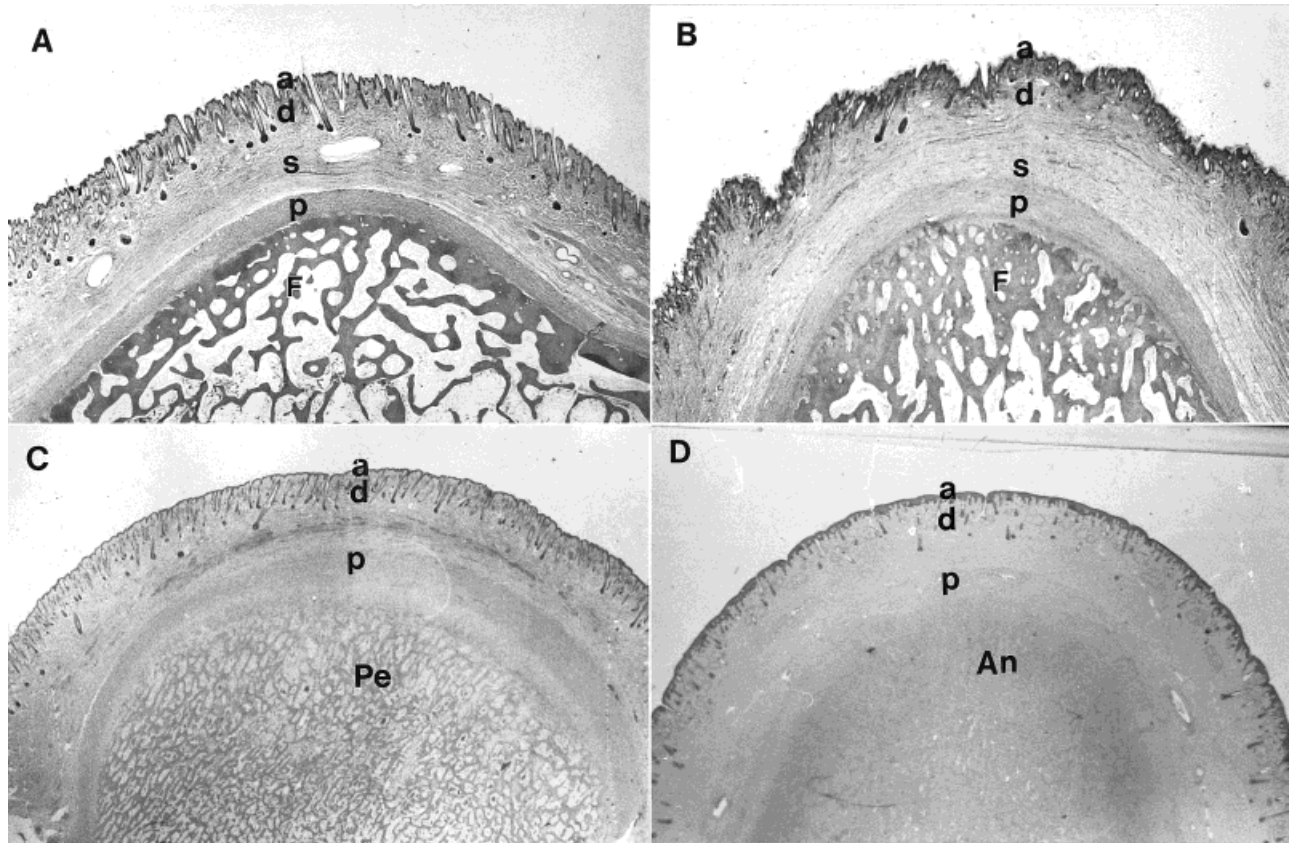


Fig. 1. Vertical sections of frontal lateral crest (FLC)/pedicle/antler cut coronally through the integument and underlying tissue. Haematoxylin and eosin. **A:** FLC from an 8-month-old male calf. Notice that the epidermis of the integument is undulated. a, epidermis; d, dermis; s, subcutaneous loose connective tissue; p, periosteum; F, frontal lateral crest. $\times 13$. **B:** FLC from an 8-month-old male calf. Notice that fine

undulations are located within the coarse ones. a, d, s, p, and F are the same as shown for A. $\times 13$. **C:** Incipient pedicle at transitional ossification stage from a 9-month-old male stag. p, periosteum/perichondrium; Pe, pedicle; a and d are the same as shown for A. $\times 10$. **D:** Incipient antler from a 10-month-old male stag. p, perichondrium; An, antler; a and d are the same as shown for A. $\times 7.5$.

Transmission Electron Microscopy (TEM)

TEM was conducted on samples from four deer at two ossification stages (two deer/stage), OPC (pedicle), and aECO (antler). A small piece of skin (approximately 1.5 mm^2) from the centre region of a pedicle or an antler apex was removed immediately after tissue sampling (see Tissue Sampling) and put into the fixative (3.125% cacodylate-buffered glutaraldehyde) for 24 hr. The samples were trimmed to about 1 mm^2 . After further processing for TEM in the same manner as described by Li and Suttie (1998), the ultra thin sections were cut from the embedded tissue samples and doubly stained with uranyl acetate and lead citrate. Examination of these stained ultra thin sections under an electron microscope (Philips EM 410LS) was mainly confined to the basement membrane region.

