Scanning Microscopy

Volume 4 | Number 3 Article 19

8-2-1990

Tumor-Like Growth of Antlers in Castrated Fallow Deer: An Electron Microscopic Study

Richard J. Goss
Brown University

Scanning Microscopy, Vol. 4, No. 3, 1990 (Pages 715-721)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/90$3.00+.00

TUMOR-LIKE GROWTH OF ANTLERS IN CASTRATED FALLOW DEER: AN ELECTRON MICROSCOPIC STUDY

Richard J. Goss

Division of Biology and Medicine, Brown University
Providence, Rhode Island 02912
Phone No. (401) 863-2130

(Received for publication March 4, 1990, and in revised form August 2, 1990)

Abstract

Male deer regenerate new sets of antlers each year. When fully grown, rising levels of testosterone promote antler ossification, cutting off the blood flow and causing the velvet integument to be shed. After the mating season, the old antlers fall off to be replaced by new ones.

When the adult fallow deer is castrated in autumn or winter, its bony antlers are shed and replaced by usually shorter regenerates that remain permanently viable and in velvet. If prevented from winter freezing, these antlers continue to grow thicker each year, eventually giving rise to amorphous outgrowths, or antleromas, from their sides. These growths mushroom out from the antler as clusters of nodules, developing in unpredictable locations, but commonly at the bases and ends of the antlers. Their integument contains numerous hair follicles. Internally, antleromas are composed of masses of collagen together with fibroblasts actively engaged in ribonucleic acid and protein synthesis. Thin basal laminae surround the blood vessels, and in the skin separate the overlying epidermis from the collagenous substance of the antleroma.

Despite their superficial resemblance to hypertrophic scars, antleromas lack many of the characters by which they are diagnosed. They may be classified as benign tumors, at least in the generic sense. Antleromas would appear to represent a sustained expression of antler regeneration uncoupled from those morphogenetic influences responsible for the configurations into which deer antlers normally develop.

KEY WORDS: antler, antleroma, velvet skin, tumor, collagen, fibroblast, hypertrophic scar, deer, castration, electron microscopy.

Introduction

Deer can be induced to grow some rather bizarre protuberances from the shafts of their antlers. These amorphous outgrowths superficially resemble cancers or hypertrophic scars, but their true nature remains to be understood. In the fallow deer, they may range from small nodules a millimeter or two in diameter to large tumors the size of one's fist (Fig. 1). They may mushroom out around the base of the antler, or hang in festoons from the ends. In the roe deer they typically expand out over the head like a wig, in which case they are referred to as "perukes" (Blauel, 1935; Bubenik, 1963; Bubenik and Weber-Schilling, 1986; Olt, 1927; Rorig, 1907; Wahlin and Alm, 1989). Alternatively, they have been called cactus antlers in the mule deer (Mearns, 1907), or antleromas in the fallow deer (Goss, 1983).

Whatever their ultimate configuration, such outgrowths are produced only in castrated deer. Normally, a deer's antlers are cast off and replaced every year (Brown, 1983; Bubenik, 1966; Fennessy and Drew, 1985; Goss, 1983). During the growing phase, antlers are said to be "in velvet" because of the fuzzy pelage that adorns these developing appendages. They sprout from pedicles after the previous set of dead bony antlers has detached from the head (usually in late winter or spring). The growing appendages elongate rapidly, giving rise to magnificent branched antlers in only a few months. As the days become shorter toward the end of summer, rising levels of testosterone promote the solid ossification of the fully grown antler, cutting off its blood supply and causing the enveloping integument to peel off. Once the velvet has been shed, the deer are ready for rut, equipped with hard, bony headpieces that are used to good effect in intimidating rival males in combat or by display.

If such a deer is castrated while in hard antler, the abrupt decline in serum testosterone titer leads to the osteoclastic erosion of bone at the base of the antler. As a result, the antlers fall off in a few weeks and new ones regenerate, sometimes even at an atypical time of the year. The antlers of castrated males are not prevented from growing by the absence of testosterone, but they are prevented from maturing. Their bone remains cancellous and the velvet skin is not shed. Such antlers remain permanently viable and in velvet. In subsequent years, they are stimulated to grow without having lost the previous year's antlers. Instead of continuing to grow in length and in morphologic complexity as is the case with normal antlers, they tend to grow laterally by thickening their shafts. If prevented from freezing in the winter, the "castrate antlers" of the fallow deer may develop nubbly surfaces, eventually giving rise to enlarged amorphous outgrowths in unpredictable locations.

