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Electron Microscopic Studies of Antlerogenic Cells From Five Developmental Stages During Pedicle and Early Antler Formation in Red Deer (*Cervus elaphus*)

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ABSTRACT

Previous studies using light microscopy have revealed that histogenesis of deer pedicle and antler has four ossification stages. The first of these stages is the development of the permanent pedicle. Initial development of the pedicle is from the cellular layer cells of the antlerogenic periosteum and these cells have been termed initial antlerogenic cells (IACs). Apart from the IACs, it has also been shown that the cellular layer cells of the apical periosteum/perichondrium, the peripheral periosteum of pedicles or antlers, and the marginal periosteum surrounding the pedicles are also capable of either partially or fully generating a pedicle or an antler. Therefore, these cells can all be considered antlerogenic cells and called apical antlerogenic cells (AACs), peripheral antlerogenic cells (PACs), and marginal antlerogenic cells (MACs), respectively. The aim of this study was to examine the ultrastructure of these antlerogenic cells, and to determine whether there were ultrastructural correlates with the changes of these antlerogenic cells and ossification stages. The ultrastructure of each type of antlerogenic cells was systematically examined using transmission electron microscopy, at each stage of pedicle and first antler growth. At the first ossification stage, the IACs were spindle-shaped and inactive. The most obvious feature was the presence of abundant intracellular glycogen. The MACs were similar to the IACs. During the early second stage, most of the AACs changed in appearance from preosteoblasts to prechondroblasts. Much less heterochromatin was found in the AACs than in the IACs. The most striking attribute of the AACs was the existence of intracellular collagen fibers. The MACs showed abnormal dilation of the rough endoplasmic reticulum (RER). During the late second stage, the majority of the AACs were prechondroblasts. AAC nucleoli were clearly discernible and the cisternae of the RER were arranged in parallel. The MACs contained a greater proportion of abnormally-dilated RER. During the third stage, the AACs were all prechondroblasts. The Golgi apparatus in these cells was well developed. Many free ribosomes in rosettes were scattered in the cytoplasm. Most cytoplasm of the majority of the MACs was occupied by abnormally-dilated RER (the lumen of the RER was extremely dilated and appeared electron-lucent). During the fourth stage, the AACs were similar to their counterparts from the third stage, but the boundaries of some AACs were ill-defined. Some MACs were found to be undergoing apoptosis. The PACs were becoming less and less

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active from distal to proximal along the shaft of the antler. It is a novel finding that antlerogenic cells change in appearance and subcellular content from preosteoblasts to prechondroblasts prior to the transition from intramembranous to endochondral ossification during pedicle formation. Therefore, the differentiation process from antlerogenic cells to chondroblasts is a matter of maturation from prechondroblasts to chondroblasts. The fact that the antlerogenic cells are rich in glycogen makes them more like embryonic cells. The local membrane deficiency of some AACs at the fourth stage and the presence of mature collagen fibrils within the AACs may reflect the unusually high demand for collagen fibrils during the period of rapid antler growth. Anat. Rec. 252:587–599, 1998.

Key words: deer; pedicle; antler; antlerogenic periosteum; perichondrium; antlerogenic cells; intramembranous ossification; endochondral ossification; electron microscopy

Deer pedicles and antlers are bony organs. Unlike horns, which are skin derivatives and grow from the base, these bony organs grow from the tips and are formed from antlerogenic periosteum overlying the lateral crest of the deer frontal bone (Hartwig and Schrudde, 1974). Although the discovery of antlerogenic periosteum was made more than 20 years ago, and is considered one of the most important landmarks in the history of deer antler research (Goss and Powel, 1985), the biological features of the antlerogenic periosteum have received little attention. Likewise, the mechanism whereby a permanent bony pedicle is derived from a thin layer of antlerogenic periosteum and then gives rise to a deciduous antler is not well understood.

It has been shown (Li and Suttie, 1994a) that antlerogenic periosteum consists of an outer fibrous layer and an inner cellular layer, both of which are much thicker than their adjacent somatic counterparts. As pedicle and first antler are mainly derived from the cellular layer cells, these cells are termed antlerogenic cells. At the early pedicle developmental stage, initial antlerogenic cells (IACs) located in the periosteum of the central part of an antler growth region start to proliferate and then to differentiate into osteoblasts. In turn, these osteoblasts form trabecular bone. In this manner, a pedicle is built up. However, when the pedicle grows to 5–10 mm in height, some of the antlerogenic cells in the apical periosteum of the pedicle begin to change their differentiation pathway from one forming osteoblasts to one forming chondroblasts, whereas the rest of the cells keep to their original fate and form bone. Therefore, osseocartilaginous tissue is formed by the apical antlerogenic cells (AACs). When the pedicle grows to 25–30 mm in height, the apical antlerogenic cells of the pedicle differentiate into chondroblasts only, and these cells maintain this transformed differentiation pathway throughout their developmental ontogeny until the first antler has fully formed.

Although the potential to grow antlers is normally held in the antlerogenic cells at the tips of developing pedicles and antlers, if either is damaged other cells are capable of partially replacing them. These include the cells in the peripheral periosteum of pedicles and growing antlers (Suttie and Fennessy, 1985), and in the surrounding periosteum of the pedicles (Li and Suttie, 1994b). Therefore, they are called peripheral and marginal antlerogenic cells (PACs, MACs), respectively.

The aim of this study was to use transmission electron microscopic (TEM) techniques to investigate initial, apical, peripheral, and marginal antlerogenic cells at different developmental stages during pedicle and early first antler formation, and to determine whether there were ultrastructural correlates with these changes in differentiation pathway and in the transformation from pedicle to antler.

MATERIALS AND METHODS

Animals and Tissues

Nineteen male red deer calves (including three castrates) and three female red deer yearlings were selected for both light microscopic (Li and Suttie, 1994a) and TEM studies. The male deer were classified into five groups based on different pedicle/antler ossification stages from the results of the light microscopic studies: Group I, Intramembranous ossification; Group II, Early transitional ossification; Group III, Late transitional ossification; Group IV, Pedicle endochondral ossification; and Group V, Antler endochondral ossification. Prepubertal castrated male deer and female deer were used for comparison with normal male deer, and were assigned to Group I as they did not grow pedicles and antlers. The details for the allocation of the animals and the tissue samples into particular groups are shown in Table 1. Initial periosteum, apical periosteum/perichondrium, marginal periosteum and peripheral periosteum, and facial periosteum (as control) were taken from the animals as follows.

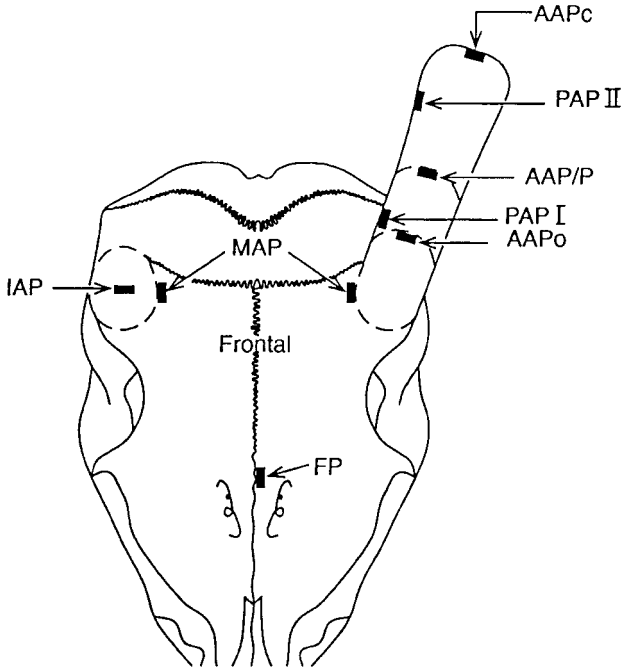
Tissue Collection Techniques

The initial and marginal antlerogenic, and facial periostea (about 1 × 2 mm) were sampled using a similar technique as described by Li and Suttie (1994a). The procedures for sampling apical periosteum/perichondrium and peripheral periosteum were as follows. After peeling off the skin of the pedicle/antler to expose the bony protuberance, a piece of full thickness apical periosteum/perichondrium (about 1 × 2 mm) was taken from the tip of the protuberance, and a piece of peripheral periosteum (peripheral periosteum I, about 1 × 1 mm) with a thin layer of underlying bone was taken from the shaft of the pedicle of Groups IV and V (Fig. 1). The sampling site was located 20 mm above the base of the pedicle, medial to the shaft. A piece of peripheral periosteum (peripheral periosteum II, about 1 × 1 mm) with a thin layer of underlying bone was taken from the shaft of antlers of Group V (Fig.

