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Histogenesis of Antlerogenic Tissues Cultivated in Diffusion Chambers In Vivo in Red Deer (*Cervus elaphus*)

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ABSTRACT In a previous study we showed that formation of deer pedicle and first antler proceeded through four ossification pattern change stages: intramembranous, transition, pedicle endochondral, and antler endochondral. In the present study antlerogenic tissues (antlerogenic periosteum, apical periosteum/perichondrium, and apical perichondria of pedicle and antler) taken from four developmental stages were cultivated in diffusion chambers in vivo as autografts for 42–68 days. The results showed that all the cultivated tissues without exception formed trabecular bone de novo, irrespective of whether they were forming osseous, osseocartilaginous, or cartilaginous tissue at the time of initial implant surgery; in two cases in the apical perichondria from antler group, avascularized cartilage also formed. Therefore, the antlerogenic cells, like the progenitor cells of somatic secondary type cartilage, have a tendency to differentiate into osteoblasts and then form trabecular bone. Consequently, the differentiation pathway whereby antlerogenic cells change from forming osteoblasts to forming chondroblasts during pedicle formation is caused by extrinsic factors. Both oxygen tension and mechanical pressure are postulated to be the factors that cause this alteration of the differentiation pathway.

Deer antlers are a unique biological feature because they annually cast and fully regenerate. Deer are not born with these deciduous bony appendages; they form from the apices of permanent bony protruberances, or pedicles, which initiate from the antlerogenic periostea (the periostea overlying frontal lateral crests) of a male deer calf skull at puberty (Hartwig and Schrudde, '74; Goss, '90). The histogenesis of the pedicle has received little study, and likewise the transition between the growth of the permanent pedicle and the deciduous antler has not been investigated. Recently Li and Suttie ('94a) studied systematically the histological progression of events during pedicle and first antler growth in red deer. They found that a change in ossification pattern occurred from intramembranous through a transition to modified endochondral ossification when the permanent pedicle transformed to the deciduous antler. That is, the antlerogenic cells in the periosteum changed their differentiation pathway from forming bone to cartilage. However, it is not known whether the alteration of the differentiation pathway shown by the antlerogenic cells is temporary or permanent. If the alteration is temporary, the antlerogenic cells must be identical with the progenitor cells of normal secondary type cartilage (such as mandibular condyle) in terms of histogenic differentiation. These progenitor cells are known to have bidifferential potentials; that is, although they normally differentiate into osteoblasts, under specific conditions they may differentiate into chondroblasts (Hall, '78; Stutzmann and Petrovic, '82; Ben-Ami et al., '93). If the alteration is permanent, the developmental stage (transitional, pedicle endochondral, or antler endochondral) at which the differentiation destiny of the antlerogenic cells become unalterable is not known.

Antler and pedicle cartilage, unlike its somatic counterpart, is well vascularized (Banks and Newbrey, '82; Li and Suttie, '94a). Banks and Newbrey ('82) reported that the vascularized tissue formed in antler growth was true cartilage, but the metabolic demands of fast growing cartilage might require the additional nutrient supply provided by this unique vascular system. However this hypothesis has not been tested experimentally.

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Rosin et al. ('63) showed that a diffusion chamber (a chamber sealed with filters at each end which allow the passage of diffusible substances but not cells) is a suitable device for studying osteocartilaginous tissue formation de novo from periosteum in vivo, and also the tissue cultivated in the chamber is free from direct connection of both vascular and nervous systems.

The aim of the study was to use the diffusion chamber technique to cultivate in vivo autografts of growing pedicle and antler cells from different ossification stages to determine whether an irreversible decision was made to transform from bone to cartilage, and if so, at which stage this occurred. A second aim was to determine whether antlerogenic cells could grow without a direct vascular or nervous supply and to test whether the vascularized cartilage formation in pedicle and antler is due to metabolic demand, by slowing down the tissue formation in a limited space (diffusion chamber).

MATERIALS AND METHODS

Animals

The experiment was carried out on 17 red deer stag calves which were allocated to one of five groups based on their pedicle/antler height and ossification stage (Li and Suttie, '94a), (Table 1). Group I (pre-pedicle, n = 3) had frontal lateral crests (the ridge from which the pedicle will grow) less than 5 mm in height (intramembranous ossification stage). Group II (visible pedicle, n = 3) had 15- to 28-mm pedicles (transitional ossification stage). Group III (pedicle, n = 4) had 30- to 45-mm pedicles (pedicle endochondral ossification stage). Group IV (antler, n = 4) had 25- to 65-mm early first antlers on the top of the fully grown pedicle (antler endochondral ossification stage). Group V (control, n = 3) had a 10- to 20-mm-high pedicle (control group), but facial periosteum only was cultivated (see below). From July 1, the deer were observed weekly, and implantation surgery took place when the pedicle/antler had reached a pre-determined height range. The medial pedicle height was measured with a ruler. During the experimental period (July 1 to March 30 of the following year), the deer were maintained in indoor pens (25 m^2/each), and fed a concentrate diet and hay ad libitum at Invermay.