RESULTS

The histological results are summarised in Table 1.

Intramembranous Ossification Stage (IMO)

The integument overlying the apex of the frontal lateral crests consisted distoproximally of epidermis, dermis, and subcutaneous loose connective tissue (SLCT; Fig. 1A,B).

The epidermis appeared to be thin and undulating in shape. The undulation of the epidermis in some cases was in such a manner that fine undulations were located within the coarse ones (Fig. 1B). The dermis contained hair follicles and their associated appendages. These appendages included an abundance of sweat glands, small and mono-lobed sebaceous glands, and arrector pili muscles (Fig. 2A). The SLCT was a very loose and thick layer within which the vascular system was located (Fig. 3A).

BrdU labelled cells were not found in the epidermis of apical skin, and were very sparsely located in the dermis and hair follicles at this stage (results not shown).

Transitional Ossification Stage (OPC)

The major difference in histological structure of the apical integument compared to that at the IMO stage was the change in thickness and density of SLCT (Fig. 1C). At the early OPC stage, the SLCT appeared substantially thinner and denser than that at the IMO stage, the boundary with underlying periosteum could not be readily defined (Fig. 3B). At the late OPC stage, the SLCT seemed to be even thinner and denser than the early OPC stage, and no longer loose in character, although away from the tip of the pedicle the SLCT was less dense. The SLCT merged imperceptibly with

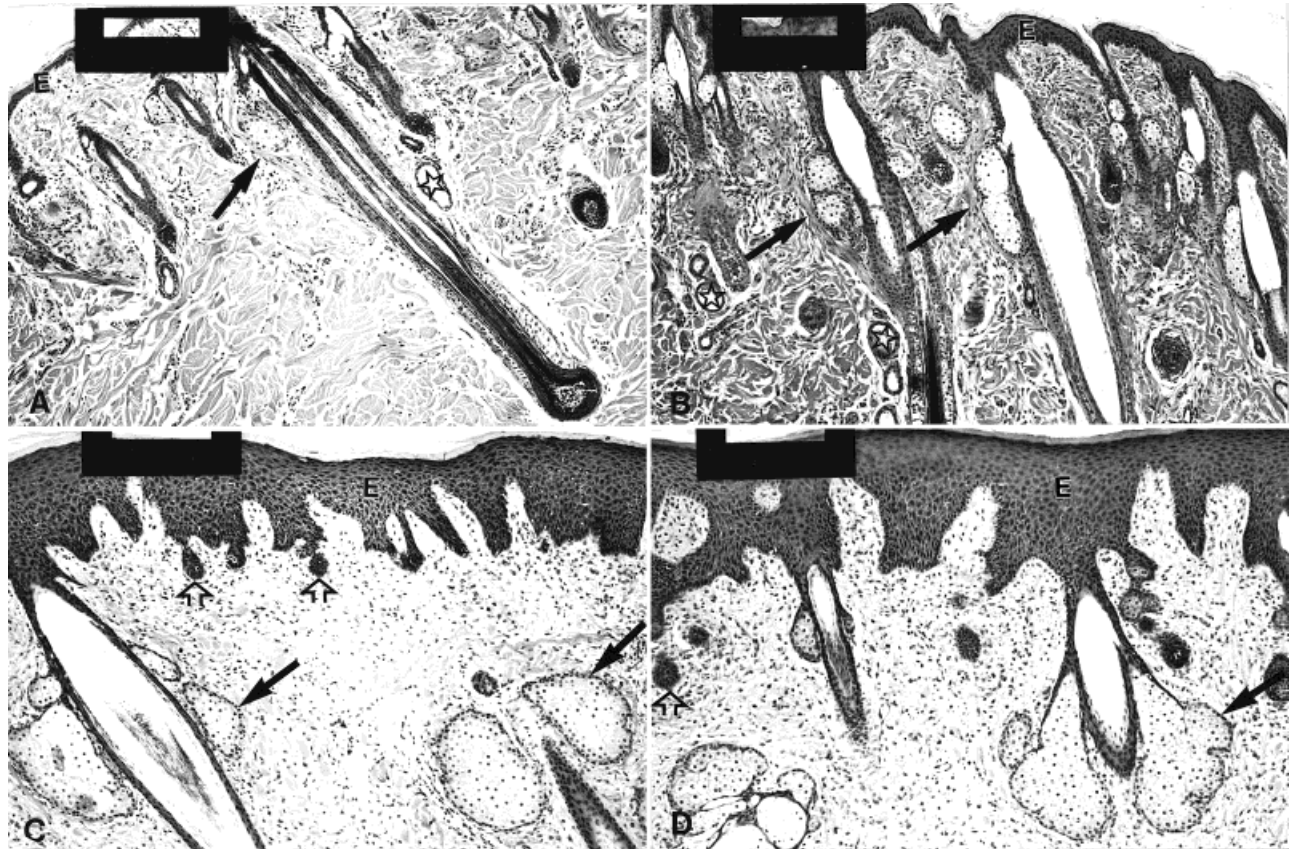


Fig. 2. Apical epidermis and its associated appendages. Haematoxylin and eosin. Scale bar = 760 μ m. **A:** FLC stage. Notice that the epidermis (E) is thin; the sebaceous gland (arrow) is small and monolobar with a obvious arrector pili muscle attached. Open star, sweat gland. **B:** OPC stage. Epidermis is thicker than at FLC stage, but still undulating. Arrows and open star are the same as shown for A. **C:** Late pECO stage. Epidermis is more or less flat and much thicker than at OPC stage. The

