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Antler Regeneration: A Dependent Process of Stem Tissue Primed Via Interaction With its Enveloping Skin

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ABSTRACT Deer antlers are unique mammalian appendages in that each year they are cast and fully regenerate from permanent bony protuberances, called pedicles. In a previous study, we found that there is a difference in the degree of association between pedicle bone and its enveloping skin: tight at the distal third and loose at the proximal two thirds of a pedicle stump. The distal part has been termed the ''potentiated'' region, and the proximal part the ''dormant'' region. In the present study, pedicle stumps were artificially created in yearling sika deer by cutting off the tissue distal to either the potentiated or the dormant region. A piece of impermeable membrane was then inserted into the space between the bone and the skin of each treated pedicle stump, while the control pedicles had the same surgery without membrane insertion. The results showed that the inserted membrane blocked pedicle skin participation in the process of antler regeneration. All three potentiated bony pedicle stumps regenerated skin-less antlers; whereas, one of the three dormant bony pedicle stumps failed to regenerate any antler tissue. The other two dormant stumps eventually regenerated normal antlers; however, this only occurred after loss of the inserted membrane. No antler tissue regenerated from the dormant stumps while the inserted membrane remained in place (up to 55 days). All control pedicle stumps regenerated normal antlers. Therefore, we conclude that it is the pedicle bone, but not pedicle skin, that gives rise to regenerating antlers, and that pedicle bone can acquire the potential to regenerate an antler only when it is primed via interaction with its enveloping skin. *J. Exp. Zool. 307A:95–105, 2007.*

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Regenerative biology is mainly investigated utilising model organisms, such as hydra, planarians and urodeles. Although these model organisms demonstrate some remarkable capabilities of epimorphic regeneration, such as growing back their partially lost body or limbs, phylogenetically they are very distant to mammals. The applicability of these findings from model animals to clinical medical science remains to be determined.

Deer antlers are unique in that they are cast and fully regenerate each year. Antler regeneration takes place in early spring each year following casting of previous hard antlers. The newly regenerating antlers are enveloped with soft skin, which is called velvet. Rapid velvet antler growth occurs during the late spring and summer. Antlers attain their full size in autumn, which is followed by full calcification and shedding of the velvet skin to expose hard bone. The bony antlers drop off in the next spring, which triggers a new round of the antler growth cycle (Li, 2003). Therefore, deer antlers offer a unique opportunity to explore how nature solves the problem of mammalian organ/appendage regeneration (Goss, '95). However, thus far our knowledge regarding antler regeneration is still fragmental. Revealing the mechanism underlying antler regeneration would

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undoubtedly place us in a better position to promote tissue/organ regeneration in humans.

Although being a cranial appendage, antler casting and regeneration do not take place directly from the deer head. Instead, it occurs from permanent bony protuberances, called pedicles. A pedicle stump consists predominantly of two tissue components: bone, including periosteum and skin. Controversy exists regarding which of the pedicle tissue components is responsible for antler regeneration. Wislocki and Waldo ('53) along with Goss ('92) stated that the cells in the dermal layer of the pedicle skin form the antler bud, and hence give rise to the whole antler. However, Gruber ('37) and Kierdorf et al. (2003) suggested that pedicle periosteum may provide the main cell source for antler regeneration. In the recent studies, we took morphological (Li et al., 2004b) and histological (Li et al., 2005) approaches to investigate this phenomenon. We found that the two growth centres (osseocartilaginous tissue) responsible for the formation of the antler main beam and brow tine resulted directly from the proliferation and differentiation of pedicle periosteal cells, and no obvious dedifferentiation and redifferentiation processes were detected. Therefore, we concluded that antler regeneration is a stem-cell-based process and the stem cells reside in the periosteum of pedicle stumps. This has set antler regeneration apart from the typical blastema-based regeneration in some model animals, such as newts (Mescher, '96). However, these morphological and histological results need to be confirmed by functional analysis.

It is recognised within the antler research field that the ability to regenerate antler is held within the entire pedicle, as antler regeneration can take place from a stump created by amputation at any level along the pedicle shaft (Goss, '83). Interestingly, Li and Suttie (2003) found that there is a difference in the degree of association between the enveloping skin and pedicle periosteum along a pedicle shaft in young deer. The skin of the proximal pedicle portion, approximately two thirds of the total pedicle length, is loosely attached to the pedicle periosteum; whereas on the distal third of the pedicle, the skin was tightly bound to the pedicle bone. As close association between the periosteum and covering skin appears to be a prerequisite for initial antler generation (Goss, '90; Li and Suttie, 2000; Li et al., 2001), they hypothesised that the distal region is in a more advanced state of preparation for antler regeneration. Therefore, the area where skin is loosely attached to the periosteum is termed ''dormant region''; whereas the area where skin is tightly associated with the periosteum, the ''potentiated region'' (Li and Suttie, 2003). They further inferred that the interactions between pedicle periosteum and the enveloping skin are a prerequisite for antler regeneration, and that the close association between these two tissue types is required only to realise these interactions. However, these hypotheses have not been tested experimentally.

This experiment was designed to determine the following two aspects. Firstly, which tissue type from a pedicle stump was required for antler regeneration; and secondly whether antler regeneration was dependent on interactions between the pedicle bone and the enveloping skin, and if so were these interactions required during the entire course of antler regeneration?