Construction of the diffusion chamber

A 30-mm-diameter Millicell-HA cultivation plate insert (PIHA03050, Millipore Corp.) was modified to serve as the main component. The insert was sealed with a 0.45-µm pore size filter membrane on one side (this pore size allows the passage of diffusible substances but not cells). A plastic rim of 5 mm in height was cut from the unsealed side of the insert in order to make a 7-mm-high chamber. A 30-mm-diameter filter membrane with 0.45-µm pores (Millipore HVLP) was used to seal the open side of the chamber. The chambers with one side sealed and the filter membranes were sterilized by ethylene bromide gas. The open side of the chamber was sealed with the filter membrane after the deer tissue was po-

TABLE 1. Allocation of the experimental animals

Group (stage)	Stag No.	LW (kg)	At surgery: Pedicle length (mm) (left/right)	At surgery: Date (day/month)
I (pre-pedicle)	31	45.5	<5	8/8
	59	47.5	<5	8/8
	9123	49.0	<5	8/8
II (visible pedicle)	20	44.5	25/25	15/8
	60	50.0	28/28	8/8
	276	52.5	15/15	8/8
III (pedicle)	215	62.0	45/45	17/9
	237	62.5	35/35	17/9
	242	69.5	35/30	12/11
	245	58.0	35/35	17/9
IV (antler)	211	69.0	120/120	17/9
	236	77.5	75/80	12/11
	243	68.5	95/100	29/10
	266	70.0	70/75	29/10
V (control)	45	62.0	20/20	15/8
	58	53.0	10/10	15/8
	281	50.0	202/20	15/8

sitioned (see below) using Selleys glue (Gel-Grip, Selley's Chemical Company PTY Limited). The volume of the chamber was approximately 4,308 mm^3. A diagram of a complete diffusion chamber is shown in Figure 1.

Surgery

The surgery took place between August 8 and December 30. The animals were sedated with xylazine (0.75–1.0 mg/kg LW) (Rompun, Bayer Ltd.) i.v. following a 24-hr fast. The anaesthesia was induced with a mixture of halothane, nitrous oxide, and oxygen after intubation. The surgery was carried out with aseptic precautions. After the surgery, the animals were given 4 ml yohimbine (0.9 w/v) to reverse the xylazine component of the anaesthesia and were monitored until fully recovered. For all animals, the right crest/pedicle/antler was the treated side, whereas the left side was allowed to develop a normal antler as a control.

Tissue biopsy and implantation

For groups I and II, a crescent shaped incision was made on the scalp medial to the frontal lateral crest/pedicle. The flap of skin was separated from bone by blunt dissection and was reflected laterally. A 22-mm-diameter sterilized hole punch (Li and Suttie, '94b) was used to mark out the presumptive antlerogenic region, centred on the frontal lateral crest, on the exposed frontal bone. First, a small piece (2×3 mm^2) of periosteum with a layer of underlying bone was taken from the central part of the marked region for histology; then the remaining antlerogenic periosteum without underlying bone was peeled off, and the wound was sutured. (The antlerogenic periosteum is considerably thicker than that round the edge and freely lifts from the underlying bone: Li and Suttie, '94b). For groups III and IV, a first incision was made for 270° around the base of the pedicle with the unincised edge laterally, and a second was made medially from the tip of a pedicle/antler to the base until it met the first incision. The skin was separated from the pedicle/antler by blunt dissection. Apical periosteum/perichondrium without underlying tissue was peeled off from the pedicle/antler after a small piece (2×3 mm^2) of periosteum/perichondrium with a layer of underlying tissue had been removed from the central part of the apex for histology. The remaining stump was detached using embryotomy wire. Remaining skin was trimmed to fit the wound, which was then closed with silk suture. For the control group (group V), a 40-mm-long skin incision was made along the midline of the nasal bone. This incision was continued laterally from both ends of the first incision to the left (Fig. 2). Taking care not to injure main blood vessels, we separated a flap of skin by blunt dissection and reflected it laterally to expose the left nasal bone. A 22-mm-diameter disk of facial periosteum was peeled off after a small piece (2×3 mm^3) of periosteum with a layer of underlying bone had been removed from the central part of the marked region for histology. The 22-mm-diameter antlerogenic/facial periosteum or apical periosteum/perichondrium disk was separated using sterile scissors into 16 pieces. The pieces of the periosteum/perichondrium were placed in the diffusion chamber, which was immersed in sterilized cultivation medium (BGJb + F12 nutrient, Sigma Corp.) with the sealed side as bottom. The upper open side of the chamber was sealed with the sterilized filter membrane. Implant surgery immediately followed biopsy as follows. A skin incision about 40 mm in length was made caudal to the

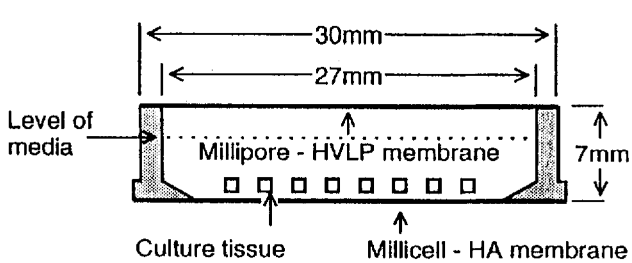

Fig. 1. Diagram of a completely constructed diffusion chamber.