TABLE 1. Allocation of the Experimental Animals and Tissue Samples

Group	Sex	Age (month)	Number (n = x)	Pedicle length (mm)	Antlerogenic periosteum/ perichondrium (n)				Facial periosteum (n)
					Initial or apical	Marginal	Peripheral I	Peripheral II	
I	Male	4	4	unpalpable	4	4	—[c]	—	2
	Male[a]	6	3	unpalpable	3	—	—	—	—
	Female	14	3	unpalpable	3	—	—	—	2
II	Male	6	3	8–15	3	2	—	—	1
III	Male	8	3	20	3	3	—	—	—
IV	Male	8.5	3	25–40	3	3	3	—	—
V	Male	8–9	3	60–70[b]	3	3	3	3	—

[a]Castrated 3 weeks prior to the biopsy.
[b]With 10 mm antler.
[c]Absent.



AAPc	Apical antlerogenic perichondrium
AAPo	Apical antlerogenic periosteum
AAP/P	Apical antlerogenic periosteum/perichondrium
PAP I	Peripheral antlerogenic periosteum I
PAP II	Peripheral antlerogenic periosteum II
MAP	Marginal antlerogenic periosteum
IAP	Initial antlerogenic periosteum
FP	Facial periosteum

Fig. 1. Illustration of the biopsy sites.

1). The sampling site was just above the junction of pedicle and antler.

Techniques for Transmission Electron Microscopy (TEM)

Following removal from the deer, tissue samples were immediately put into 3.125% cacodylate-buffered glutaraldehyde solution (Dickson, 1984) for 24 hr and were then trimmed into small blocks (approximately 1 mm^2). The trimmed tissue blocks were placed in decalcification solution (0.013 M disodium EDTA + 1.032 M NaOH) for approximately 5 weeks at 4°C with the solution changed every two days (Dickson, 1984), washed using 0.15 M cacodylate buffer containing sucrose for 48 hr at 4°C with the solution changed every half day, and post-fixed in 1% OsO_4 in 0.15 M cacodylate buffer containing sucrose for 2 hr at 4°C. In order to enhance glycogen staining for tissue in Group 1, 1% OsO_4 + 1.5% potassium ferrocyanide in 0.1 M cacodylate buffer was used. After three 15 min distilled water washes, the blocks were dehydrated in a graded ethanol series, passed through propylene oxide, and were then embedded in resin (Agar 100). Ultra thin sections were cut, doubly stained with uranyl acetate and lead citrate, and observed using an Akashi 002A electron microscope.

The antlerogenic cells observed in this study were morphologically assigned to either preosteoblasts or prechondroblasts based on the criteria set by Tang and Chai (1986) for preosteoblasts, Rifkin et al. (1980) and Silberberg (1968) for prechondroblasts.

Techniques for Glycogen Staining in Light Microscopy

The periodic Acid-Schiff (PAS) reagent method with diastase control (Drury and Wallington, 1967) was employed for staining glycogen in thick sections (5 µM). Four sections were cut from each tissue block, two for diastase control and two for the treatment. Treated sections were placed in distilled water while control sections were incubated in distilled water plus 10% human saliva for 30 min at 37°C. The control sections were washed in running tap water for 5 min and then both treatment and control sections were stained using the PAS reaction and counterstained with haematoxylin.

RESULTS

Ultrastructurally, the initial (Group I), marginal (Groups I–V), peripheral I (Groups IV and V), and peripheral II (Group V) antlerogenic periostea, and facial periosteum could be divided into three layers: outer, mid, and inner. The midlayer (cellular layer) varied considerably in width. The inner layer overlying the bone surface was made up of ovoid to cuboidal osteoblasts 1 to 2 cells in thickness. The cellular layer had a looser structure and contained undif-

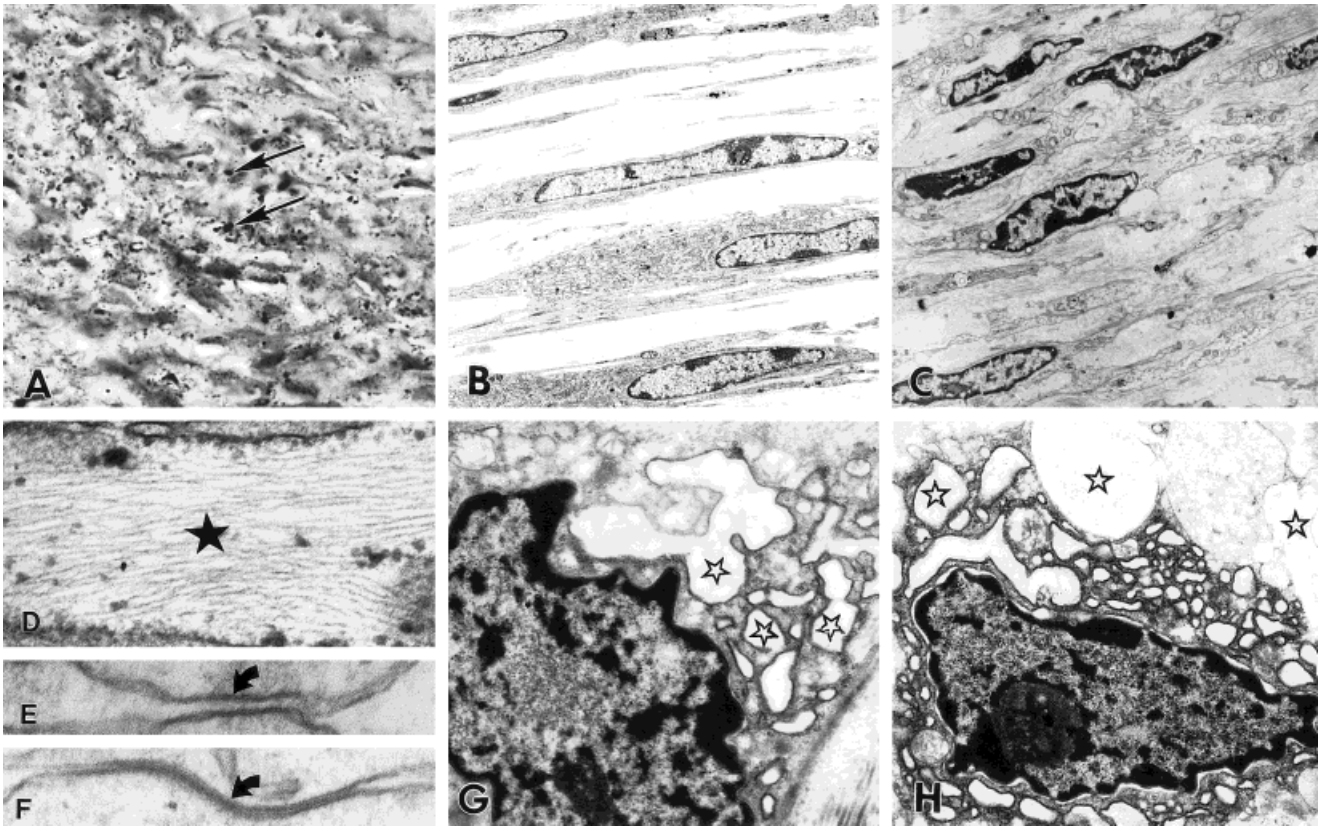


Fig. 2. Light (A) and electron (B–H) micrographs of the cells from male (except for G) calves of Group I. **A**: A section from initial antlerogenic periosteum stained with PAS reaction and haematoxylin counterstain. Note that glycogen granules were scattered all over the section. Two examples of densely stained intracellular glycogen are indicated by arrows. ×1,000. **B**: Osteogenic cells from the facial periosteum. Note the absence of intercellular collagen fibers. ×5,063. **C**: A group of initial antlerogenic cells (IACs) with large blocks of marginated heterochromatin, which occupied most of the nuclear space. ×3,893. **D**: Abundant cytoplasmic filaments (star) in an IAC running in parallel with the cisternae of the RER. ×91,200. **E**: A desmosome-like junction (arrow) between two adjacent osteoblasts. ×137,500. **F**: A tight junction (arrow) between two adjacent osteoblasts. ×128,125. **G**: A part of an IAC from a female calf. Note that nearly all the RER were abnormally-dilated (open star). ×19,400. **H**: An IAC from a castrated male deer with an irregular nucleus, greatly abnormally-dilated RER (open stars). ×20,945.