MATERIALS AND METHODS

Animals

Six 12-month-old male sika deer (*Cervus nippon*) calves were selected for the membrane insertion study. The procedure was undertaken when incipient spike antlers had developed from the top of each pedicle (Fig. 1A). The deer were then randomly divided into two groups: Group I, potentiated region (Fig. 1B) membrane insertion, and Group II, dormant region (Fig. 1F) membrane insertion. One pedicle from each deer was randomly chosen for the membrane insertion, and the other was sham-operated serving as a control (Table 1). Membrane insertion surgery was conducted on 28 May in the Northern Hemisphere. Considering animal welfare, this superficial surgery, which is not much different from commercial velveting, was carried out when each animal was placed under general anaesthesia.

Membrane insertion surgery

The detailed procedure for the identification and exposure of the potentiated and dormant regions has been reported elsewhere (Li and Suttie, 2003). Briefly, to undertake the membrane insertion within the potentiated region, a skin incision was made around the pedicle just below the junction between pedicle and antler. Antler tissue was then sawn off along the skin incision to create a potentiated pedicle stump (Fig. 1B). The second skin incision was made on the medial side of the pedicle starting from the first incision and termi-

Fig. 1. Membrane insertion surgery. (**A**) Pedicle of 5 cm and incipient spike antler of 3 cm (arrow) from a 12-month-old male sika deer (*Cervus nippon*). (**B**) Potentiated pedicle stump was created by sawing off the incipient antler just below the junction of pedicle and antler. A vertical skin incision was made on the medial side of the stump. (**C**) Pedicle skin was separated from the bone through the skin incision by blunt dissection and reflected laterally. (**D**) A piece of impermeable Teflon membrane was used to wrap the exposed stump bone and the membrane was then tied in place by a Teflon thread. (**E**) After suturing of the skin incision, any excess membrane was trimmed away. (**F**) A vertical pedicle skin incision allows identification of the very point where transition occurs from tight to loose contact of the skin with the underlying bone. This point marks the boundary between the potentiated and the dormant pedicle regions.

nating at the junction of the pedicle and deer skull (Fig. 1B). Both mechanical force and a scalpel were used to separate the pedicle skin from its bone within the distal pedicle (Fig. 1C). Subsequently, a piece of impermeable Teflon membrane (3 wide PTFE tape, Alltech, AUS/NZ) was used to wrap

TABLE 1. Allocation of animals and pedicles

Deer no	Region of membrane insertion	Height (pedicle+antler, cm) at surgery	
		Left	Right
22	Potentiated	MI (3+7)	Control (3+7)
151	Potentiated	Control (5+3)	MI (5+3)
183	Potentiated	MI (3.5+6)	Control (3.5+6)
24	Dormant	MI (5+10)	Control (5+10)
33	Dormant	MI (5+3)	Control (5+3)
179	Dormant	Control (5+4.9)	MI (4.5+3)

MI: membrane insertion; Control: sham operation, without membrane insertion; Potentiated region: distal part (1/3) of a pedicle; Dormant region: proximal part (2/3) of a pedicle.

the exposed pedicle bone stump (Fig. 1D). After the membrane was secured in place by tying a Teflon thread around the membrane, the reflected pedicle skin was then returned to its original position. The skin incision was closed using a silk suture and the excess Teflon membrane was trimmed (Fig. 1E).

For membrane insertion at the dormant region, the boundary between the dormant and the potentiated regions along each pedicle shaft was delineated by examination along a vertical pedicle skin incision. The point of transition between tight and loose association of the skin with the underlying periosteum was then identified (Fig. 1F). Subsequently, dormant pedicle stumps around 2–2.5 cm in height were created by cutting off the distal part of each pedicle together with its connected antler at the transition point. Membrane insertion in the dormant region was carried out following the exact procedure used for the potentiated region.

Observations

After their initial recovery from surgery, the experimental deer were observed at weekly intervals using binoculars. The animals were photographed from distance fortnightly to record the growth status of the antlers.

Tissue sampling and histology

Antlers that grew from the membrane-inserted pedicle stumps, including both skin-less and normal antlers (enveloped with skin), were sawn off from the pedicle base on 5 August. This date was selected as by then the growth rate of the control side antlers had substantially decreased. Extra care was taken during removal of the antlers so as not to dislocate or damage the inserted membrane. The removed antlers were then carefully examined and measured. Tissue samples were collected from the tip, shaft and the junction area between the pedicle skin and antler scab of the skin-less antlers. For the normal antlers formed from the membrane-inserted dormant pedicles (♯24 and ♯179), a dissection was conducted to find out whether any membrane remained at the site of insertion in these antlers. The sham-operated antlers on the control sides were also cut off and examined. Tissue samples were also collected from the tip and shaft of these normal antlers for histology.

The sampled tissues were histologically processed following the procedure used in our previous study (Li and Suttie, '94). They were sectioned at 5 μm and stained with haematoxylin and eosin (HE) prior to counterstaining with alcian blue (AB).

RESULTS

Morphology

Potentiated region

All the inserted membranes in this group remained at the original site thus effectively blocked the participation of pedicle skin in antler regeneration. Nevertheless, spike antlers still regenerated from these potentiated pedicle stumps. The antlers had no skin and were instead covered with a scab (Fig. 2). At the initial stages of regeneration, the antler buds were covered with a fresh blood coloured covering. During the period of antler elongation, the fresh blood colour gradually turned into the dark red coloured scab beginning at the most proximal region and gradually moving upwards toward the distal tip. However, the tip of each skin-less antler remained a fresh blood colour until the time of approaching tissue sampling. This clearly indicates that growth