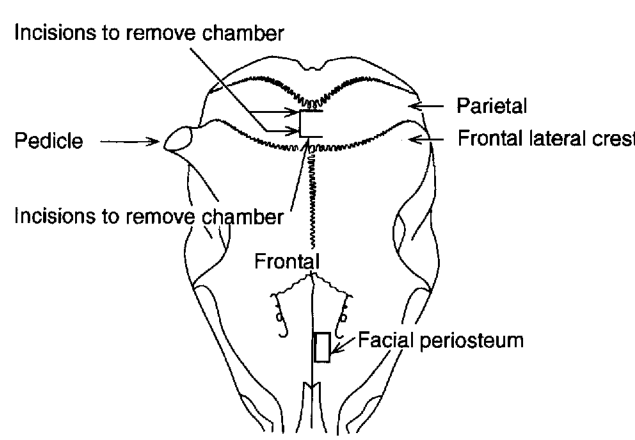

Fig. 2. Illustration of the sampling sites and the implanting site for the diffusion chamber.

pedicle above the suture between the parietal and frontal bones. A mid-line pocket was made by separating the scalp from the parietal bones through the incision caudally. Then the sealed chamber with the antlerogenic tissues in place was inserted in the pocket between the skin and periosteum. The wound was closed with sutures (Fig. 2).

Removal

The chambers were recovered from the deer after a 42-day (groups III and IV), 63-day, or 68-day (groups I, II, and V) cultivation period (see below for reasons). A 30-mm-long incision was made about 10 mm caudal to the chamber. A second incision of the same length was made perpendicular to the first and about 10 mm right to the chamber (Fig. 2). The skin was bluntly dissected from the chamber, and the connective tissue surrounding the chamber was cut along the base edge of the chamber. Care was taken to leave membrane/chamber seals intact. Then the chamber was removed and the skin incision closed with a silk suture. After removal, a connective tissue capsule which wrapped the chamber was circumferentially cut around the polystyrene ring of the chamber. The cultivated tissue with the filter membranes and the connective tissue capsule was weighed after it was removed from the polystyrene ring. The weight of cultivated tissue was derived from the total weight subtracting the two filter membranes. The length of the pedicle and antler (if present) from the control side as well as the treated side, if any, was measured. At the end of the antler growing season, the lengths of the pedicles and antlers grown on the experimental deer were measured.

Determination of the cultivation period

Determination of the cultivation period was mainly based on the developmental stage of the cultivated antlerogenic tissue and the speed of the cultivated tissue formation. For groups I (periosteum), II (periosteum/perichondrium), and V (periosteum, control), the cultivation periods were long enough at least to allow the control side pedicle to proceed to the endochondral ossification stage, but not so long that the tissue growing in the chamber would break the membranes. For groups III (pedicle perichondrium) and IV (antler perichondrium), the cultivation period was sufficiently long for the cultivated tissue (which could be palpated through the skin) to reach the final volume of the cultivated tissue from groups I, II, or V.

Histology

All the tissues cultivated in the diffusion chamber in vivo and the tissues taken at initial implantation surgery were fixed in 10% buffered formalin immediately after surgery. Following a minimum of 24 hr in the fixative, the tissues were then decalcified in Raymond Lamb "R.D.C." commercial decalcification solution for about 10 hr and washed in tap water for 2–4 hr. The tissues were embedded in paraffin wax and sectioned at 5 μm. Two stains were used: Gill's haematoxylin and alcoholic phloxine/eosin for general histological interpretation and alcian blue and haematoxylin/eosin for confirming the presence of cartilaginous tissue. The tissue sections were observed and photographed using a Zeiss Axioplan Microscope. The ossification patterns by which the tissues were formed were evaluated by comparison with the formation of somatic bone (Ham, '69) and antler bone (Banks and Newbrey, '82).

RESULTS

General

At the time of chamber removal all the control pedicles of the stags in the experiment, including the control group (group V), had proceeded to antlers (maximum length 290 mm), except for these of stags 59 (35-mm pedicle) and 9123 (30-mm pedicle) in group I (Table 2). Following the cultivation period from days 42 to 68, the mean weight of the cultivated antlerogenic tissues plus the wrapping connective tissue capsules formed during cultivation (groups I–IV) was 5.89 (SD 0.91)g, whereas the mean weight of the cultivated facial periosteum plus the connective tissue capsules (group V) was 3.49 (SD 0.10) g. By the end of the antler growing season, five of the treated (i.e., groups I–IV) animals had formed pedicles and antlers (55–375 mm in length) at the site of operation, no regeneration had occurred in eight of the animals, and the remaining stag had died. The control side pedicles/antlers grew to 210–510 mm in length.

Histology

The histological results showed that all the chambers were wrapped by a layer of fibrous connective tissue. The tissue components contained in the chambers and the ossification patterns through which the tissue formed are shown in Table 3.