sebaceous glands are bi- or multi-lobar (arrows) and do not have associated arrector pili muscle. Sweat glands are absent. Notice the generation of new hair follicles (open arrows). **D:** aECO stage. Epidermis is flat and thick. The sebaceous glands (arrow) are bigger and more complicated than at the pECO stage, and without associated arrector pili muscle. Open arrow is the same as shown for C.

the underlying fibrous layer of the periosteum/perichondrium.

Compared to the IMO stage, the apical integument at the OPC stage had a thicker ($P < 0.01$) epidermis, more abundant sweat glands, bigger sebaceous glands, and similar arrector pili muscles. However, the sebaceous glands were still mono-lobar in shape. The epidermis was undulating, although the undulations were smaller in amplitude and frequency (Fig. 2B).

BrdU localisation results were very similar to those at IMO stage, but more labelled cells were found in the dermis (results not shown).

TEM results showed that the basement membrane along the interface of the epidermis and the underlying dermis was intact. Inactive dermal fibroblasts adjacent to the basement membrane were located (Fig. 4C).

Pedicle Endochondral Ossification Stage (pECO)

Early pECO. The main distinguishing feature in histological structure of the integument was the formation of new hair follicles. The newly formed hair follicles were only located in the central region of the pedicle apices.

However, the generation of new hair follicles could be found from the tip to the shoulders in more advanced pedicles.

Compared to the OPC stage, the apical integument had a thicker epidermis ($P < 0.01$), bigger sebaceous glands and no obvious change in sweat glands. However, the epidermis was no longer undulating, instead the apex of the pedicle integument was covered by more or less flat epidermis. Most of the sebaceous glands were bi-lobar.

Mid pECO. The outstanding characteristic of the apical integument was that a different type of hair follicle which had big sebaceous glands, no arrector-pili-muscle, and no sweat glands (Fig. 2C) in the central apical region was observed for its first time. In addition, the bi- or multi-lobed sebaceous glands associated with the newly formed hair follicles were much bigger than those from the early pECO stage and occupied most of the dermis.

Compared to the early pECO stage, the apical integument had a thicker epidermis ($P < 0.01$), bigger sebaceous glands, and thinner SLCT (Fig. 3C). The epidermis covering the apical integument was even and flat. The density