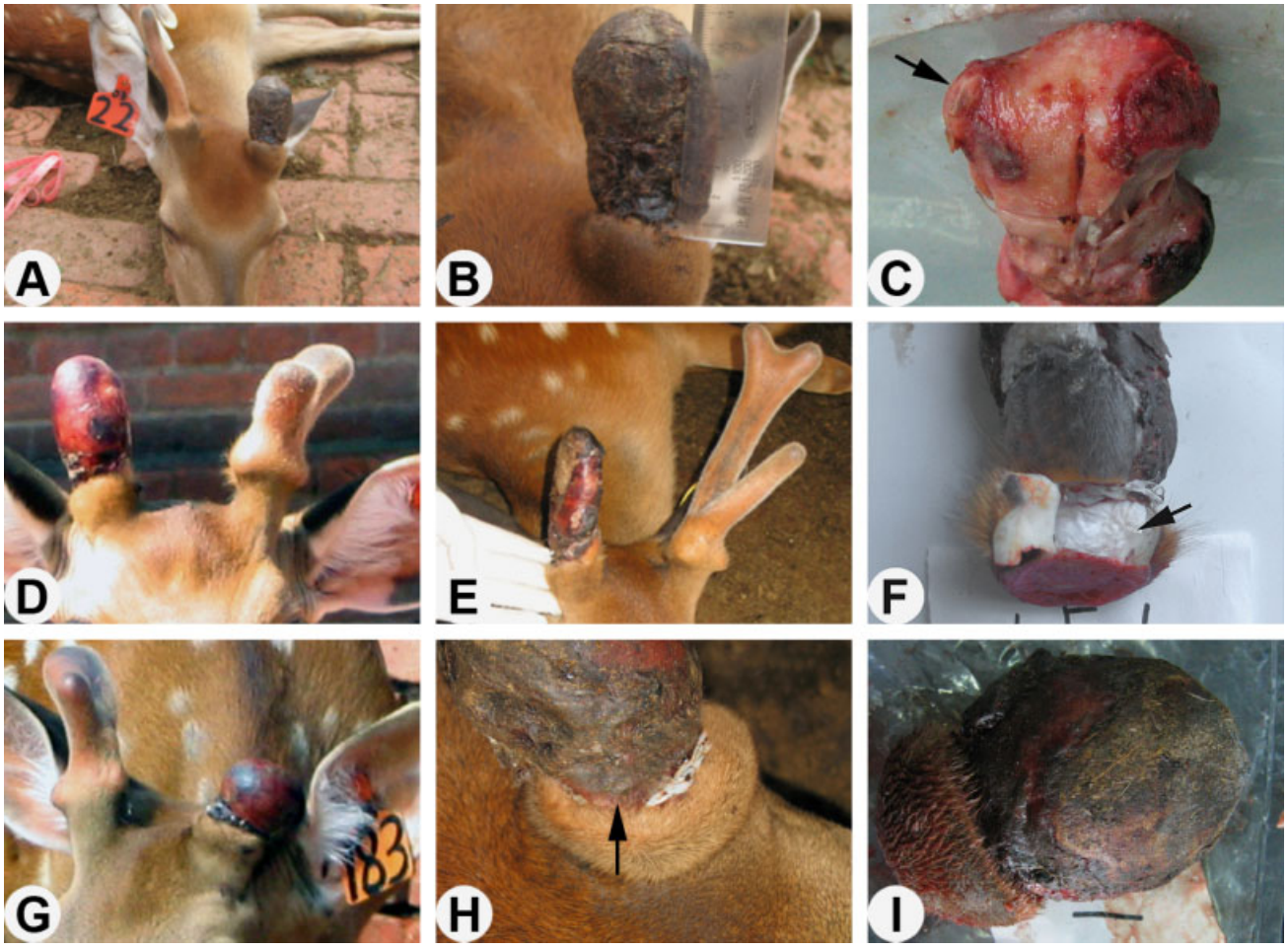

Fig. 2. Morphological observations of antler formation from the potentiated pedicle stumps. (**A**) A skin-less antler (left side of deer) from deer ♯22. Notice that the antler was covered with a dark coloured scab which clearly delineated it from the pedicle skin. Normal antler developed from the control sham-operated pedicle. (**B**) Closer examination of the skin-less antler from deer ♯22 showing it to be 8.2 cm in height. (**C**) Skin-less antler after removal from deer ♯22. Notice that a rudimentary branch (arrow) was found after the removal of the layer of scab. (**D**) Skin-less antler at mid-regeneration stage from deer ♯151 (right side of animal, cf. control side antler). Note that the antler was broader and that most parts were covered with a fresh blood coloured scab. (**E**) The same skin-less antler as in (D) but at a later stage of regeneration (10.0 cm in length; cf. control side antler). Note that the antler became darker brown coloured. (**F**) Skin-less antler removed from deer ♯151. Note that the inserted membrane (arrow) was retained in its original location. (**G**) Skin-less antler at a mid-regeneration stage from deer ♯183 (left side, cf. control side antler). Notice that the antler resembles a ball and was fresh blood coloured. (**H**) The same skin-less antler in (G) but at a later stage of regeneration (5.0 cm in length). Note that the antler became a darker blood colour and that some parts of the very distal end of the pedicle skin were observed to be in direct contact with the bone tissue (arrow). (**I**) Skin-less antler removed from deer ♯183. Note that the antler resembles a rugby ball and was covered with a dark coloured scab.

of these skin-less antlers took place from the tip as occurs in normal antlers. The skin-less antlers varied in length from 5 cm (♯183), 8.2 cm (♯22) to 10 cm (♯151). Interestingly, one skin-less antler (♯22) formed a rudimentary branch (Fig. 2C), which was discovered after removal of the scab. On the control side, all the sham-operated pedicle stumps regenerated either spike (Fig. 2A) or branched normal antlers (Fig. 2E and G).