TABLE 2. Measurements at the removal of the diffusion chambers and at the end of the antler growing season (left side = control)

Group (stage)	Stag No.	Pedicle+Antler length(mm) (L/R) At chamber removal	Pedicle+Antler length(mm) (L/R) At end of growing season	Weight of the cultivated tissue (g)[1]	Cultivation period (day)
I (pre-pedicle)	31	70/0	440/0	5.1	68
	59	35/15	380/375	4.0	68
	9123	30/0	210/0	4.0	68
II (visible pedicle)	20	160/40	400/170	4.9	63
	60	290/145	510/365	6.6	68
	276	195/55	360/55	6.6	68
III (pedicle)	215	95/0	430/0	5.6	42
	237	135/0	510/0	6.3	42
	242	60/0	Dead	6.2	42
	245	75/0	430/15	5.6	42
IV (antler)	211	310/0	360/0	4.4	42
	236	170/0	370/0	6.0	42
	243	215/0	490/0	4.5	42
	266	125/0	440/0	5.6	42
V (control)	45	110/115	390/380	3.4	63
	58	150/140	510/460	3.2	63
	281	115/125	460/410	3.3	63

[1]Including the connective tissue capsule around the implant but subtracting the two filter membranes. The weight of the two filter membranes at implantation averaged 0.2g.

Group I (pre-pedicle)

All the tissue samples biopsied at the initial implantation surgery consisted of two horizontal portions, periosteum and underlying cancellous trabecular bone (Fig. 3). The periosteum was composed of two layers, the cellular layer and the fibrous layer. The trabecular bone surfaces were lined with active osteoblasts. The osseous tissue was formed by intramembranous ossification.

All the chambers harvested after the 68-day cultivation period contained cancellous trabecular bone and fibrous connective tissue (Fig. 4). The osseous tissue was formed by means of intramembranous ossification de novo. Trabecular bone was found on the outer surface of the chamber membrane. This was associated with osseous tissue at the inner surface of the membrane in all the chambers. The explants cultivated initially in the chambers had grown together at the time of the chamber removal. Three types of fusion of the explants were encountered in all the chambers of the group. The first was the fusion of periosteal fibrous layers to periosteal fibrous layers (Fig. 5A). This type had a very clear demarcation at the junction formed by the fusion of the two fibrous layers, and trabecular bone had been formed de novo from both cellular layer sides. The second was the fusion of the periosteal fibrous layers to trabecular bone tissue which was formed from the periosteal cellular layers (Fig. 5B). In this type, one side of the junction was of cellular layer and newly formed bone spicules, and the

TABLE 3. Histological results[1]

Group (stage)	Tissues biopsied at implantation: Component	Tissues biopsied at implantation: Ossification	Tissues formed in the chambers: Component	Tissues formed in the chambers: Ossification
I (pre-pedicle)	BF (3/3)	IMO	BF (3/3)	IMO
II (visible pedicle)	BCF (3/3)	TO	BF (3/3)	IMO
III (pedicle)	CF (4/4)	MECO	BF (4/4)	IMO
IV (antler)	CF (4/4)	MECO	BCF (2/4) BF (2/4)	IMO, ECO
V (control)	BF (3/3)	IMO	BF (1/3) F (2/3)	IMO

[1]B, membranous bone; C, cartilage; F, fibrous connective tissue; IMO, intramembranous ossification; MECO, modified endochondral ossification; TO, transitional ossification.

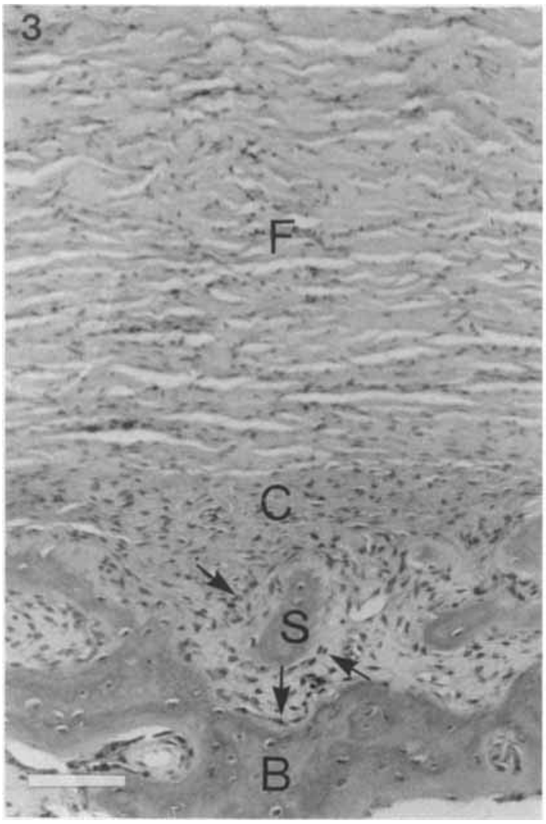

Fig. 3. Antlerogenic periosteum and underlying cancellous bone taken from a deer in group I. The surfaces of the spicules (S) were covered with active osteoblasts (Arrows). F, fibrous layer; C, cellular layer; B, bone. H & E. Bar = 0.1 mm.