In the present investigation, the histologic and ultrastructural nature of antleromas has been examined. The purpose has been to determine what cells and tissues are present in these outgrowths and in what morphological relationships to each other. It is hoped that such information might shed light on the basic nature of these tumorlike outgrowths. Ultimately, one would like to determine their histogenesis, if not the physiological conditions that promote their development in the first place.

Materials and Methods

This study has been conducted on five male fallow deer (*Dama dama*). Animals were immobilized with succinyl choline (Anectine: Burroughs-Wellcome) administered intramuscularly by darts propelled from a gun. When necessary, xylocaine was injected locally prior to surgical interventions. Castration was achieved by "elastration", i.e., by placing a rubber band around the neck of the scrotum. The latter, together with the testes, shrivelled up and dropped off in due course. The bony antlers were shed within a few weeks and new permanent ones then regenerated.

The deer were held indoors during the four coldest months of the year (from late November to late March) to prevent frostbite and subsequent necrosis of the living antlers. Otherwise, they were kept outdoors under natural conditions. At least twice a year they were immobilized in order to photograph their antlers at close range, and to examine them for signs of incipient antleroma development. After two or more years, tumorlike outgrowths eventually developed in all deer, growing larger each year. It was from these nodules that biopsies were recovered for electron microscopic study. This investigation is based on specimens derived from three different nodules grown on two of the deer. Although ultrastructural observations were consistent among these limited samples, the possibility of significant changes with age and size of antleromas cannot be excluded.

Specimens were diced to about 1 mm dimensions and fixed in Karnovsky's solution. They were then postfixed in OsO_4 in Na cacodylate buffer, dehydrated in a series of ethanols and embedded in SPURR embedding media. They were thin sectioned on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and viewed in a Philips 410 transmission electron microscope.

For scanning electron microscopy (SEM), specimens were also fixed as above. They were then coated using the osmium-thiocarbohydrazide-osmium method, dehydrated in 2,2-dimethoxypropane, and dried in a LADD critical point dryer. After coating with gold-palladium in a S.P.I. sputter coater, they were photographed in an AMRay 1000 scanning electron microscope.

Results

Antleromas developed at the earliest during the second year after castration, but in some cases new ones sprouted in the third or fourth year. They appeared in unpredictable locations, but in general tended to develop around the base of the antler or near the distal ends. They frequently grew in clusters. Occasional nodules became pendulous on elongating stalks of skin (Fig. 1). In some instances, antleromas were rounded outgrowths with a smooth surface. In others, they became convoluted when deep clefts were created by protrusions of adjacent foci of growth. These clefts were lined with skin and became filled with sebum, keratinized cells, and hairs. The consistency of the antleromas was firm but rubbery. Neither bone nor cartilage was encountered.

Internally, the outgrowths consisted of copious quantities of collagen (Fig. 2). Bundles of fibers can be seen to criss-cross throughout the substance, interspersed with widely scattered fibroblasts (Fig. 3). These cells typically contain conspicuous nucleoli and an extensive endoplasmic reticulum (ER). Dilated cisternae of the ER are evident, presumably indicative of active collagen synthesis.

Antleromas appear to be well vascularized. They bleed profusely when

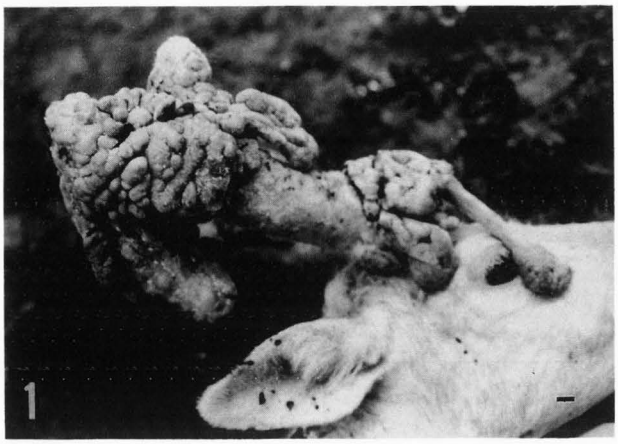

Fig. 1: Male fallow deer 4 years after castration. Massive antleromas have developed around the base and on the end of the antler, including a pendulous nodule growing down over the right eye. Bar = 1 cm.