We noted that around the time of tissue sampling, 2–3 mm of distal pedicle skin could be observed to directly contact the pedicle bone (Fig. 2H). This may have been due to distal growth of the pedicle skin or retraction of the inserted membrane.

Dormant region

Two of the three pieces of inserted membrane were lost: one (♯24) on 18 June (22 days after insertion), and the other (♯179) on 21 July (55 days after insertion). Membrane loss was attrib-

uted to the fact that the dormant pedicle stumps were too short (2–2.5 cm, half length of a potentiated pedicle stump) to firmly hold the inserted membranes, which were quite slippery, for a long period. No visible antler regeneration was observed from the dormant pedicle stumps prior to the loss of the membrane, nor did these stumps change in thickness. Following the loss of the inserted membrane, the wounds over these stumps quickly healed and antler regeneration took place rapidly. These antlers with delayed regeneration were shorter than those of the control sides but were otherwise normal (Fig. 3A–D). The earlier the membrane lost from the dormant pedicle stump, the bigger the antler regenerated (Fig. 3A and C). Careful dissection revealed that no residual membrane was found at the insertion region in these two antlers (Fig. 3B and D).

The inserted membrane in the third antler remained in its original place through until the time of tissue sampling. This effectively blocked the pedicle skin from participating in antler regeneration. Interestingly, no antler regeneration took place from this dormant pedicle stump, although the stump did increase to a diameter of 6 cm (Fig. 3E and F). Careful dissection found that the thickened pedicle resulted from the formation of soft tissue around the existing pedicle bone (Fig. 3G). On the control side, all the sham-operated pedicle stumps regenerated either spike (Fig. 3E) or branched (Fig. 3A and C) normal antlers. The very distal end of pedicle skin (2–3 mm) also directly contacted to the pedicle bone without intervention of the inserted membrane.

Histology

Potentiated region

In the shaft region of each skin-less antler, adjacent to the shell formed by the scab, a layer of transitional zone was located. The tissue of the transitional zone stained somewhat darker by HE/AB staining than the adjacent tissue types and resembled granulation tissue (Fig. 4A). Cells within this layer, particularly close to the scab side, did not appear to be viable. Adjacent to the transitional zone was a layer of fibrous connective tissue (equivalent to subcutaneous connective tissue), within which blood vessels were found

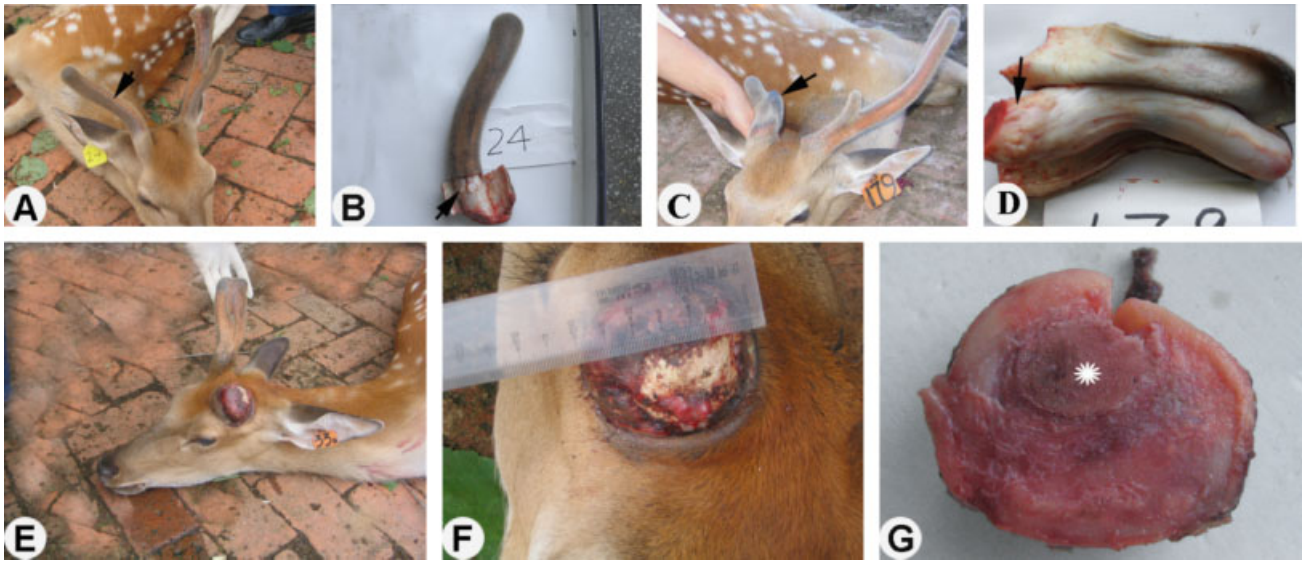

Fig. 3. Morphological observations of antler formation from the dormant pedicle stumps. (**A**) Normal antler (enveloped with velvet skin, arrow) was regenerated from the dormant pedicle stump of deer ♯24. The membrane was lost from the stump 22 days after insertion. (**B**) Pedicle and antler removed from the membrane-inserted side of deer ♯24 after growing for 58 days. Notice that no residual membrane was left in the original insertion site (arrow). (**C**) Antler (arrow) regenerated from the membrane-inserted side of the dormant pedicle stump of deer ♯179. The membrane was lost from the stump 55 days after insertion. (**D**) The pedicle and antler were removed from the membrane-inserted side of deer ♯179 after growing for 15 days after loss of the membrane. Notice that no residual membrane was left in the original insertion site (arrow). (**E**) Antler-less pedicle from the membrane-inserted side of deer ♯33 at late stage regeneration (cf. control side antler). (**F**) Closer examination of the same antler-less pedicle as in (E). Note that the pedicle increased in thickness (up to 6.0 cm). (**G**) Cut surface of the proximal end of the antler-less pedicle in (E). Note that the thickened pedicle resulted from the formation of soft tissue around the existing pedicle bone (asterisk).