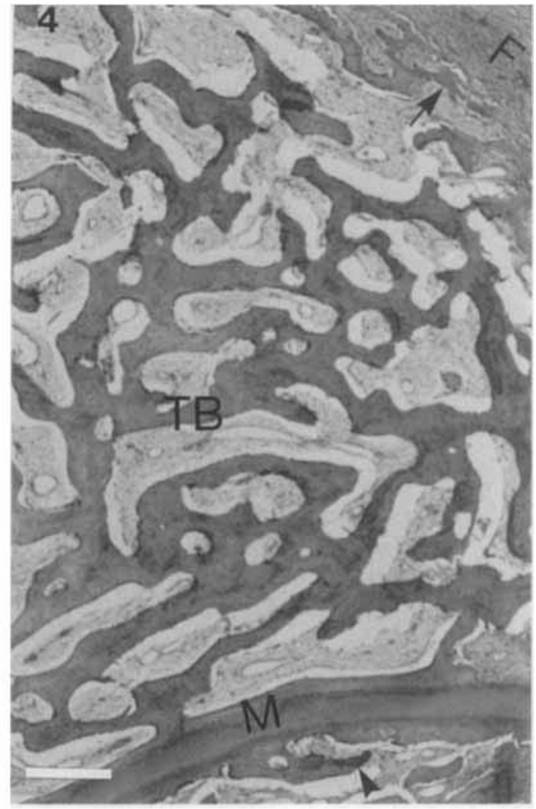

Fig. 4. Part of a vertical section through a diffusion chamber from a deer in group I. Trabecular bone (TB) and fibrous tissue (F) were found. The bone tissue was formed through intramembranous ossification de novo (arrow). The same kind of trabecular bone was induced at the outer surface of the chamber membrane (arrowhead). M, membrane. H & E. Bar = 0.3 mm.

other was mature bone spicules. The third type, trabecular bone tissue, involved the fusion of two layers; although both sides were mature bone spicules, they were different in size, shape, and orientation (Fig. 5C).

Group II (visible pedicle)

All the tissue samples taken at the initial implantation surgery consisted of two horizontal portions, periosteum/perichondrium and vascularized osseocartilaginous tissue (Fig. 6). The discrete clusters of mature chondrocytes were scattered within the bony trabeculae. The underlying osseocartilaginous tissue was formed through both intramembranous and modified endochondral ossifications.

All the chambers harvested after the 63- or 68-day cultivation period contained the same components, and the osseous tissue had been formed through the same ossification pattern as with group I. Trabecular bone tissue was found at the outer surface of the membrane associated with osseous tissue formed in the inner layer surface of the membrane in all cases (Fig. 7). The three types of the explant fusion encountered in all the chambers of the group were the same as in group I.

Group III (pedicle)

All the tissue samples taken at the initial implantation surgery consisted of two horizontal portions, perichondrium and vascularized cartilaginous tissue (Fig. 8). Underlying the hyperplastic perichondrium, all the continuous cartilaginous trabeculae were parallel to each other. This cartilaginous tissue had been formed through modified endochondral ossification. All the chambers harvested after the 42-day cultivation period contained the same components, and the osseous tis-