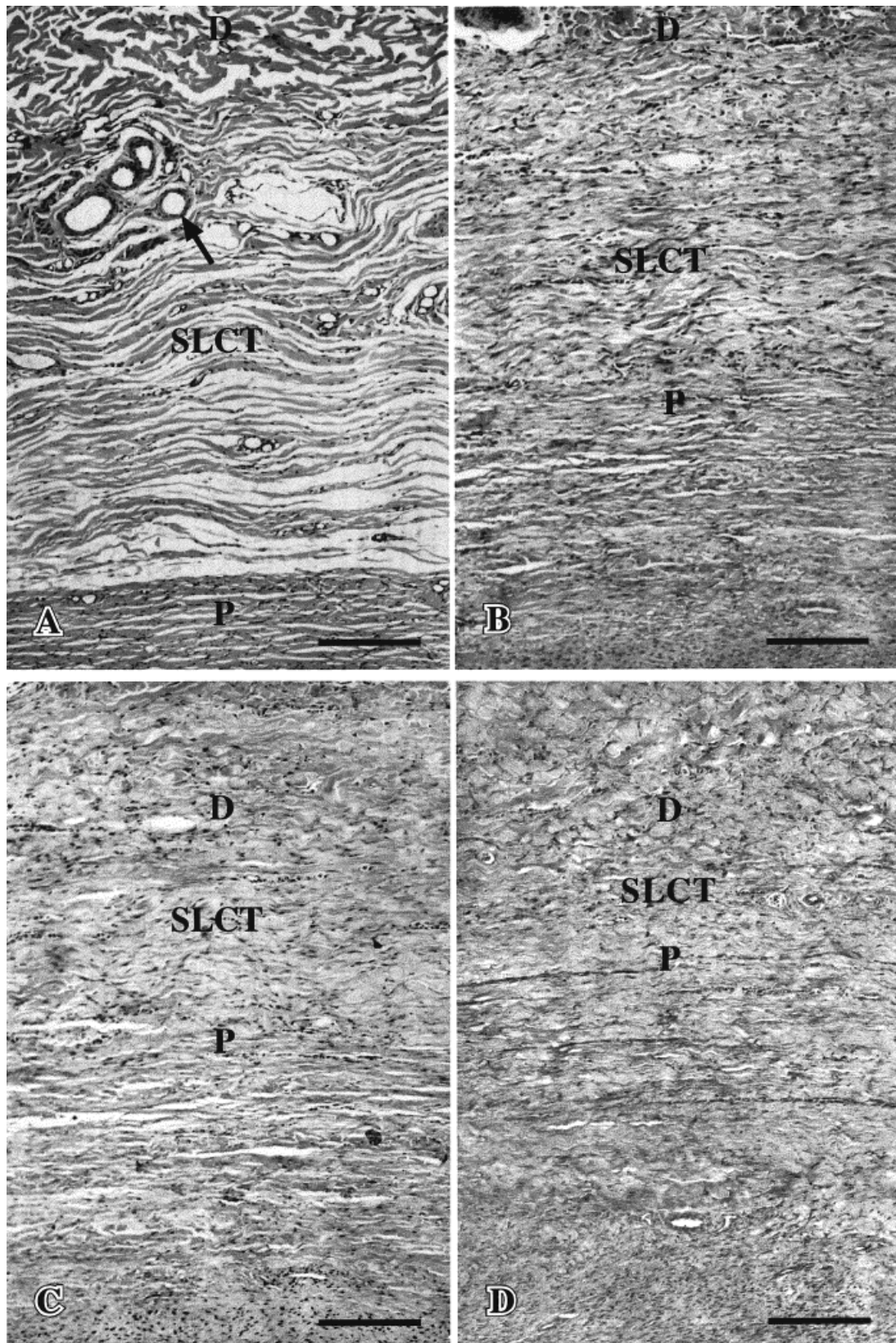


Fig. 3. Subcutaneous loose connective tissue (SLCT). Haematoxylin and eosin. Scale bar = 760 μ m. **A:** FLC stage. Notice that the SLCT is a very loose and thick layer. P, periosteum; D, dermis; arrow, blood vessels. **B:** OPC stage. Notice that the SLCT layer is substantially compressed, but the boundary between SLCT and P is still distinguishable. D, SLCT, and P are the same as shown for 3A. **C:** pECO stage.

Notice that the SLCT layer is fully compressed and the boundary between the SLCT and the fibrous layer of the perichondrium cannot be readily distinguished. P, perichondrium; SLCT and D are the same as shown for 3A. **D:** aECO stage. Notice that SLCT is only a narrow strip. P, SLCT, and D are the same as shown for 3C.

of the newly formed hair follicles was less than at the early pECO stage.

BrdU-labelled cells were found mainly in sebaceous glands, hair follicles and epidermis, but sparsely in dermis, of apical pedicle skin (Fig. 4A,B).

Antler Endochondral Ossification Stage (aECO)

The apical integument at the aECO stage (Figs. 1D and 2D) was essentially the same in histological structure as that at the late pECO stage. The epidermis did not differ significantly in thickness ($p > 0.05$) compared to that at late pECO stage. However, the SLCT layer was only a thin strip (Fig. 3D), and generation of altered hair follicles from the thick and flat epidermis could be found from the tip to the distal shaft.

TEM results showed that only fragmented pieces of the basement membrane were found. Most dermal fibroblasts along the fragmented basement membrane were active, although no direct cell-cell contact through these gaps of the fragmented membrane was detected in this examination (Fig. 4D).

DISCUSSION

Deer pedicles and growing antlers are made up of two components, interior osseocartilage and exterior integument. The histogenesis of the interior osseocartilage in red deer has been systematically studied by Li and Suttie (1994). However, the results from the present examination are the first histological description of the formation of the exterior integument. The results show that pedicle skin forms from the apex of a frontal lateral crest and proceeds through three distinct stages. These stages are characterised by compression of the subcutaneous loose connective tissue (SLCT) at the OPC stage, stretching of the undulated epidermis at the early pECO stage, and neogenesis of the skin associated appendages at the mid pECO stage. Antler velvet transformation, which occurs at the late pECO stage, is mainly associated with alteration in the skin appendages. This alteration includes the loss of arrector pili muscle and sweat glands, and the gain of the large bi- or multi-lobed sebaceous glands. Therefore, histological transformation from pedicle skin to antler velvet is a change mainly in hair follicle type. Pedicle skin is typically associated with primary hair follicles which have sebaceous glands, sweat glands and arrector pili muscles, whereas antler velvet possesses secondary hair follicles which only have sebaceous glands (for primary and secondary hair follicles refer to Ryder and Stephenson, 1968). The results of BrdU localisation confirm that besides neogenesis of skin appendages, skin itself also starts to form at mid pECO stage. Prior to this stage the epidermis and the appendages are essentially devoid of mitotic cells, but after this stage abundant mitotic cells are found in these structures.