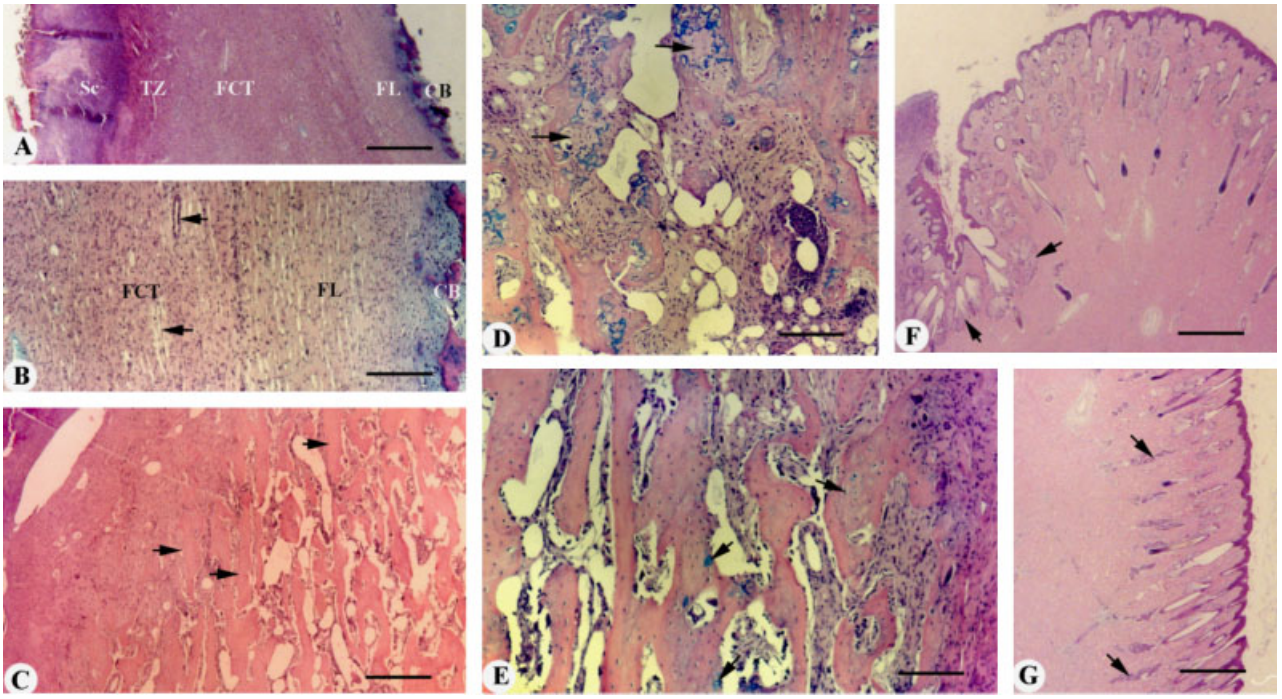

Fig. 4. Histological examination of the skin-less antlers which regenerated from the membrane-inserted potentiated pedicles. Haematoxylin and eosin (HE) staining, and counterstained with alcian blue (AB). (**A**) Outer shaft tissue. Tissue layers were identified from outermost to innermost: scab (Sc, formed mainly from blood), transition zone (TZ, likely granulation tissue), fibrous connective tissue (FCT, equivalent to subcutaneous connective tissue), periosteal fibrous layer (FL) and periosteal cellular layer plus cancellous bone (CB). Note that the layers of FCT, FL and CB are comparable to those of the control side normal antlers. Scale bar = 0.59 mm. (**B**) Higher magnification from (A). Note that fewer blood vessels (arrows) were encountered in the FCT layer than that of the control side antlers. Scale bar = 0.15 mm. (**C**) Shoulder tissue. Well-organised bone trabeculae (arrows) run parallel with the longitudinal axis of the antler. Scale bar = 0.42 mm. (**D**) Tip tissue. Notice that much less cartilage tissue was detected than in control antlers, and that cartilage tissue was being destroyed by chondroclasia (arrows). Scale bar = 0.16 mm. (**E**) Subtip tissue. Most of the cartilage tissue had been replaced by cancellous bone, although some cartilaginous cores (arrows) were still detectable by HE/AB staining. Scale bar = 0.16 mm. (**F**) Distal pedicle skin. Notice that the very distal end of the skin acquired velvet skin-like features including the acquisition of large multi-lobed sebaceous glands (arrows). Scale bar = 0.93 mm. (**G**) Subdistal pedicle skin. Notice that 2–3 mm distally to the pedicle/antler junction, the pedicle skin retained its original scalp skin features including sweat glands (arrows). Scale bar = 0.83 mm.

(Fig. 4A and B). Quantitatively, fewer blood vessels were encountered in the connective tissue layer of the skin-less antlers than those of intact ones. The connective tissue layer seamlessly merged with the inner more typical periosteal fibrous layer (Fig. 4B). The periosteal cellular layer and subperiosteal bone were typical when compared with their counterparts in normal intact antlers. The periosteal fibrous layer differed from the cellular layer which was composed of more cells and less fibres, and the fibres were finer. A discrete row of osteoblasts was found lining the surfaces of the trabecular bone. Subperiosteal cancellous bone was formed through intramembranous ossification (Fig. 4B).