ferentiated cells (antlerogenic or osteogenic cells), mononuclear phagocytes, and occasional blood vessels in a sparse collagenous matrix. The most superficial layer consisted of collagen bundles and elongated active fibroblasts aligned parallel to the bone surface. Comparing with facial periosteum or initial antlerogenic periosteum, the apical antlerogenic periosteum/perichondrium from Groups II–V had a much wider cellular layer with a smooth transition from undifferentiated cells to osseocartilaginous tissue distoproximally. Therefore, only the cells from the outer part of the wide cellular layer were examined as the aim in this study was to investigate the ultrastructure of antler stem cells. Gap junctions were observed between the cells in all three layers of these periostea/perichondria.

Glycogen granules were found in all the midlayer antlerogenic cells. These granules were confirmed to be glycogen using the periodic acid-Schiff (PAS) reagent method with diastase control at the light microscopic level. The results showed that all the antlerogenic cells contained PAS positive cytoplasmic masses (Fig. 2A) and these stained masses were removed after diastase digestion (not shown). The glycogen masses in antlerogenic tissues from different developmental stages varied in size, shape and content of deposits (Table 2). The highest content of glycogen was found in the antlerogenic cells of the apical antlerogenic periosteum/perichondrium (AAP/P) from both early and later transitional ossification stage pedicles. The initial antlerogenic periosteum (IAP) contained a moderate amount of glycogen. The apical antlerogenic perichondrium (AAP) from both pedicle and antler showed less glycogen than the IAP, whereas the osteogenic cells from the facial periosteum contained a barely detectable level of glycogen.

Group I—Intramembranous Ossification Stage

Facial osteogenic cells.

Male. The facial osteogenic cells were of elongated or irregular spindle-shape with the long axis parallel to the bone surface, and had spindle-shaped nuclei. These nuclei were predominantly euchromatic with little heterochromatin which was mainly located along the inner surface of the nuclear membrane. One to two nucleoli could be identified in these nuclei. Moderately developed rough endoplasmic reticulum (RER) and Golgi apparatus were found in these cells. A few mitochondria were scattered in the cytoplasm. There were few collagen fibers within the intercellular

TABLE 2. Density of Intracellular Glycogen in Antlerogenic Cells From Different Pedicle and Antler Developmental Stages[a]

Group		I		II	III	IV	V
Tissue	IAP	MAP	FP	AAP/P	AAP/P	AAP	AAP
Density of glycogen	++	+	+−	++++	++++	+++	++

[a]+−: barely detectable; +: low density; ++: moderate density; +++: high density; ++++: very high density; IAP: initial antlerogenic periosteum; MAP: marginal antlerogenic periosteum; FP: facial periosteum; AAP/P: apical antlerogenic periosteum/perichondrium; AAP: apical antlerogenic perichondrium.

space among these osteogenic cells, indicating that cells were not synthesizing and secreting matrix materials (Fig. 2B).

Female. In comparison with the male, female osteogenic cells had fewer organelles and more intercellular mature collagen fibers. Short rod-like RER was scattered in the cytoplasm, and the spindle-shaped nuclei had scant heterochromatin lying along the nuclear membrane (not shown).

Initial antlerogenic cells (IACs).

Male. The IACs were preosteoblastic in appearance, and spindle-shaped, but shorter and wider than the facial osteogenic cells. The irregularly oval shaped nuclei of the IACs were located centrally. Large blocks of heterochromatin were distributed around the periphery in the nucleoplasm (Figs. 2C and 4B). Most cells appeared to be inactive. There were no obvious nucleoli. Most of the IACs had few cytoplasmic organelles, some had moderately developed RER and Golgi apparatus, but nearly all had abundant cytoplasmic filaments (Fig. 2D). The lumen of some parts of the RER contained dense amorphous granular or microfibrilar materials. Desmosome-like junctions between IAC processes were encountered. Long osteoblast processes penetrating into osteoid were a common feature. Two types of junction complexes (desmosome-like and tight) between adjacent osteoblasts were observed (Fig. 2E,F).

The most obvious feature of the IACs was the intracellular glycogen, which varied in density, appearance, and distribution. These granules appeared either in rosettes or in monoparticulate form. In the cells with less abundant intracellular glycogen, some of the granules were found in close proximity to the RER (Fig. 3A), but in others they were randomly scattered in the cytoplasm. Some glycogen granules were associated with mitochondria (Fig. 3B) and some with recognizable lipid droplets (Fig. 3C). Some cells contained so much glycogen that usual cellular organelles were displaced (Fig. 3D). Some IAC processes were fully filled with glycogen granules (Fig. 3E).

Female. Most IACs and osteoblasts were inactive. The RER from most of these cells was extremely dilated. This dilation was quite different from that in the normal cells, as the lumen of the extremely dilated RER was electronlucent and appeared empty (Fig. 2G). This phenomenon therefore was termed abnormal-dilation or abnormally-dilated. In these IACs and osteoblasts, swollen mitochondria often accompanied the abnormally-dilated RER. Therefore, these cells were likely to be degenerating cells. Osteoclasts and mononuclear phagocytes were frequently observed. The phagocyte surface was characterized by ruffle and smooth areas with phagocytic vacuoles.

Castrated male. The majority of the cells had greatly abnormally-dilated RER and many vacuoles (Fig. 2H). The nucleus was of an irregular oval shape and had large blocks of heterochromatin either marginated along the periphery or scattered within the nucleoplasm.

Marginal antlerogenic cells (MACs). MACs were similar to IACs and were spindle to oval shaped. Their nuclei had irregular oval or bizarre shapes with big blocks of heterochromatin distributed marginally (Fig. 4A). Moderately developed RER occupied the major part of the cytoplasm within which a few mitochondria were scattered. A few glycogen granules in rosette form were randomly distributed in the cytoplasm.

Group II—Early Transitional Ossification Stage

Apical antlerogenic cells (AACs). Most of the AACs of the group were preosteoblasts (Figs. 4B and 2C), but some were prechondroblasts (Figs. 4C and 6B). The prechondroblasts were polarized and had homogeneous nucleoplasm. The nuclei of these prechondroblasts had smooth nuclear envelopes and prominent nucleoli.

The most striking feature of these AACs was the presence of intracellular collagen fibers. These fibers were found within smooth single membrane-limited vacuolar structures and were identical to their extracellular counterparts in diameter and periodicity (640–700Å; Fig. 5A–D). The fiber-containing vacuolar structures varied in size and shape. These structures were associated with the Golgi apparatus (Fig. 5A–C). However some fiber-containing vacuoles were located in the vicinity of the plasma membrane (Fig. 5D). Nonbounded intracellular collagen fibers were not found.