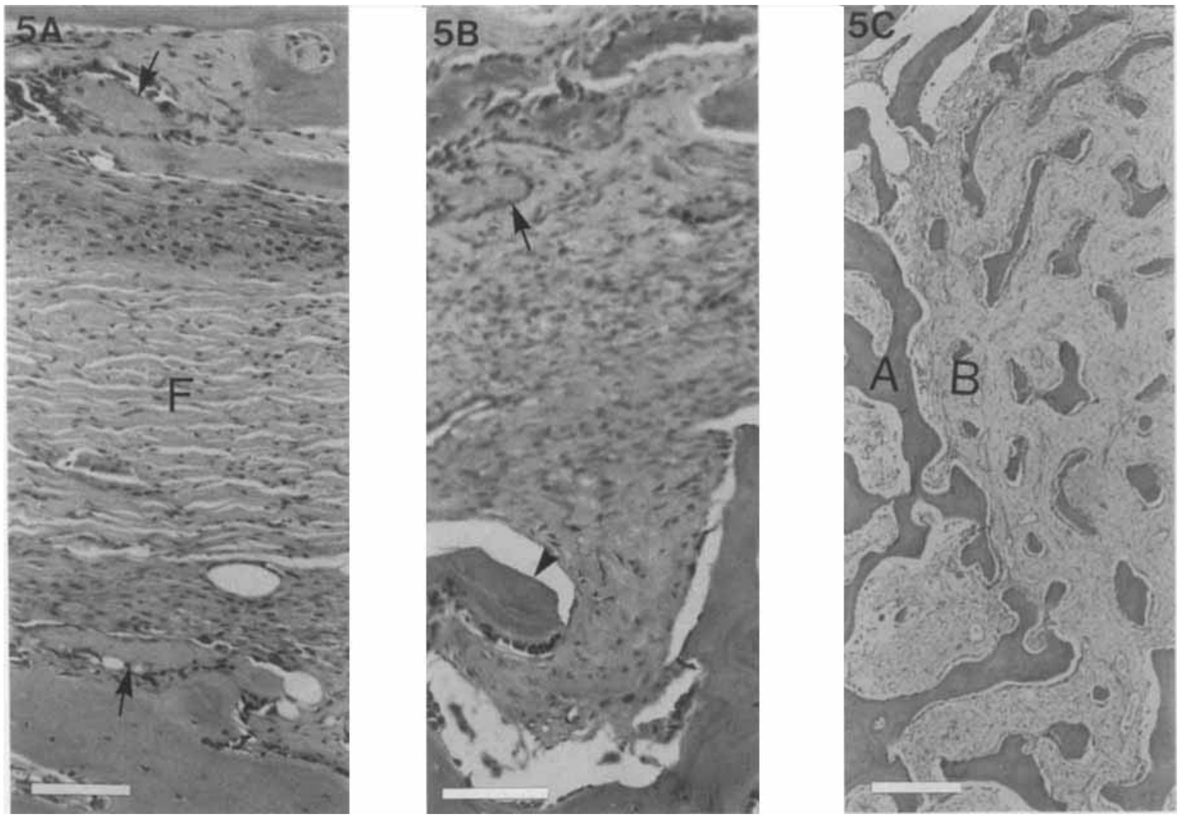

Fig. 5. Part of a verticle section through a diffusion chamber from a deer in group I. Three fusion types were encountered in group I. H & E. **A:** Periosteal fibrous layer to periosteal fibrous layer. Both fibrous layers fused together (F), and trabecular bone was formed de novo from both cellular layer sides (arrows). Bar = 0.1 mm. **B:** Periosteal fibrous layer to trabecular bone formed from periosteal cellular layer. One side of the fusion was cellular layer and newly formed bone spicules (arrow), and the other was mature bone trabeculae (arrowhead). Bar = 0.1 mm. **C:** Trabecular bone to trabecular bone. Notice at the fusion junction that the bone spicules from explant A and explant B were different in size, shape, and orientation. Bar = 0.3 mm.

sue was formed through the same ossification pattern as group I (Fig. 9). However, there was much less osseous tissue and much more fibrous tissue than for groups I and II. No osseous tissue was found at the outer surface of the membrane in any chamber, although the osseous tissue was formed at the inner surface of the membrane in all the chambers. The explants had already grown together at the time of chamber removal, but only one type of explant fusion was encountered, fibrous tissue to fibrous tissue.

Group IV (antler)

All the tissue samples taken at the initial implantation surgery consisted of two horizontal portions, perichondrium and vascularized cartilaginous tissue, which were similar to those of group III. The cartilaginous tissue was formed through the same ossification pattern as for group III.

Of the four chambers harvested after the 42-day cultivation period, two contained two components, osseous tissue and fibrous tissue. The remaining two contained three portions, osseous tissue, cartilaginous tissue (Fig. 10), and fibrous tissue. The osseous tissue and the cartilaginous tissue were formed through intramembranous ossification and endochondral ossification, respectively. No bone formation was found at the outer surface of the membrane in any chamber in the group, although trabecular bone was formed along the inner surface of the membrane in all the chambers. The explants in all the chambers had grown together. The fusion type encountered was the same as in group III.

Group V (control)

All the tissue samples taken at the initial implantation surgery consisted of two horizontal

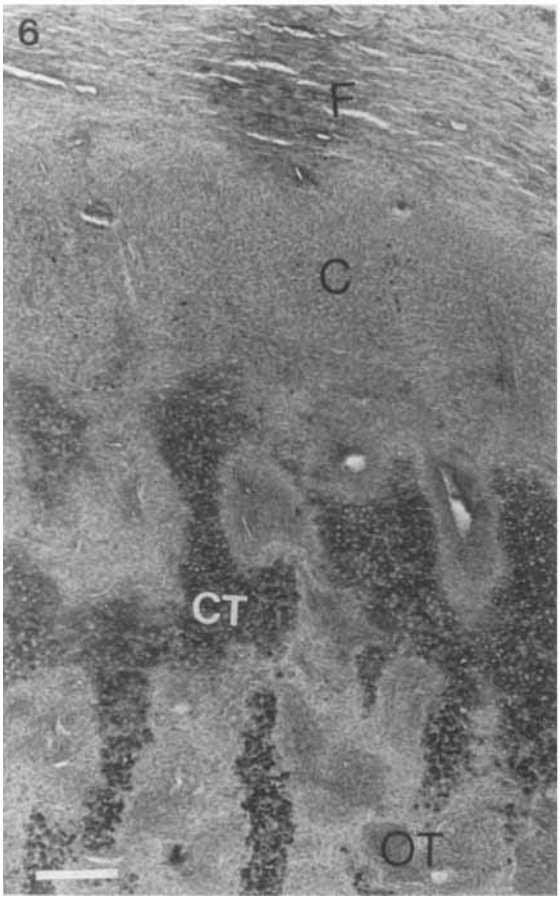

Fig. 6. A vertical section of a 25-mm-high pedicle from a deer in group II. The osseocartilaginous tissue was formed underlying the antlerogenic periosteum/perichondrium. OT, osseous tissue; CT, cartilaginous tissue. F and C are the same as shown in Figure 1. Alcian blue/H & E. Bar = 0.3 mm.