On the shoulder of each antler tip, well-organised bony trabeculae were formed running parallel to the antler's longitudinal axis (Fig. 4C). At the very tip of each skin-less antler, however, much less cartilage tissue was detected than that of the control side antlers (data are not shown). The cartilage in the tip region was disorganised and showed evidence of destruction by chondroclasia (Fig. 4D). In the further proximal region most of the cartilage tissue was replaced by cancellous bone, only some cartilaginous cores were still detectable by HE/AB staining (Fig. 4E).

Some parts of the very distal end of the pedicle skin (2–3 mm) acquired velvet-skin-like features even though this skin was not participating in the process of antler regeneration, which is reminiscent of the distal skin of a pedicle stump shortly after casting of the hard antler. These new features include the acquisition of large multi-lobed sebaceous glands and the loss of arrector pili muscles and sweat glands (Fig. 4F). However, the

rest of the pedicle skin still retained its original scalp skin features (Fig. 4G).

Dormant region

The dormant pedicle stump from animal number 33 was the only one in Group II that retained its Teflon membrane, but failed to regenerate a skin-less antler. Distoproximally a shell of scab covered distal end of the pedicle stump and overlies the granulation tissue. Immediately under the granulation tissue slender bony trabeculae were located above the more matured counterparts (Fig. 5A). The overall picture is reminiscent of the very initial stages of normal antler regeneration when only a scab covers the central region of a pedicle stump (Li et al., 2005). The nature of the soft tissue (Fig. 5B) formed around the pedicle bone was not ascertained but resembled a mixture of connective and granulation tissue. Interestingly, like in the potentiated pedicle, some parts of the distal end of the dormant pedicle skin also acquired velvet skin-like features (Fig. 5C), although the rest of the pedicle skin retained its original scalp skin attributes (Fig. 5D).

DISCUSSION

The findings from the present study are important in understanding antler regeneration and stem cell activation. Firstly the data show that pedicle bone including the periosteum, but not pedicle skin, is the tissue that gives rise to antler regeneration. Secondly that interactions between the pedicle bone and the pedicle skin are indispensable for antler regeneration. Thirdly, these interactions are only required to prime pedicle bone for antler regeneration and not to drive the subsequent regenerative process.

Whether the histogenesis of antler regeneration relies on pedicle bone (periosteum) or pedicle skin has been a significant controversy within the field of antler research (Goss, '95; Kierdorf et al., 2003). In recent studies, Li et al. have used both morphological (2004b) and histological (2005) approaches in order to identify the stem cell/tissue responsible for antler regeneration. They have convincingly demonstrated that antler regeneration is derived from stem cells resident within the pedicle periosteum. The present membrane insertion study is a functional in vivo confirmation of the finding that it is the pedicle bone containing the periosteum and not pedicle skin that gives rise to regenerating antlers. Consequently, the controversy over the role of pedicle skin in antler regeneration has been resolved by this study. However, we cannot conclude from this experiment whether regenerating antlers are derived from the whole pedicle bone, or solely from pedicle periosteum. Pedicle periosteum has been considered to be the sole candidate of stem tissue with rest of the pedicle bone not appearing to be required for antler regeneration (Li et al., 2004a,b, 2005). Whether or not this is truly the case can only be determined by deletion of the pedicle periosteum prior to antler regeneration and then subsequent observation of whether antler regeneration can take place from pedicle stumps without periosteum.

It is known that heterotypic tissue interactions are crucial for organ development (Carlson, '99) and epimorphic regeneration (Mescher, '96). Interestingly, Li and Suttie (2003) found that the whole shaft of a pedicle stump can be classified into two regions based on the degree of association between the bone and the enveloping skin. The tightly contacted region has been termed potentiated region, whereas the loosely attached region the dormant region. They have further inferred that the interactions between pedicle periosteum and the enveloping skin are a prerequisite for antler regeneration, and these interactions are realised through the close association between these two tissue types. If this hypothesis is true, the potentiated region must have progressed one step further toward antler regeneration than the dormant region. The present experiment provided convincing evidence for this hypothesis. All three potentiated bony pedicle stumps regenerated antlers after separation of the pedicle bone from its enveloping skin by insertion of the impermeable Teflon membrane. In contrast, one of the three dormant bony pedicle stumps did not give rise to antler tissue after separation of the bone and skin by a Teflon membrane. In the other two cases, no antler tissue regenerated from the pedicles while the membranes remained in position (up to 55 days). Only after loss of the membrane did the two dormant pedicle stumps eventually regenerate normal antlers (enveloped by velvet skin). Therefore, we conclude that tissue interactions between pedicle bone and its enveloping skin are indispensable for antler regeneration. It is also clear that once the potentiated stage has completed association between the two interactive tissue types, it is no longer required for antler regeneration and thus the essential interactions are only transient in nature. In other words,