The heterochromatin in the nucleus of these cells was distributed along the periphery in a thin layer interrupted at the sites of the pores of the nuclear envelope. In addition, small clumps of heterochromatin were associated with the nucleolus or widely scattered throughout the nucleoplasm. The nucleolus could be identified, but the components (Pars amorpha and nucleolonema) from which the nucleolus is constructed could not be clearly distinguished (data not shown).

Marginal antlerogenic cells (MACs). The MACs from normal males were similar to those from Group I. However, some RER in the cytoplasm was moderately abnormally-dilated (Fig. 4D).

Facial osteogenic cells (FOCs). The FOCs from the normal male were similar to those of males or females of Group I. However, the FOCs of this group had more heterochromatin and fewer organelles (data not shown).

Group III—Late Transitional Ossification Stage

Apical antlerogenic cells (AACs). The majority of the AACs of this stage were prechondroblasts (Fig. 6A and 6B), although some preosteoblasts were still occasionally found. The oval or round nucleus of these AACs had 2–4

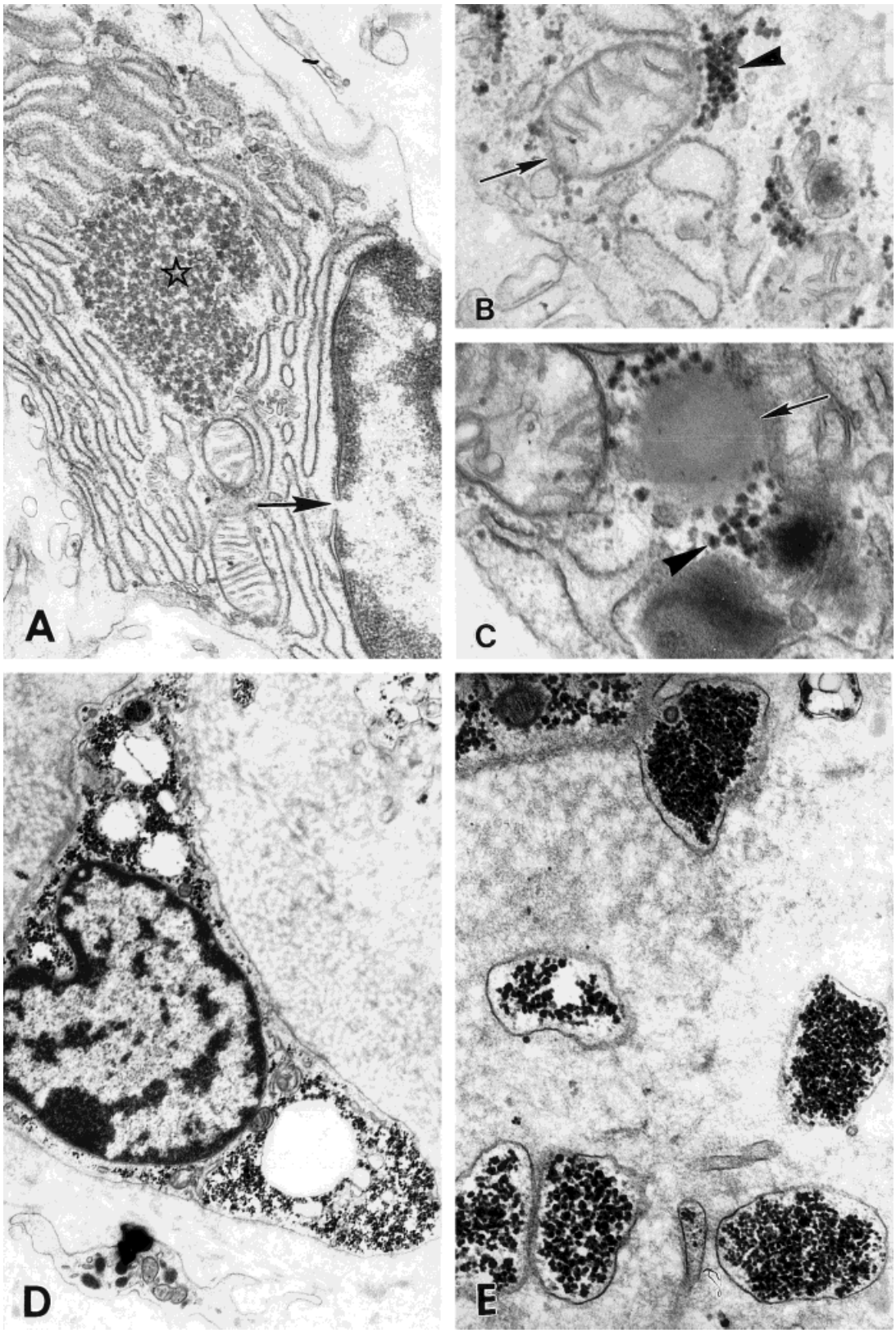


Fig. 3. Intracellular glycogen of antlerogenic cells of the initial antlerogenic periosteum from Group I. **A**: A large lake of glycogen (open star) in rosettes is located within the region of RER. Note the unusual nuclear pore (arrow) without a septum to close the open channel. ×23,075. B: A cluster of glycogen granules (arrow head) is closely associated with a mitochondrion (arrow). ×32,000. **C**: A group of glycogen granules (arrow head) associated with a lipid droplet (arrow). ×19,840. **D**: The cytoplasm of a cell densely occupied by glycogen granules. In the cell only a few organelles were visible. ×10,762. **E**: A cross-section of a group of glycogen-filled processes of a cell or cells. ×25,257.