One striking feature of pedicle skin and antler velvet is their capacity for rapid expansion, which is commensurate with the elongation of the interior component (Billingham et al., 1959; Goss, 1964). However, the question of what triggers and maintains pedicle skin expansion has never been proposed. The histological examination in the present study showed that when a pedicle starts to grow, the first sign of change in the overlying skin is the compression of the SLCT, followed by stretching of the overlying undulated epidermis. Formation of new pedicle skin

and its associated appendages is not initiated until the overlying epidermis is fully stretched and starts to increase in thickness. These results suggest that the factor driving pedicle skin expansion may be the mechanical stretch resulting from the rapid expansion of the underlying antlerogenic tissue. It has been shown that new skin is produced, in experimental condition, after stretching by subcutaneous insertion of an inflation-controlled tissue expander (Austad et al., 1982; Brandy, 1991; Timmenga et al., 1989). Austad et al. (1982) concluded that skin expansion is a physiological process to accommodate an enlarging mass beneath it by increasing surface area. Francis and Marks (1977) found that stretching the skin stimulates epidermal proliferation only sufficiently to relieve tension. The present study showed that newly formed pedicle skin is histologically comparable to artificially induced skin. Both newly formed apical pedicle skin (present study) and artificially induced skin have thicker epidermis (Austad et al., 1982; Johnson et al., 1988) and thinner SLCT (Pasyk et al., 1982) than the skin from which they are originally derived. Therefore, pedicle skin expansion could be caused by mechanical tension, which is created by the underlying actively forming antlerogenic tissue. In this regard, the fast forming antlerogenic tissue could be considered as a special kind of "tissue expander." It is likely then that at the initial stage pedicle skin stretches to accommodate this expanding "expander" and that pedicle skin formation at the later stage releases the tension resulting from the continuously expanding expander.

Li and Suttie (1994) hypothesised that one of the main factors causing the change in ossification type of the interior component during pedicle formation is mechanical compression. The present study supports this notion, as the change in ossification type from IMO to OPC happens only when the overlying SLCT is fully compressed, and from OPC to pECO only when the overlying skin is sufficiently stretched. The force built up in the skin would create mechanical pressure on the underlying antlerogenic tissue. Besides providing evidence in support of our previous hypothesis, the present study also suggests that pedicle skin formation is caused by mechanical stretch which is derived from the expanding interior component. Therefore, there may be reciprocal mechanical interactions between apical pedicle skin and the underlying antlerogenic tissue. The rapidly expanding antlerogenic tissue may drive pedicle skin expansion and formation, and in turn the stretched pedicle skin may exert mechanical pressure on the underlying antlerogenic tissue causing it to change in ossification type.

The factors that cause the transformation from pedicle skin to antler velvet are not known. Mechanical stretch, which may drive pedicle skin formation, cannot itself cause this transformation to happen. Mechanical stretch can only stimulate skin neogenesis but cannot alter skin type (Austad et al., 1982; Johnson et al., 1988). Goss (1990) thought that putative inductive molecules from the underlying antlerogenic tissue must play a pivotal role in this skin transformation. Further, he concluded that intimate association between antlerogenic tissue and the overlying skin is indispensable in allowing these molecules to reach and effect the overlying skin. Histological examinations of the exterior component formation from the present study support Goss's conclusion. Antler velvet transformation in normal first antler generation did not