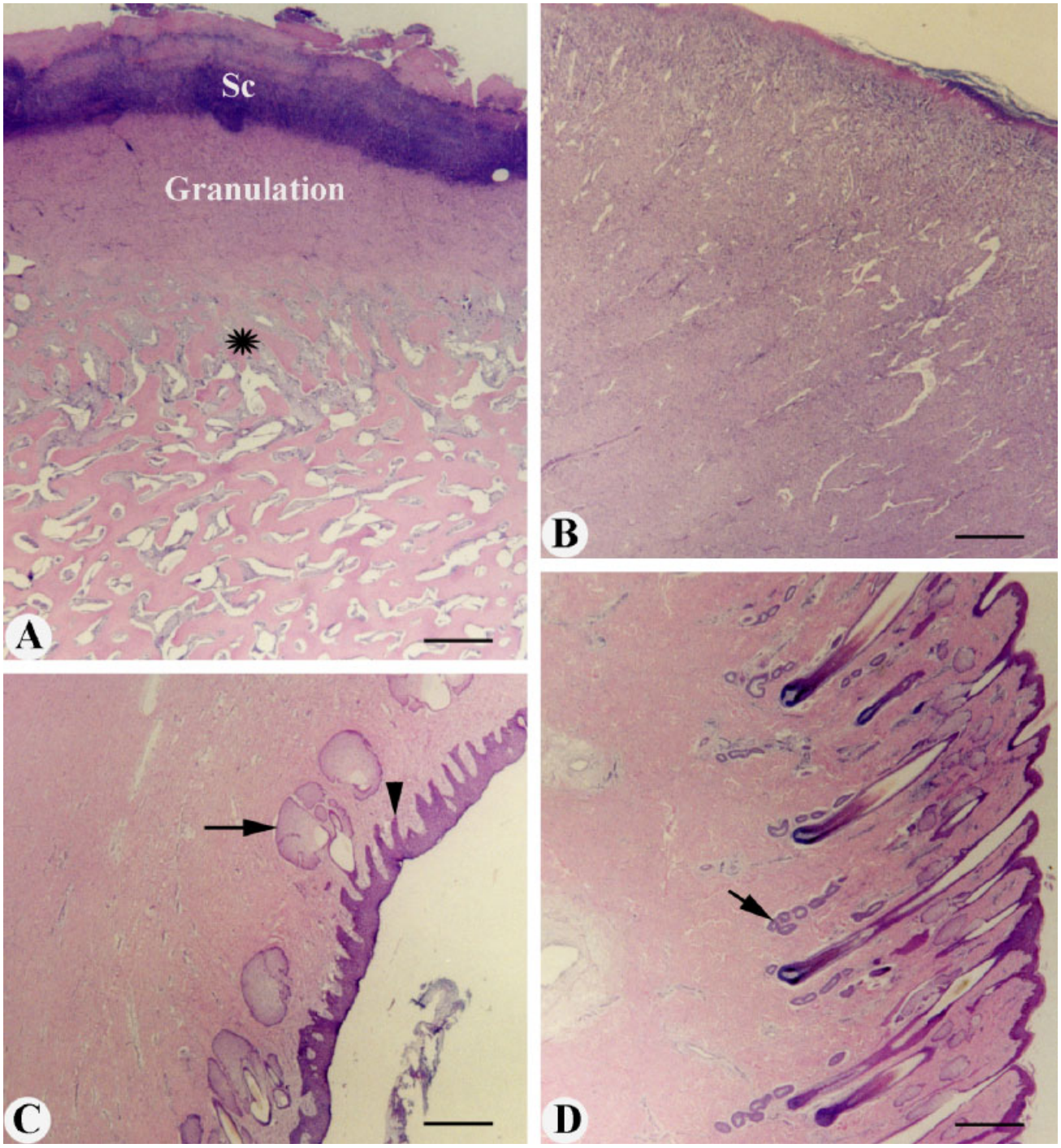

Fig. 5. Histological examination of the antler-less pedicle from deer ♯33. HE staining, and counterstained with AB. (**A**) Apical tissue. Distoproximally, a layer of scab (Sc) was observed overlying the granulation tissue. Under the granulation, slender bony trabeculae (asterisk) were located above the more matured counterparts. The overall picture is reminiscent of the very initial stage of normal antler regeneration. Scale bar = 0.51 mm. (**B**) Soft tissue formed around the existing pedicle bone. The nature of the tissue is not typical of any particular tissue type but rather resembles a combination of connective and granulation tissue. Scale bar = 0.54 mm. (**C**) Distal pedicle skin. Notice that the very distal end of the skin acquired velvet skin features including the acquisition of the large multi-lobed sebaceous glands (arrow) and formation of hair follicles de novo (arrow head). Scale bar = 0.48 mm. (**D**) Subdistal pedicle skin located 2–3 mm away from the distal end. Notice that the pedicle skin retains its original scalp skin features including sweat glands (arrow). Scale bar = 0.47 mm.

antler regeneration only requires the pedicle bone to be primed via interactions with its enveloping skin, and that actual antler growth is independent of the skin. This appeared to also apply to the process of antler branching, as one of the skin-less antlers showed evidence that it had initiated branching. Therefore, antler patterning information appears to be programmed either when the pedicle bone is being primed at the potentiated stage, or at an even earlier stage.

Goss ('83) stated that no known external mammalian organs/appendages can survive without enveloping by an integument. Interestingly, skin-less antlers in this study not only survived, but regenerated de novo from the potentiated bony pedicle stumps. Undoubtedly, the outmost layer of scab effectively protected the skin-less antlers from desiccation. To sustain growth, the left-over residual blood vessels in the vascular layer (between skin and bone) would have surely contributed to the supply of nutrients and the removal of wastes during the growth of the regenerating antlers. However, the blood vessels within the antler bone should not be ignored. Rolf and Enderle ('99) reported that sufficient blood supply is maintained to the major parts of the antler bone after velvet shedding and up to 3–4 weeks prior to antler casting. In this regard, both hard and skin-less antlers bear a similar analogy.

Why skin-less antlers grow smaller than their control counterparts is an interesting question. There may be unknown factors affecting the growth or it may be a result of impaired blood supply. Another possibility is that alterations in ossification type during skin-less antler formation could have been involved in this growth retardation. Whereas elongation of the control side antlers was achieved via proper modified endochondral ossification, only a limited amount of cartilaginous tissue was found in the very tip of each skin-less antler before it was replaced by bone. This gives the impression that skin-less antlers may grow only partially through endochondral ossification and complemented with intramembranous ossification, at least when approaching the final stage of growth (tissue sampling stage). Because bone growth through intramembranous ossification is much slower than those through endochondral ossification (Li and Suttie, '94), it is conceivable that the resulting structure will be smaller if formed through a mixture of intramembranous and endochondral ossification than exclusively through endochondral ossification.