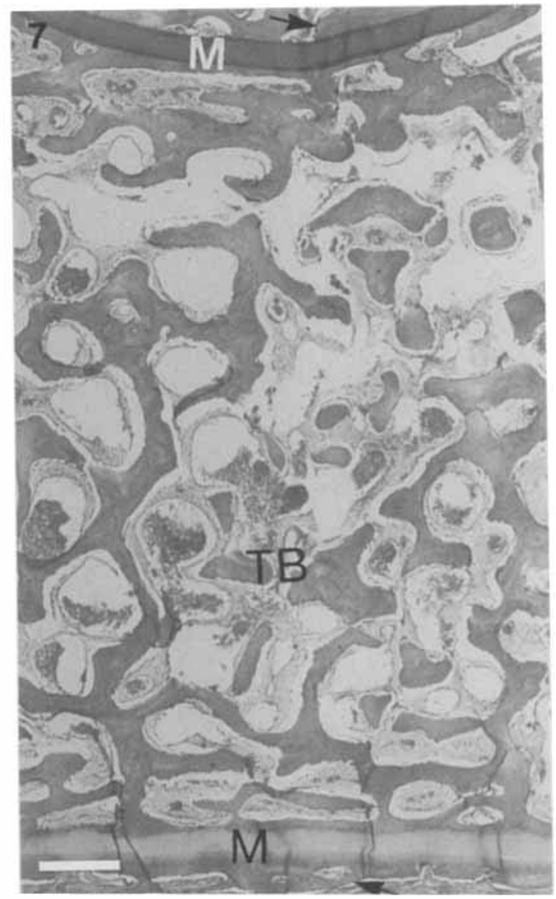

Fig. 7. Part of a vertical section through a diffusion chamber from a deer in group II. The chamber was full of trabecular bone (TB). The same kind of bone was induced at the outer surfaces of both side filter membranes (arrows). M, membrane. H & E. Bar = 0.3 mm.

portions, periosteum and osseous tissue, which was similar to that of group I. The osseous tissue was composed of compact bone, which was formed through intramembranous ossification.

Of the three chambers harvested after the 63-day cultivation period, one contained two components, osseous tissue and fibrous tissue. The osseous tissue was formed through intramembranous ossification. The remaining two were exclusively fibrous tissue (Fig. 11). No bone formation was found at the outer surface of the membrane in any chamber in the group. The explants in all the chambers had grown together. The fusion type encountered was periosteal fibrous layer to periosteal fibrous layer, which was similar to that of group III.

DISCUSSION

The histogenesis of a pedicle and a first antler covers two distinct phases: an internal change phase by which pedicle tissue is formed, and an external change phase, which indicates the beginning of antlerogenesis (Li and Suttie, '94a). The internal change phase is associated with changes in the ossification pattern from intramembranous through transitional to endochondral. The external change phase is defined by the integument change from typical scalp pelage to antler velvet. The tissues of both the pedicle and antler are formed through the proliferation and differentiation of the antlerogenic cells, which reside initially in the antlerogenic periosteum. The results of this experiment showed that all the cultivated antlero-

Fig. 8. A vertical section of a 35-mm-high pedicle from a deer in group III. The continuous cartilaginous trabeculae (CCT) were formed underlying the hyperplastic perichondrium (PC). Alcian blue/H & E. Bar = 0.3 mm.

Fig. 9. Part of a vertical section through a diffusion chamber from a deer in group III. Only a small piece of trabecular bone (TB) was encountered in the chamber. M, membrane. H & E. Bar = 0.1 mm.

genic tissues (antlerogenic periosteum from the intramembranous ossification stage, apical periosteum/perichondrium from the transitional ossification stage, and apical perichondria from both the pedicle and antler endochondral ossification stages) without exception formed trabecular bone tissue de novo, irrespective of whether they were forming osseous, osseocartilaginous, or cartilaginous tissue at the time of the initial surgery, although in two cases in the antler group, cartilaginous tissue was also formed. At the time of chamber removal, the control pedicles had grown to at least 30 mm in length, which means that they had already proceeded to the modified endochondral ossification stage and were forming cartilage exclusively (Li and Suttie, '94a). Therefore it clearly indicates that the antlerogenic cells, like the progenitor cells of the somatic secondary type cartilage (Hall, '78; Stutzmann and Petrovic, '82), have a tendency to differentiate into osteoblasts, which then form trabeculr bone. Accordingly, the alteration of the differentiation pathway from one forming osteoblasts to one forming chondroblasts, which is the characteristic change in the pattern of ossification during pedicle formation, must be caused by extrinsic factors, rather than being genetically pre-determined to do so. It is interesting that antlerogenic cells retain this intrinsic tendency of differentiating into osteoblasts through all their developmental ontogeny, including the transformation from a permanent pedicle to a deciduous antler.

So far a few extrinsic factors have been reported to alter the differentiation pathway of the progenitor cells of the secondary type cartilage, but the two most important are mechanical factors (Pritchard, '72; Stutzmann and Petrovic, '82; Ben-Ami et al., '93) and oxygen tension (Ham, '69). What are the extrinsic factors causing the change of the differentiation pathway of the antlerogenic cells during pedicle formation? The histological studies support the hypothesis that the ossification pattern changes are caused by the same extrinsic factors which have the capacity to alter the differentiation pathway of the progenitor cells of secondary type cartilage. Although at the onset of the transitional ossification stage the regular waved fibrous periosteum covers the apex of the