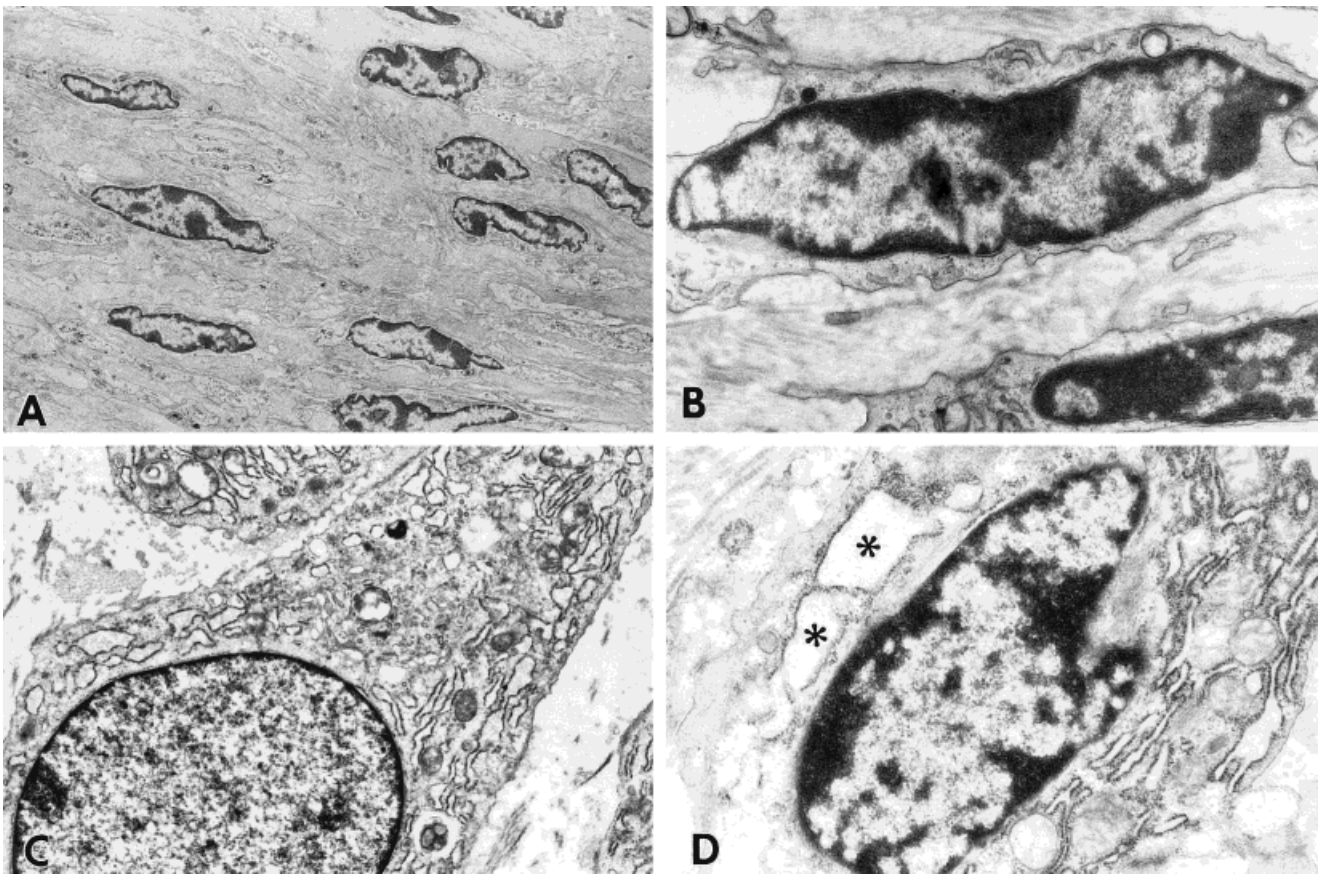


Fig. 4. Antlerogenic cells from Group I (A) and Group II (B–D). **A**: Marginal antlerogenic cells (MACs) from Group I. Note the large blocks of marginated heterochromatin which occupied most of the nuclear space. ×2,613. **B**: A preosteoblast-like apical antlerogenic cell (AAC) with few cytoplasmic organelles from Group II. ×14,254. **C**: A part of a prechondroblast-like AAC with moderately developed RER and perinuclear Golgi apparatus filling the cytoplasm from Group II. Note the cell was polarized. ×9,712. **D**: A MAC from Group II. Note that some RER in the cytoplasm was moderately abnormally-dilated (stars).

prominent nucleoli. The components of these nucleoli, pars amorpha and nucleolonema, were clearly discerned (Fig. 6A). The groups of associated cisternae of the RER in the cytoplasm were arranged more or less in parallel, but they were less closely spaced and less precisely ordered than in osteoblasts or chondroblasts. The Golgi apparatus was not very well developed. Enormous ribosome rosettes could be seen scattered in the cytoplasm.

Intracellular collagen fibers were also found in these prechondroblasts, although less prominent than in the AACs of Group II.

Marginal antlerogenic cells (MACs). Although the ratio of nucleus to cytoplasm decreased and less heterochromatin occupied the nuclear space compared with those of Group II, most of the RER was abnormally-dilated and the heterochromatin was more electron-dense (Fig. 6E).

Group IV—Pedicle Endochondral Ossification Stage

Apical antlerogenic cells (AACs). The AACs encountered at this stage were all prechondroblasts (Fig. 6A). However, the Golgi apparatus was very well developed (Fig. 6C) compared with that of Group III. Except for prominent nucleoli, the nucleoplasm of the cells was homogeneous. The nucleolus was similar to Group III. Many free ribosomes in rosettes were scattered in the cytoplasm (Fig. 6D). Intracellular collagen fibers were frequently observed in the AACs (not shown).

Peripheral antlerogenic cells (PACs). The PACs were spindle shaped and had oval nuclei, moderately developed Golgi apparatus and RER. A few mitochondria were distributed within the region of the RER in the PACs. Microgranular material could be identified in the lumen of these RER.

Marginal antlerogenic cells (MACs). The majority of the MACs were of an irregular oval shape and their nuclei had numerous indentations. Most cytoplasm was occupied by abnormally-dilated RER (Fig. 6F). Very sparse microamorphous material could be identified in the lumen of some parts of the RER, whereas other areas were totally empty.

Group V—Antler Endochondral Ossification Stage

Apical antlerogenic cells (AACs). Most of the AACs at this stage were similar to those of Group IV. However, some unusual AACs were encountered occasionally. The boundaries of these unusual AACs were ill-defined in

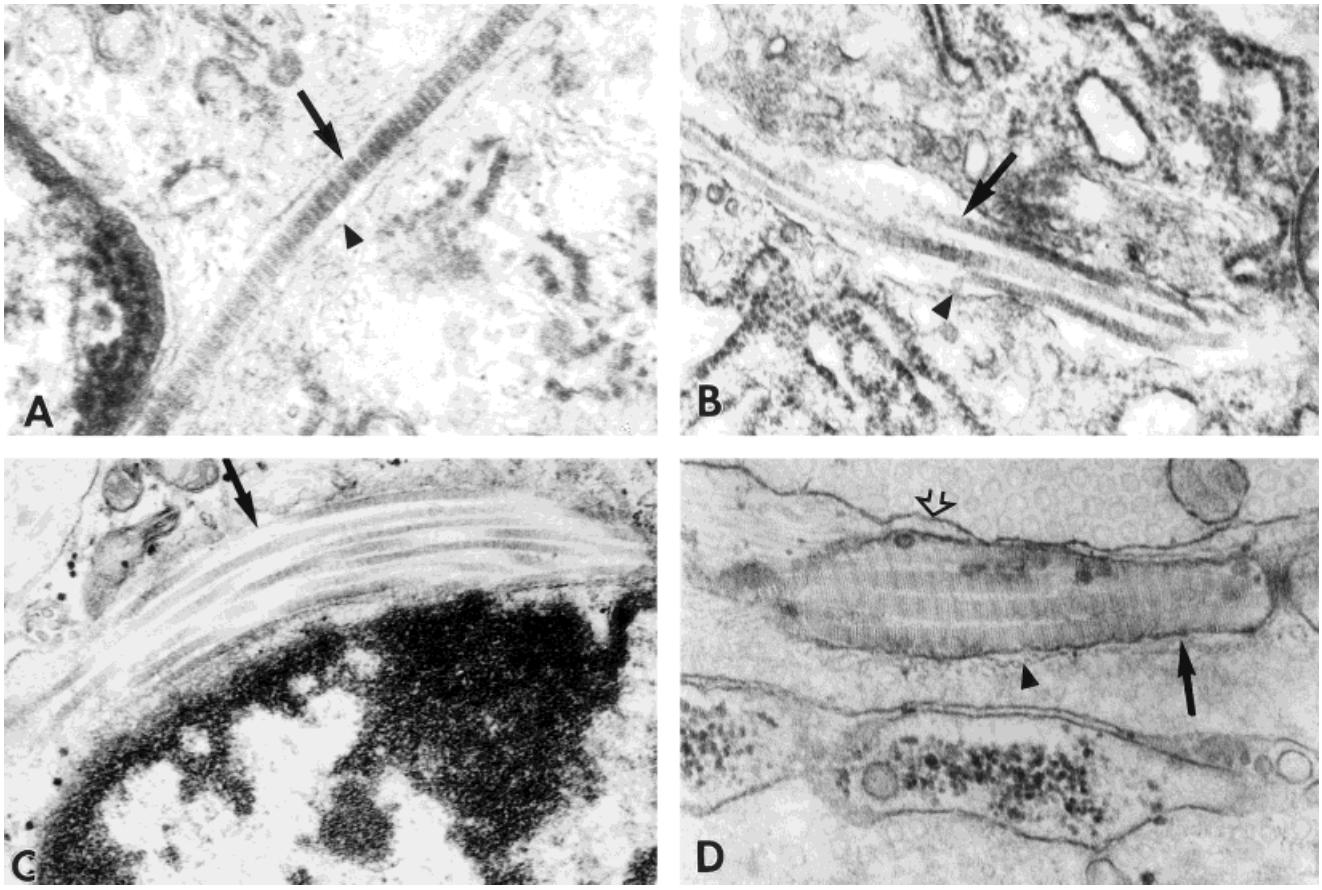


Fig. 5. Intracellular collagen-like fibres in the apical antlerogenic cells (AACs) from Group II. **A**: A single fiber (arrow) within a smooth membrane-bounded vacuolar structure (arrow head) located in the perinuclear Golgi region. ×81,667. **B**: Three fibers (arrow) within a vacuole (arrow head) located in the Golgi region. ×45,675. **C**: A bundle of fibres within a vacuole located in the vicinity of the nucleus of an AAC. ×40,863. **D**: A bundle of fibers (arrow) within a vacuole (arrow head) located in the vicinity of an AAC membrane (open arrow). ×127,600.

places where long segments of cell membrane appeared to be lacking, although the rest of the organelles of these AACs seemed to be normal (Fig. 7A,B).