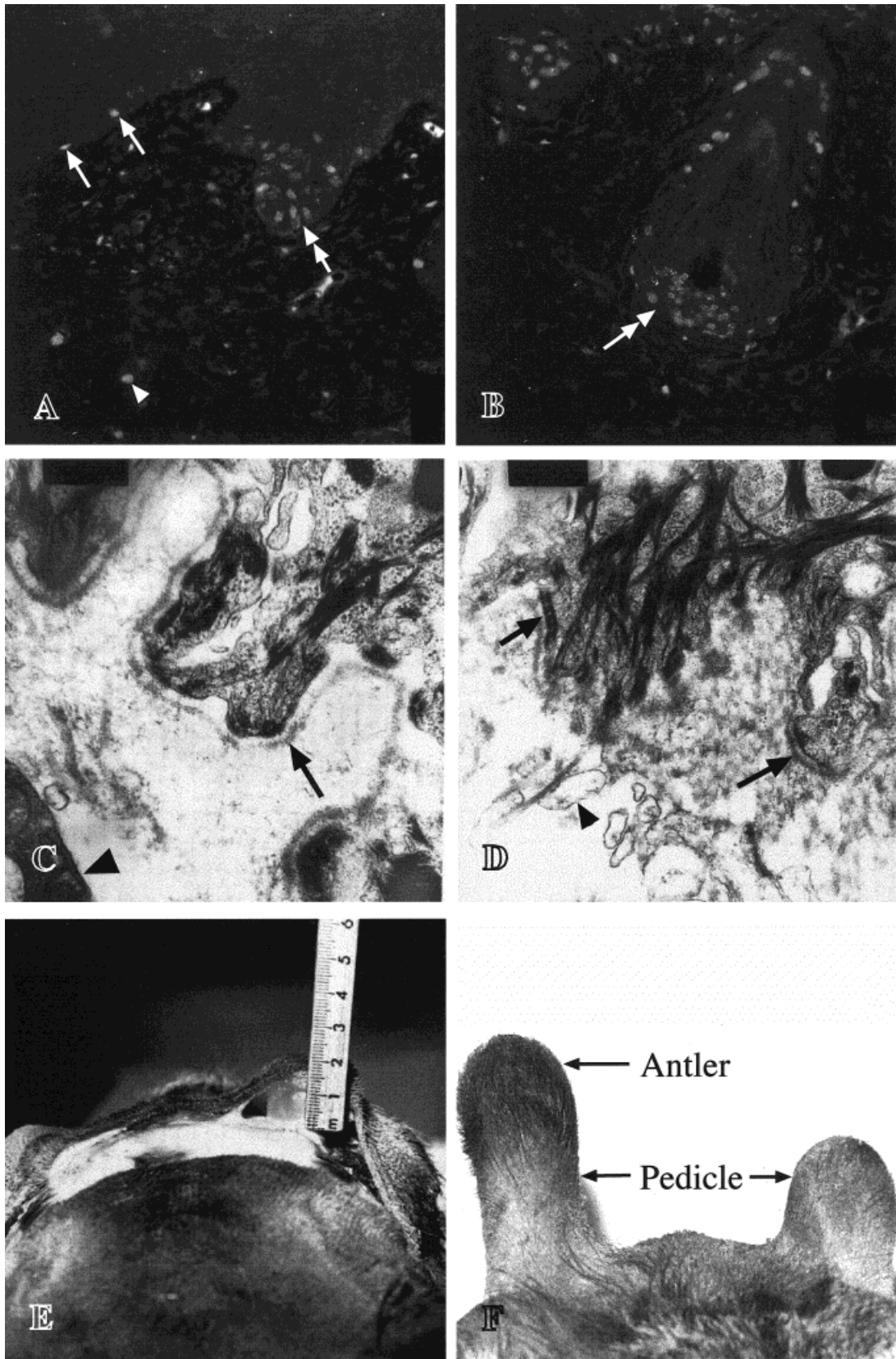


Fig. 4. A,B: Mitotic cells in the apical pedicle skin at pECO stage. $\times 205$. **A:** A neoforming hair follicle (double-head arrow), mitotic cells in the epidermis (arrows), and dermis (arrowhead). **B:** A hair papilla (double-head arrow). Note intensive mitotic cells are localised in the papilla. C,D: Electron microscope micrographs of the apical skin basement membrane. **C:** From a OPC stage pedicle. Note a well intact basement membrane (arrow), and adjacent to it were the inactive dermal fibroblasts (arrowhead). $\times 31,000$. **D:** From an early aECO stage antler. Note a fragmented basement membrane (arrow) was found, and most dermal fibroblasts adjacent to the membrane were active and had their pro-

cesses approaching the gaps of the fragmented membranes (arrowhead). $\times 21,000$. **E:** Posterior view of a deer head from a 4-month-old male calf. Showing that the skin becomes stretched when a 20 mm high pedicle-shaped object is inserted into the space between frontal lateral crest and the overlying scalp skin. **F:** Pedicles from a stag of our previous denervation experiment. Note that although the denervated pedicle on the left side had given rise to an antler bud, the untreated pedicle on the right side was still at pedicle stage as defined by a lack of velvet-like skin.

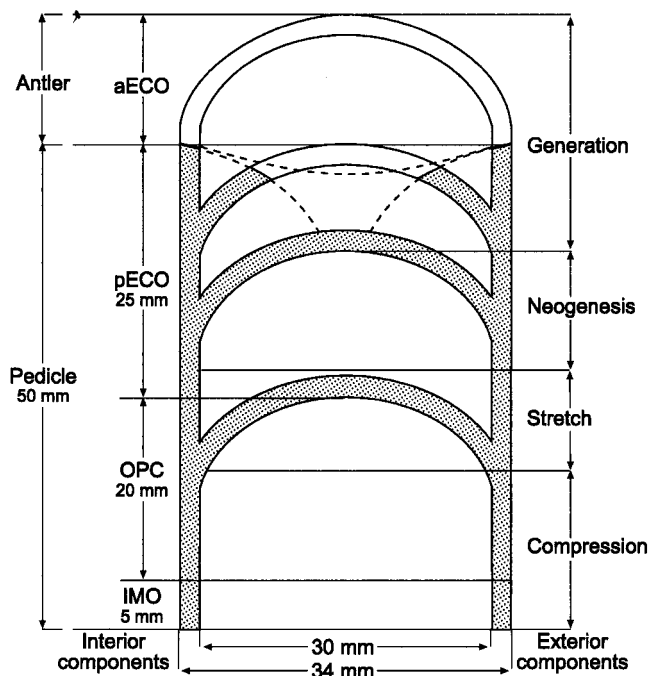


Fig. 5. Illustration of formation of pedicle interior and exterior components and their transformation to antler counterparts. IMO, intramembranous ossification; OPC, transitional ossification; pECO, pedicle endochondral ossification; and aECO, antler endochondral ossification.

start until a pedicle developed into the late pECO stage, at which the SLCT has been fully compressed and the two interactive tissues had become intimately associated. Compression of SLCT would substantially shorten the distance between these two tissues, and hence facilitate putative inductive molecules to reach the target tissue. The tissue association theory is also supported by the fact that, no matter how late a pedicle initiates, its full length is always greater than 20 mm in red deer (personal observation). This is probably because the space between a frontal lateral crest and the overlying scalp can accommodate that much volume, but beyond this the overlying skin becomes tightly stretched, hence close association can be achieved. A pedicle-shaped object, if less than 20 mm in length, can be manually inserted into the space between a frontal lateral crest and the overlying scalp, but, if 20 mm or more, cannot do so without seriously stretching the overlying scalp (Fig. 4E).