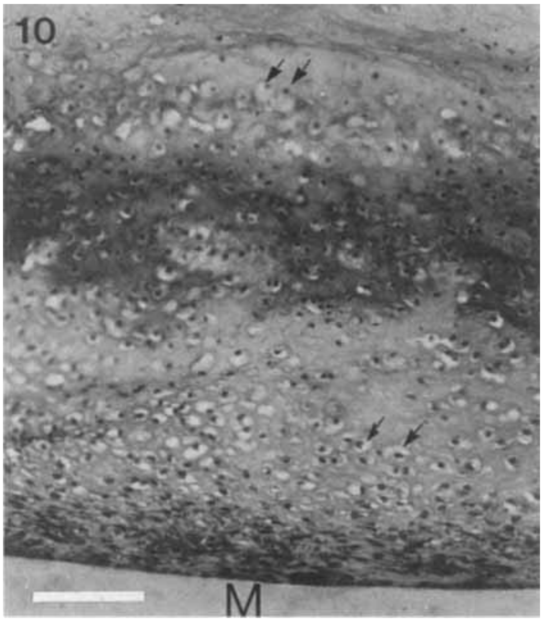

Fig. 10. Part of a vertical section through a diffusion chamber from a deer in group IV. Avascular cartilaginous tissue was encountered. Hypertrophied chondroblasts (arrows) were differentiated from the progenitor cells, which were adjacent to the membrane. M, membrane. Alcian blue/H & E. Bar = 0.1 mm.

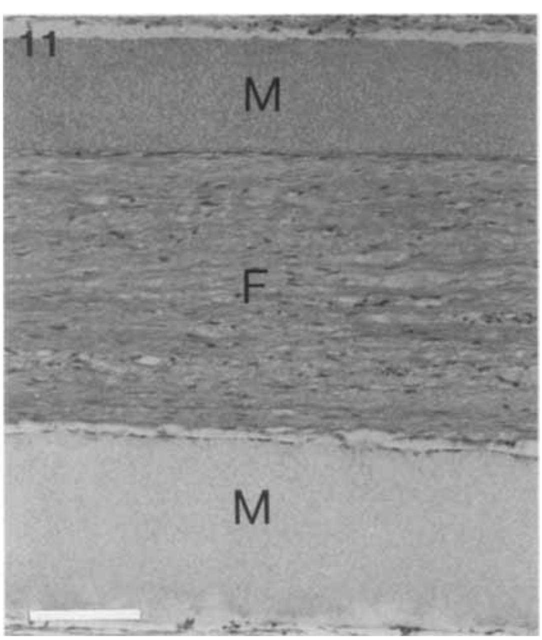

Fig. 11. Part of a vertical section through a diffusion chamber from a deer in group V (control group). Fibrous connective tissue (F) was exclusively found in the chamber. M, membrane. H & E. Bar = 0.1 mm.

incipient pedicle (Li and Suttie, '94a), the differentiation of the antlerogenic cells cannot be under significant pressure, so the mechanical factors probably do not play an important role in this step. However, as only the antlerogenic cells in the rapidly forming areas located in the tip of a pedicle differentiate into chondroblasts, a functional vascular system may not have been formed in these areas. Thus the antlerogenic cells could differentiate in a very low oxygen tension environment. As the pedicle grows, the amplitude of the regular waved fibrous periosteum covering the apex of the pedicle gradually decreases and eventually vanishes when the ossification pattern approaches the modified endochondral pattern. From then on the antlerogenic cells of the pedicle probably proliferate and differentiate in a very low oxygen tension environment as well as under mechanical pressure. Speer ('82) also found that the proliferating and differentiating cell mass at the tip of the antler tines was under pressure provided by the axially oriented collagen fibres.

Because the alteration of the ossification pattern in pedicle formation is dependent on the environment, this change is a reversible one. As the antlerogenic cells have the same feature as the progenitor cells of somatic secondary type cartilage in terms of differentiation behaviour, deer pedicle development should be a very good model for studying the mechanism of developmental changes in the pattern of ossification.

The result that the avascularized cartilage formed de novo within the limited space of the chambers by the apical perichondrium of the antler supports the hypothesis that the formation of cartilage with a vascular network during antler growth is due to the metabolic demands of the rapidly growing tissue.

Bone formation from the antlerogenic periosteum occurred de novo in the diffusion chambers (which were free from direct nervous connection). This is consistent with previous data which indicated that pedicles could form from aneural antlerogenic periostea (Li et al., '93). Therefore, the present results reinforce the conclusion that a nervous input is not required for pedicle initiation.

Only one animal from the control group in which the facial periosteum was cultivated developed osseous tissue in the diffusion chamber. Also the bone which formed did not induce additional bone formation at the outer layer of the membrane. This is consistent with the results of Rosin et al. ('63). However, all cultivated antlerogenic tissues in the experimental groups formed trabecular bone, and all antlerogenic periostea in groups I and II induced bone formation at the outer layer of the membranes. This means that the antlerogenic cells in the periosteum/perichondrium must have a stronger potential to form bone than the cells of the somatic periosteum. Also, the antlerogenic cells in the antlerogenic periosteum from the early pedicle developmental stages produced inductive materials which could penetrate the filter membrane (pore size, 0.45 μm) to induce osseous tissue formation at the outer surface of the membrane. However the antlerogenic tissues from groups III and IV did not show this inductive property, perhaps because the cultivation period in groups III and IV (42 days) was shorter than that for groups I and II (68 days).

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