The cisternae of the RER were dilated and contained a microgranular or microfibrilar electron-opaque substance. Besides RER, free ribosomes in rosette form were also located in the cytoplasm of these AACs (not shown).

Peripheral antlerogenic cells I (PAC I). The nuclei of PAC I were of an irregular round shape and had prominent nucleoli. The RER of these cells was moderately dilated, and the microfibrilar or microgranular materials could be discerned within the lumen of the RER (Fig. 7C). Mitotic figures were occasionally encountered (not shown).

Peripheral antlerogenic cells II (PAC II). In comparison with PAC I, some of the PAC II were in early stage of degeneration and had more irregular nuclei with condensed chromatin. Some abnormally-dilated RER were found in the cytoplasm (Fig. 7D).

Marginal antlerogenic cells (MACs). MACs undergoing degeneration were frequently encountered (Fig. 7E). These degenerating cells had condensed chromatin, extremely abnormally-dilated RER and swollen mitochondria. However, most MACs appeared healthy, although inactive with a few short segments of rod-like, abnormally-dilated RER. No material could be discerned in the lumen of the RER.

DISCUSSION

Antlerogenic cells were designated by Li and Suttie (1994a) as those in the cellular layer of the periosteum located in the central part of the antler growth region. However, marginal and peripheral periostea also have the potential to form antler tissue. The marginal and pedicle peripheral periostea have the full capacity to regenerate an antler, while the antler peripheral periosteum has a partial capacity to do so (Suttie and Fennessy, 1985; Li and Suttie, 1994b). Therefore, in this study the cellular layer cells from the marginal, pedicle peripheral, and antler peripheral periostea were all classified as antlerogenic cells.

This is the first ultrastructural demonstration that antlerogenic cells change in appearance and subcellular content from preosteoblasts to prechondroblasts prior to the transition from intramembranous to endochondral ossification pattern during pedicle formation. Li and Suttie (1994a) reported that the change in ossification pattern started when the antlerogenic cells began to alter their differentiation pathway from one of forming osteoblasts to

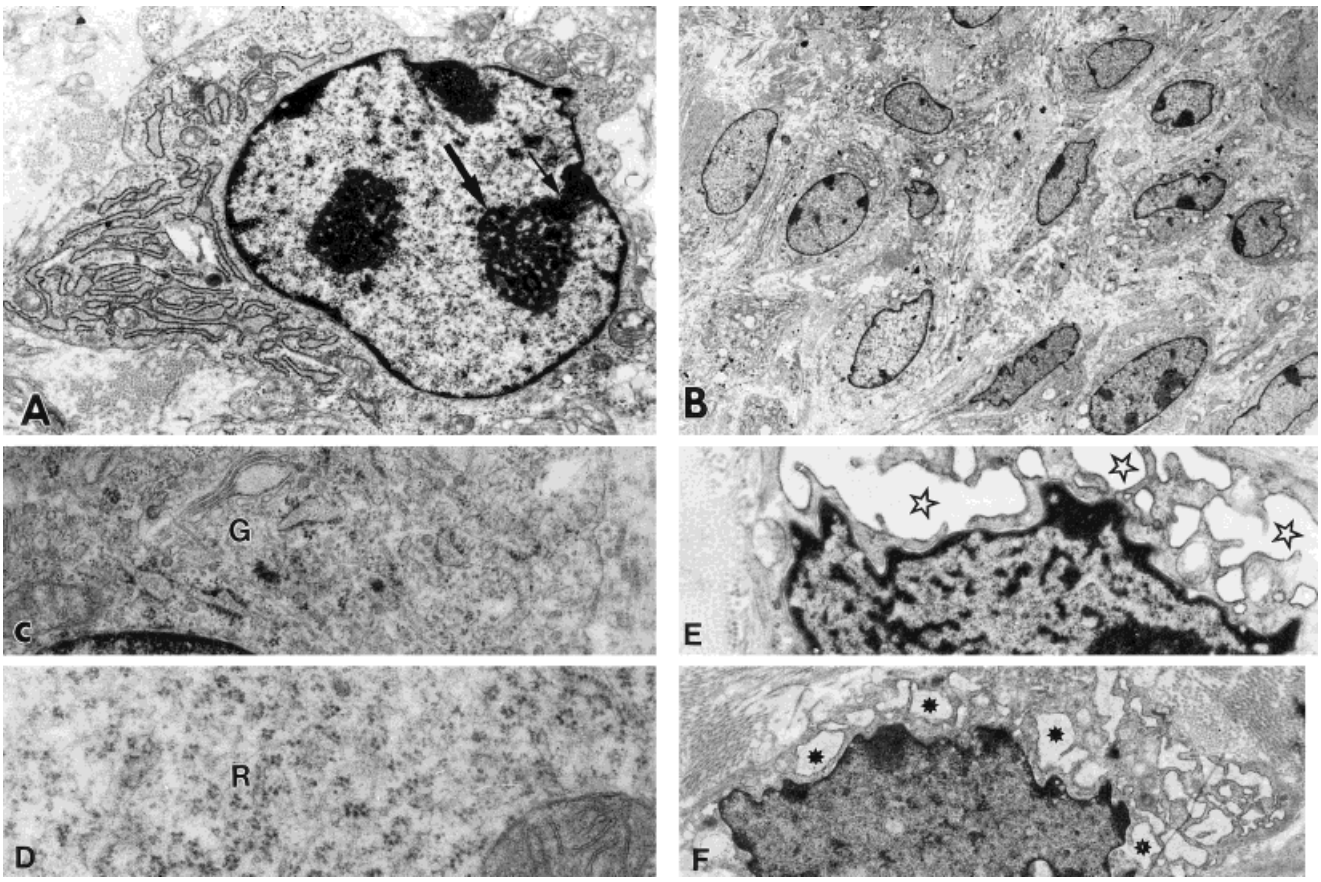


Fig. 6. Antlerogenic cells from Group III and Group IV. **A**: A prechondroblast-like apical antlerogenic cell (AAC) from group III with a round nucleus and three prominent nucleoli, and abundance of euchromatin. Note that the components of the nucleolus, pars amorpha (small arrow), and nucleolonema (large arrow) could be clearly discerned. ×13,867. **B**: A group of AACs from Group III. Note that most of these cells were prechondroblast-like. ×2,613. **C**: A part of an AAC from Group IV with well-developed perinuclear Golgi apparatus (G). ×27,390. **D**: A part of an AAC from Group IV with an abundance of free ribosomes in rosettes (R) arranged peripherally along the cell membrane. ×34,752. **E**: A part of a marginal antlerogenic cell (MAC) from Group III with the abnormally-dilated RER (open stars). ×7,754. **F**: A MAC from Group IV. Note nearly all the cisternae of the RER in the cell were abnormally-dilated (asterisks). ×6,023.

one of forming chondroblasts. However, it was not clear at the light microscopic level whether antlerogenic cells kept their original preosteoblast-like appearance through their ontogeny. In the present study the use of electron microscopy has permitted a detailed analysis to be made of the cellular morphology underlying these changes, and the results showed that antlerogenic cells change in appearance from preosteoblasts to prechondroblasts before the antlerogenic cells differentiate into chondroblasts. Therefore, the differentiation process from antlerogenic cells to chondroblasts is simply a matter of maturation from prechondroblasts to chondroblasts, rather than transformation from preosteoblasts to chondroblasts. It has been demonstrated that the change in ossification pattern from intramembranous to endochondral during pedicle formation is caused by extrinsic factors (Li et al., 1995). Therefore, these extrinsic factors could function not only in the maturation process from prechondroblasts to chondroblasts, but also influence the progeny of antlerogenic cells themselves.