Nonetheless, chemical induction alone, without mechanical stimulation, may not be able to accomplish the whole process of antler velvet transformation. Chemical induction may only be able to alter the type of newly forming skin, but not the type of existing skin, as demonstrated by our previous study (Li et al., 1995). In that study, diffusion chambers were loaded with antlerogenic periosteum and were subcutaneously implanted onto deer nasal bone for more than 2 months. No visible change in the overlying skin type was detected. The chambers were sealed with a 0.45 μ m pore size membrane, which allows passage of molecules but limits antlerogenic tissue outgrowth. By so doing, it would not create any mechanical tension to the overlying skin, hence, would not promote new skin formation. Therefore, antler

velvet transformation may be accomplished by a combination of mechanical stimulation and chemical induction. It is likely that mechanical stimulation drives skin formation, whereas chemical induction determines the type of neoforming skin.

One may argue our topologic induction hypothesis has over-emphasised the roles of local factors (mechanical and chemical factors) and ignored the systemic factors (such as endocrine profiles, etc.) in first antler generation. However, the fact that the timing of first antler generation among individuals in the same deer group varies considerably does not support the notion that systemic factors play an important role in this generation. Although some systemic factors like testosterone may play a role in modifying the formed antler velvet. Our denervation study (Suttie et al., 1995) adds further evidence to our hypothesis. In that study, in the same deer when the denervated side pedicle had already given rise to an antler bud, the control side pedicle had just reached the early pECO stage and was still covered by typical scalp skin (Fig. 4F).

Our electron microscopy results from this study also support the theory that antler velvet transformation has resulted from topological induction. Disruption of the basement membrane integrity of apical skin was detected at the early antler stage, but not at the pedicle stage. It is known that an intact basement membrane between epithelium and mesenchyme inhibits tissue regeneration (Neufeld and Aulhouse, 1986). Neufeld et al. (1996) reported that basal lamina (the top layer of basement membrane) is initially absent from the amputation surface of an animal appendage and re-established to continuity by the late bud stage of regeneration. It forms generally from base to apex. They concluded that the ability to delay basal lamina closure until after a blastema has formed is the feature that distinguishes regenerating from nonregenerating appendages. Nevertheless, it seems clear that physically breaking the basement membrane is the prerequisite for regenerating regenerative appendages. First antler generation is considered unique because it is not an example of regeneration (Goss, 1983). The finding that disruption of the basement membrane integrity of apical skin during the transformation from a pedicle to an antler would at least partially explain why this process can proceed without having anything lost to be replaced.

Transformation in ossification from pedicle type to antler type in the interior component in red deer is completed when a pedicle grows to about 25 mm in length (Li and Suttie, 1994). However, the full length of a grown pedicle is about 50 mm as defined by the difference in the exterior skin type. Why the interior component and the exterior component transform so asynchronously is unknown. The present examination revealed that the transformation in the exterior component from pedicle skin to antler velvet takes place when a pedicle grows to about 40 mm long (late pECO stage), rather than at the time a pedicle reaches its full length (50 mm). However, at the very first stage antler velvet emerges only at the central region of a growing pedicle apex. Thereafter, velvet increases in area as the pedicle elongates. This results in the boundary of the pedicle skin and velvet migrating distally and laterally. Once the boundary has moved to the shoulder of the pedicle, the newly formed velvet will cover the entire apex and the pedicle reaches its full length. The moving distance of the boundary from its first appearance to reaching the pedicle shoulder is pedicle diameter dependent. There-

fore, there is an overlap period between the termination of pedicle skin formation and the initiation of first antler velvet (Fig. 5). As velvet formation cannot be easily observed macroscopically until the boundary has migrated to the shoulder of a pedicle, it is generally thought that antler transformation in red deer takes place when a pedicle grows to about 50 mm in length.

Discovery of how antler velvet initially generates has only partially explained the phenomenon of asynchronous transformation in the two components. A 15 mm growth period between the completion of the interior component transformation and the commencement of velvet generation has not yet been explained. It is known that first antler generation, as defined by the change in skin type, is the result of the induction from antlerogenic tissue to the overlying skin (see above). The putative inductive molecules must traverse the fibrous periosteal layer, subcutaneous connective tissue and dermis in order to effect the transformation of overlying epidermis into antler velvet. This represents an induction across a distance of over 1 mm, an exceedingly long-range effect in histological terms (Goss, 1995). Consequently, it may take time to effectively establish this induction, hence the time lapse between the transformations of the interior component and the exterior component results. Pedicle length, which varies among different deer species, may depend on how quickly this effective induction can be established.