What causes an ossification type change in the skin-less antlers could only be speculated. Based on their previous studies, Li and Suttie (2000) concluded that the change in ossification type from intramembranous to endochondral during pedicle/antler formation is caused by the mechanical pressure which is mainly derived from the mechanically stretched enveloping skin. Skin-less antlers mean that the main mechanical force has been lifted from the forming antler bony structure, although some residual mechanical pressure from the subcutaneous connective tissue and fibrous layer of perichondrium will still exist. This reduced mechanical pressure may have been the cause for the partial change in ossification type.

Velvet skin formation during first antler generation has previously been thought (Li and Suttie, 2000) to be caused by the combination of mechanical tension and chemical induction, both of which are derived from the underlying and rapidly expending antlerogenic tissue. The former driving skin growth and the latter determining skin type. The results from the present study on antler regeneration support this hypothesis. Firstly, once mechanical tension was lost, due to insertion of the membrane, the pedicle skin stopped further growth; this occurred even if the pedicle skin had transformed to antler velvet at the distal end. Secondly, only the pedicle skin at the very distal end, where it was in direct contact with pedicle bone, transformed into antler velvet skin. The rest of the pedicle skin remained unchanged when separated from pedicle bone by the inserted membrane. Therefore, close contact is necessary to convey the inductive signal from pedicle bone to the enveloping skin and neither mechanical stimulation nor chemical induction are dispensable for normal antler velvet skin formation.

Interestingly, feedback from the transformed velvet skin at the distal end of the antler-less dormant pedicle stump did not trigger the formation of antler from the stump bone. This may be due to several factors including the proximity of the rutting season and hence the high level of androgen hormones acting in an inhibitory manner and/or the very limited area of velvetised skin being insufficient to stimulate antler formation.

Lastly, one may argue that the skin-less regenerated antlers are simply an extension of the pedicles, rather than regenerated antlers as we have claimed. It would have been useful to retain these skin-less structures until the next spring to ascertain if they will become calcified and cast.

The benefits of keeping these structures were however outweighed by recovering the skin-less antlers for histological analysis and an examination of the mode of growth (endochondral and/or intramembranous ossification). The decision was also based on previous research (Li and Suttie, '94), where we found that the pedicle itself does regenerate and pedicles can only grow to their species-specific heights (5 cm in sika or red deer). Also, the distal end of each potentiated pedicle stump skin was found to transform to velvet skin and one of the skin-less regenerates initiated the branching process. Consequently, we have confidence in concluding that these skin-less regenerates are true antlers and not pedicles.

Overall, the present study demonstrates that it is the bone component including periosteum of a pedicle stump, but not the skin component, that gives rise to regenerating antler. Bone component can acquire the potential to regenerate an antler only when it is primed via interaction with its enveloping skin.

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LITERATURE CITED

Carlson BM. 1999. Human embryology and developmental biology, 2nd edition. St. Louis: Mosby. p 184–207.

Goss RJ. 1983. Deer antlers. Regeneration, function and evolution. New York, NY: Academic Press.

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. 1992. The evolution of regeneration: adaptive or inherent? J Theor Biol 159:241–260.

Goss RJ. 1995. Future directions in antler research. Anat Rec 241:291–302.

Gruber GB. 1937. Morphobiologische Untersuchungen am Cerviden-Geweih. Werden, Wechsel und Wesen des Rehgehorns. Nachr Ges Wiss Gottingen, Math-Physik KI NF Fachgr VI 3:9–63.

Kierdorf U, Stoffels E, Stoffels D, Kierdorf H, Szuwart T, Clemen G. 2003. Histological studies of bone formation during pedicle restoration and early antler regeneration in roe deer and fallow deer. Anat Rec 273A:741–751.

Li C. 2003. Development of deer antler model for biomedical research. Recent Adv Res Updates 4:256–274.

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 J. 2000. Histological studies of pedicle skin formation and its transformation to antler velvet in red deer (*Cervus elaphus*). Anat Rec 260:62–71.

Li C, Suttie JM. 2003. Tissue collection methods for antler research. Eur J Morphol 41:23–30.

Li C, Harris A, Suttie J. 2001. Tissue interactions and antlerogenesis—new findings revealed by a xenograft approach. J Exp Zool 290:18–30.

Li C, Suttie JM, Clark DE. 2004a. Deer antler regeneration: a system which allows the full regeneration of mammalian appendages. In: Suttie JM, Haines SR, Li C, editors. Advances in antler science and product technology. Queen's Town, New Zealand: Taieri Printery p 1–10.

Li C, Suttie JM, Clark DE. 2004b. Morphological observation of antler regeneration in red deer (*Cervus elaphus*). J Morphol 262:731–740.

Li C, Suttie JM, Clark DE. 2005. Histological examination of antler regeneration in red deer (*Cervus elaphus*). Anat Rec A: Discov Mol Cell Evol Biol 282A:163–174.

Mescher A. 1996. The cellular basis of limb regeneration in urodeles. Int J Dev Biol 40:785–795.

Rolf HJ, Enderle A. 1999. Hard fallow deer antler: a living bone till antler casting? Anat Rec 255:69–77.

Wislocki GB, Waldo CM. 1953. Further observations on the histological changes associated with the shedding of the antlers of the white-tailed deer (*Odocoileus virginianus borealis*). Anat Rec 117:353–375.