IACs and MACs of Group I are ultrastructurally similar to each other at the pre-pedicle stage. However, as the development of pedicle and antler proceeds, the divergence in cell ultrastructure between the progenies of IACs and MACs becomes more and more obvious. At the antler stage, MACs contain predominant abnormally-dilated RER and heterochromatin, and some MACs even begin to degenerate, in contrast, AACs (progeny of IACs) had well-developed organelles and prominent nucleoli (Table 3). An interesting phenomenon is observed at the antler stage that the partial cytoplasm of some AACs is directly exposed to the extracellular matrix. This phenomenon may be artificially created due to either poor osmium fixation or the AACs are cut tangentially. However, the similar phenomenon has not been observed at the pedicle growth stages, although the same methodology is used for the whole experiment. This observation may be a true fact, then this phenomenon can be considered as a kind of holocrine secretion. If this is the case, the fate of these holocrine secreting AACs would demise as this is obviously an irreversible process. What role would these holocrine secreting AACS play in antler formation if this phenomenon is a true fact? Chai and Tang (1979) reported a similar phenomenon in the osteogenic cells from the bone fracture callus. They thought that this was possible only when these osteogenic cells exfoliated the cell membrane

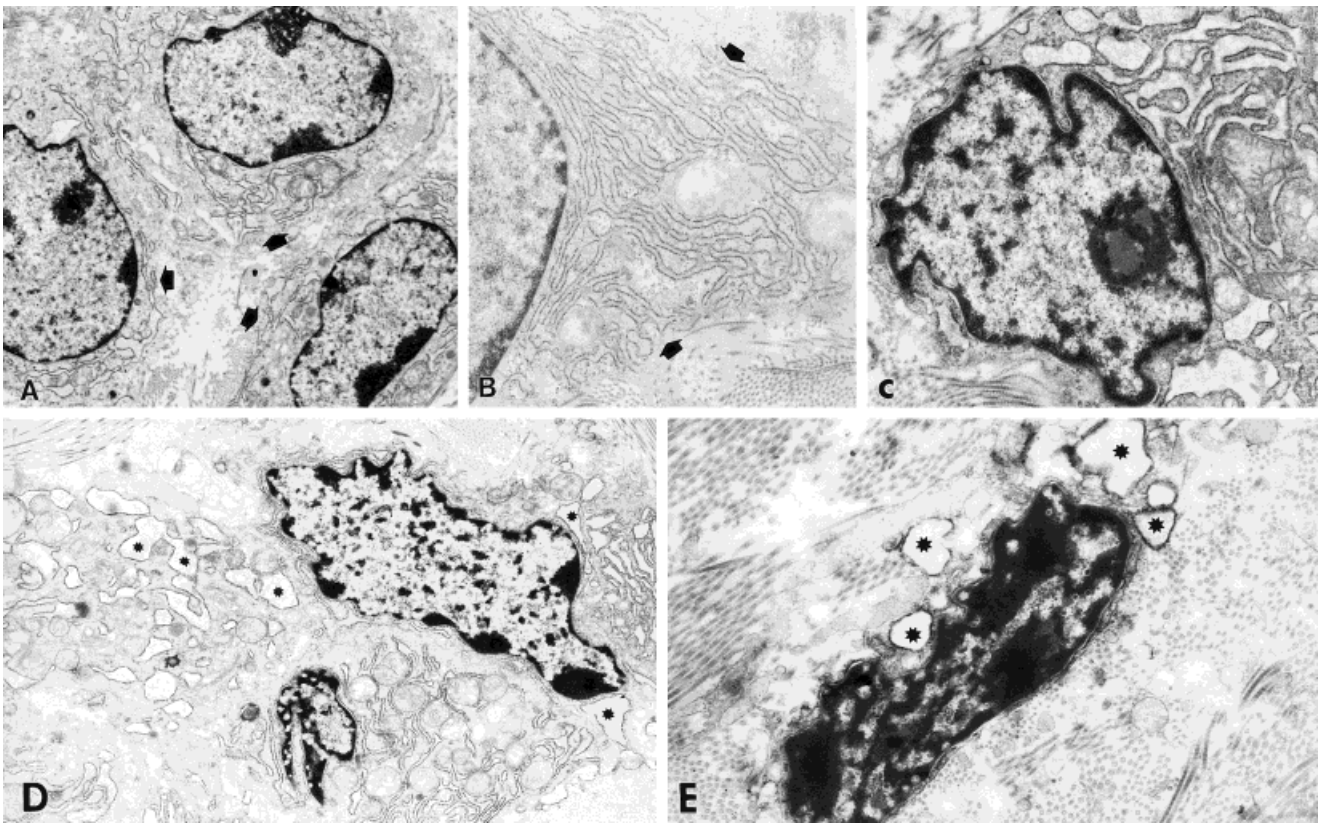


Fig. 7. Antlerogenic cells from Group V. **A**: A group of apical antlerogenic cells (AACs) in which most parts of the cell membrane surrounding these cells were ill-defined (arrows). ×5,200. **B**: A part of an AAC with the cytoplasmic organelles directly exposed to the intercellular matrix (arrows) where, otherwise, a limiting membrane should be located. ×13,907. **C**: A peripheral antlerogenic cell (PAC) from peripheral antlerogenic periosteum I. Note the irregular shaped nucleus. ×14,784. **D**: Two PACs from peripheral antlerogenic periosteum II were in early stage of degeneration. Note the irregularity of the nuclei with condensed chromatin, and abnormally-dilated RER (asterisks). ×8,778. **E**: A marginal antlerogenic cell undergoing degeneration, with condensed chromatin and very abnormally-dilated RER (asterisks). ×35,728.

and extruded RER at the same time, probably to provide as much procollagen as possible to meet the demands from fast forming tissue. In this way, a local deficiency of cell membrane may result. As antler is a very fast growing tissue and rich in collagen, it is reasonable to postulate that the local membrane deficiency of AACs is a result of the extrusion of procollagen directly into the extracellular environment to meet the high demands for collagens during fast antler growing period.

The antlerogenic cells from all pedicle and antler developmental stages were rich in glycogen, which makes them more like embryonic cells or the osteogenic cells from osteogenesis imperfecta tissue (Doty and Mathews, 1971). However, glycogen abundance varies between the different types of antlerogenic cells and between the same cell types at different developmental stages. Scott and Glimcher (1971) reported that intracellular glycogen is mainly used as an energy source as it is closely associated with mitochondria or involved in lipid droplet formation. The glycogen molecule is in a constant dynamic state so that there is rapid synthesis and degradation of glycogen even when the total amount of glycogen is constant in the cell. When endogenous glucose is needed, the level of glycogen in the cell falls very rapidly. When glucose is replaced the glycogen stores return to their original level. In addition to its use as an energy source, it is reported (Cabrini, 1961) that glycogen or its breakdown products also serve as a source for the intracellular synthesis of mucosubstances in the fetal osteoblast. Bonucci (1965) considered that the acquisition of a high rate of synthesis by bone cells seems to be connected with glycogen and lipid depletion, and glycogen may be involved in the accumulation of lipids. He concluded that glycogen and lipids may both breakdown in osteoblast cytoplasm during the synthesis of ossein, perhaps to satisfy the energy requirements of the cells involved in the secreting metabolism. On the basis of these conclusions and suggestions, the variation in glycogen abundance in these antlerogenic cells might be explained as follows. As antlerogenic cells become more and more actively involved in synthesis, so glycogen is gradually depleted for energy and cellular synthetics. The glycogen abundance in AACs at the endochondral stage did not return to the level of AACs at the transitional stage, probably because the metabolic rate of AACs at later stages is higher.