In conclusion, pedicle skin expansion may occur in order to release the mechanical tension created by underlying actively forming antlerogenic tissue, initially in response to it by mechanical stretch, and then by neogenesis of skin. In turn, the stretched pedicle skin may exert mechanical pressure on the underlying antlerogenic tissue causing it to change in ossification type. Antler velvet generation may be accomplished by both mechanical stimulation and chemical induction from the underlying pECO stage antlerogenic tissue. If this hypothesis is correct it is likely that mechanical stimulation drives skin formation and chemical induction determines skin type. Furthermore, asynchronous transformation of the interior and exterior components during pedicle formation and antler generation may result from the delayed chemical induction and the way antler velvet initially generates.

ACKNOWLEDGMENTS

We wish to thank Dr. Jim Webster and Mr. Ian Corson for helping with the perfusion of deer heads, Mr. Ken Turner for his assistance with histological preparations, Richard Easingwood for helping editing some of the figures using Photoshop, and Bronwyn Smaill for the assistance with the preparation of electron microscopy.

LITERATURE CITED

- Austad ED, Pasyk KA, McClatchey KD, Cherry GW. 1982. Histomorphologic evaluation of guinea pig skin and soft tissue after controlled tissue expansion. *Plastic Reconstruct Surg* 70:704-710.
- Billingham RE, Mangold R, Silvers K. 1959. The neogenesis of skin in the antlers of deer. *Annals NY Acad Sci* 83:491-498.
- Brandy DA. 1991. A special tissue expander in combination with extensive scalp-lifting. *Aesthetic Plastic Surg* 15:349-354.
- Bubenik GA. 1993. Morphological differences in the antler velvet of cervidae. In: Ohtaishi N, Sheng HL, editors. *Deer in China*. Amsterdam: Elsevier. p 56-64.
- Fennessy PF, Suttie JM. 1985. Antler growth: Nutritional and endocrine factors. In: Fennessy PF, Drew KR, editors. *Biology of Deer Production*. New Zealand: Royal Society of New Zealand Bulletin 22. p 239-250.
- Francis AJ, Marks R. 1977. Skin stretching and epidermopoiesis. *Br J Exp Pathol* 58:35-39.
- Goss RJ. 1964. The role of skin in antler regeneration. In: Montagna W, Billingham RE, editors. *Advances in biology of skin wound healing*. New York: Pergamon Press. p 194-207.
- Goss RJ. 1983. Deer antlers. Regeneration, function and evolution. New York: Academic Press.
- Goss RJ. 1987. Induction of deer antlers by transplanted periosteum. II. Regional competence for velvet transformation in ectopic skin. *J Exp Zool* 244:101-111.
- Goss RJ. 1990. Of antlers and embryos. In: Bubenik G, Bubenik A, editors. *Horns, pronghorns, and antlers*. New York: Springer-Verlag. p 299-312.
- Goss RJ. 1995. Future directions in antler research. *Anat Rec* 241: 291-302.
- Johnson PE, Kernahan DA, Bauer BS. 1988. Dermal and epidermal response to soft-tissue expansion in the pig. *Plast Reconst Surg* 81:390-395.
- Li C, Suttie JM. 1994. Light microscopic studies of pedicle and early first antler development in red deer (*Cervus elaphus*). *Anat Rec* 239:198-215.
- Li C, Suttie JM. 1998. Electron microscopic studies of antlerogenic cells from five developmental stages during pedicle and early antler formation in red deer (*Cervus elaphus*). *Anat Rec* 252: 587-599.
- Li C, Waldrup KA, Corson ID, Littlejohn RP, Suttie JM. 1995. Histogenesis of antlerogenic tissues cultivated in diffusion chambers in vivo in red deer (*Cervus elaphus*). *J Exp Zool* 272:345-355.
- Neufeld DA, Aulthouse AL. 1986. Association of mesenchyme with attenuated basement membranes during morphogenetic stages of newt limb regeneration. *Am J Anat* 176:411-421.
- Neufeld DA, Day FA, Settles HE. 1996. Stabilizing role of the basement membrane and dermal fibers during newt limb regeneration. *Anat Rec* 245(1):122-127.
- Pasyk KA, Austad ED, McClatchey KD, Cherry GW. 1982. Electron microscopic evaluation of guinea pig skin and soft tissues "expanded" with a self-inflating silicone implant. *Plast Reconst Surg* 70:37-45.
- Ryder M, Stephenson S. 1968. *Wool growth*. London: Academic Press.
- Suttie JM, Li C, Sheard PW, Corson ID, Waldrup KA. 1995. Effects of unilateral cranial sympathectomy either alone or with sensory nerve sectioning on pedicle growth in red deer (*Cervus elaphus*). *J Exp Zool* 271:131-138.
- Timmenga EJ, Schoorl R, Bos JD, Kloppe PJ. 1989. An improved model for tissue expansion and flap research in the rabbit. *Br J Plast Surg* 42:301-305.