A striking feature of the AACs of the pedicle and antler is the presence of periodic collagen fibers in the cytoplasm. These fibers, although varying in number, are always found within membrane-limited vacuolar structures. It is reported that intracellular periodic collagen fibers do not

TABLE 3. Comparison of Antlerogenic Cells From Different Developmental Stages or Different Sites[a]

Group	IAC/AAC	MAC	FOC	PAC I	PAC II
I					
Male	High nucleus:cytoplasm ratio, rich in glycogen	Similar to IAC, but smaller amount of glycogen	Elongated spindle shape and absence of extracellular fibers	[c]	[c]
Female	RER abnormally-dilated	[b]	With some extracellular fibers	[c]	[c]
II					
Male	With intracellular periodic collagen fibres	RER abnormally-dilated, and nuclei were bizarre in shape	Fewer organelles and more heterochromatin than male FOC of I	[c]	[c]
Castrated male	RER were greatly abnormally-dilated	[b]	[b]	[c]	[c]
III	Nuclei were round or oval in shape with 1–4 prominent nucleoli, heterochromitin was rare. Mitotic figures were observed	RER more abnormally-dilated and heterochromatin more electron-dense than MACs of II	[b]	[c]	[c]
IV	Similar to the AACs of III, but Golgi apparati were well developed	Highly abnormally-dilated RER and indented nucleus	[b]	Moderately developed RER and Golgi aparatus	[c]
V	Similar as the AACs of IV, but some of the AACs had ill-defined boundaries	Degenerating MACs were frequently encountered	[b]	Mitotic figures were encountered	Irregular shaped nucleus and abnormally-dilated RER

[a]IAC/AAC: Initial antlerogenic cells/apical antlerogenic cells; MAC: Marginal antlerogenic cells; FOC: Facial osteogenic cells; PAC I or II: Peripheral antlerogenic cells I or II.
[b]Absent.
[c]Not relevant.

exist in the fibroblasts or osteogenic cells of normal tissue (Ross, 1968). Therefore, these fibers may seem to be intracellular, but are in fact extracellular. They may have occupied indentations of the cell surface, and the limiting membrane of a vacuole containing collagen fibers may be an invaginated portion of the plasmalemma. However, our findings do not seem to support this notion, as the intracellular fibers are only found in the AACs, but not in the IACs, PACs, or MACs. The former cells are actively involved in pedicle and antler formation, and have regular oval or round shapes with a smooth cell membrane, particularly the AACs in pedicle endochondral or antler endochondral stages. The latter cells are quiescent, and have irregular shapes with numerous indentations. It has been reported that intracellular periodic collagen fibers do exist in neoplastic fibroblasts (Welsh, 1966; Henderson et al., 1986) and in the osteogenic cells of the fracture callus (Gothlin and Ericsson, 1970; Chai and Tang, 1979), although in much less extent compared with the case of the pedicles and antlers. Gothlin and Ericsson (1970) found that although the fiber-containing bodies varied greatly in size and shape, the bordering membranes were always smooth. However, Chai and Tang (1979) reported that intracellular collagen fibers were found in the RER. Our results are similar to those of Gothlin and Ericsson. Opinions differ in regard to the pathway through which the newly synthesised molecules in the collagen-producing cells are extruded to the extracellular environment, but the majority support the RER-Golgi apparatus pathway (Scherft and Groot, 1990). The present finding that the collagen fiber containing vacuoles is closely associated with Golgi apparatus are compatible with this view. It is known that the formation of collagen fibers involves two steps: an intracellular step which includes synthesis and extrusion of procollagen helix, and an extracellular step of assembling procollagen into collagen fibers. Gothlin and Ericsson (1970) suggested that the occurrence of the fiber-containing elements represents a deviation in the normal maturation and structural reorganization process of collagen, perhaps dependent upon the demands for rapid and extensive collagen production in the callus. In the case of antler formation the presence of intracellular mature collagen fibers coincides with the local membrane deficiency of the AACs. Therefore these phenomena may both reflect the unusually high demand for collagen during the period of fast antler growth.

Nucleus and RER of the antlerogenic cells are the two organelles which undergo most notable changes in appearance at successive developmental stages in the course of pedicle and antler formation. The present studies clearly show that as pedicle development proceeds, in the progeny of IACs and AACs the ratio of euchromatin to heterochromatin increases, the characteristic structure of the nucleolus becomes clearer, and both RER and free ribosomes in

the cytoplasm are getting more predominant. It is generally accepted that the more euchromatin the nucleus contains, the more active the cell is, and vice versa (Bubel, 1989). The nucleolus is especially prominent in rapidly growing embryonic cells and has been noted that the functional activity of the nucleolus and its characteristic structure are interdependent (Fawcett, 1966). In addition, the nucleolus is considered a ribosome-producing machine (Alberts et al., 1983). Ribosomes, either free or as part of RER, are active participants in the synthetic activities of the cell. Bound ribosomes synthesize proteins mainly for secretion. In contrast, free ribosomes, which are abundant in rapidly dividing cells, are active in the synthesis of proteins for use within the cell (Flickinger et al., 1979). Therefore, the progeny of IACs and AACs might be becoming increasingly active in both division and synthesis, which in turn would result in rapid pedicle and antler formation.

In the present study, an extremely dilated RER with an electron-lucent lumen was frequently encountered in the antlerogenic cells, particularly in the IACs from castrated male and female deer, and in the MACs from intact stag calves with later growth stage pedicles or early antlers. As the density of the contents in the RER lumen should be higher than that of the cytoplasmic matrix, these RERs have been named abnormally-dilated RER. A similar phenomenon was also found in follicular carcinoma of thyroid and juxtaglomerular cell tumors (Henderson et al., 1986). Because the lumen of abnormally-dilated RER in the pedicle or antler tissue either appears empty or the density is lower than that of the cytoplasm matrix, the extreme dilation of the RER may not be attributed to the accumulation of cell products.

A possible explanation for the formation of these abnormally-dilated RERs in antlerogenic cells from the castrated male or intact female deer is as follows. Antlerogenic cells are androgen hormone target cells, and the formation of abnormally-dilated RER may result from the low levels of androgen hormones. Helminen and Ericsson (1971) reported that the ultrastructural reaction of prostatic epithelial cells (androgen hormone target cells) to castration is disappearance of the long cisternae of RER and then replacement by small "empty" vesicular or short tubular elements. They thought that the autophagy does not hit the cytoplasm "blindly," but rather is discriminative with regard to the RER. The underlying cause might be the cessation of hormone action on the protein synthesizing apparatus of the cytoplasm, i.e. ribosomes and the RER.

The abnormally-dilated RER was also found in most of MACs from stag calves with later stage pedicles or early antlers. These MACs are inactive in both division and synthesis. The abnormally-dilated RER may be the structural basis for the observation that MACs are synthetically inactive or even degenerative cells. What factor causes RER in MACs to become abnormally-dilated is not known.

It is of interest that mitotic figures were rarely seen in the AACs in this examination, as late growth stage pedicles and antlers are fast growing tissues and the growth center in these tissues is located in the cellular (mesenchymal) layer of the apical periosteum/perichondrium (Banks and Newbrey, 1982; Li and Suttie, 1994a). The results from an experiment of localization of mitotically active cells in pedicle and antler using the BrdU (5'-bromo-2'-deoxyuridine) labeling technique in vivo (Li, 1997) reveal that dividing cells are mainly located within the junction region between the inner part of the cellular layer of the apical antlerogenic perichondrium and subcellular layer tissue, with few dividing cells in the outer part of the cellular layer. Li (1997) hypothesized that these mitotically quiescent cells in the outer part of the cellular layer may serve as a stem cell reservoir to continuously supply committed progenitor cells which in turn will divide and differentiate either into osteoblasts or chondroblasts to form pedicle or antler tissue. Therefore, the failure to find mitotic figures in the apical perichondrium from these late growth stage pedicles and antlers may be solely due to the sampling procedure used in the study, as only the outer part of the cellular layer was examined in order to study the ultrastructure of the antler stem cells.

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