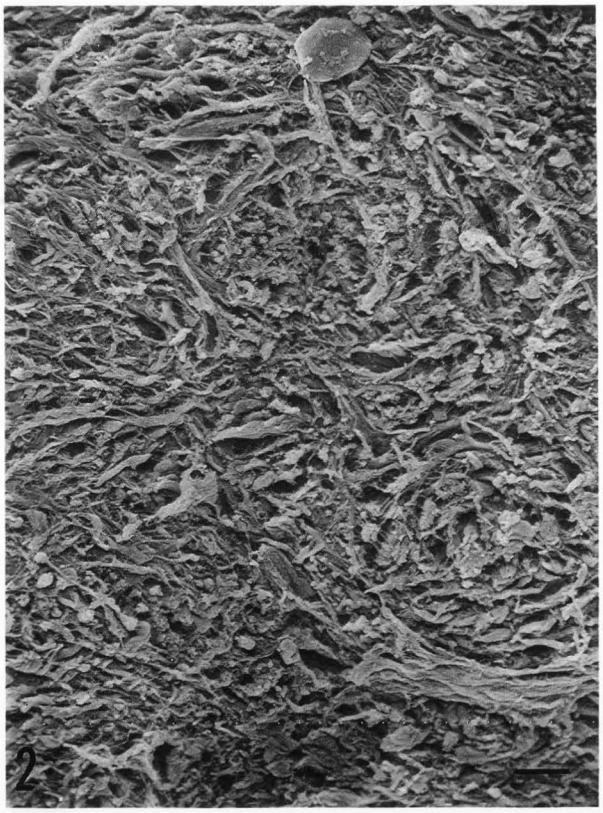

Fig. 2: SEM of central region of an antleroma, showing the nearly homogeneous configuration of the quantities of collagen that make up the nodule. Bar = 10 μm.

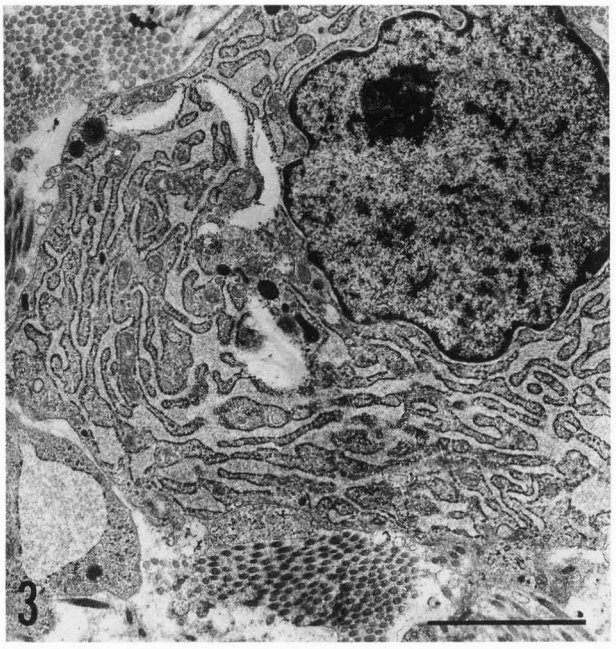

Fig. 3: Fibroblasts are embedded among bundles of collagen. Note distended cisternae of the ER, suggestive of synthetic activity. Bar = 1μm.

Fig. 4: Cross section through a small blood vessel in an antleroma. A pericyte is seen in the upper right. Bar = 1μm.

incised, and blood vessels are readily found in sections (Fig. 4). The small vessels appear normal histologically, being lined with endothelium and partially surrounded by occasional pericytes. Their lumens are patent. Externally, a very thin basal lamina separates the vessels from the surrounding collagen. Small vessels are devoid of tunica media and adventitia.

At the epidermal interface there exists only a thin basal lamina, albeit one that is clearly delineated (Fig. 5). Well defined papillary dermis and reticularis are not discernible. Collagen fibers are present immediately beneath the basal lamina, but they lack the organized structure typical of dermis. Instead, they are continuous with those at deeper levels. Otherwise, the subepidermal region is distinguished only by the sparseness of its collagen and the prevalence of ground substance and open spaces.

The epidermis resembles that of the growing tip of a normal antler. There are numerous hair follicles in various stages of development (Fig. 6), each one accompanied by a sebaceous gland.

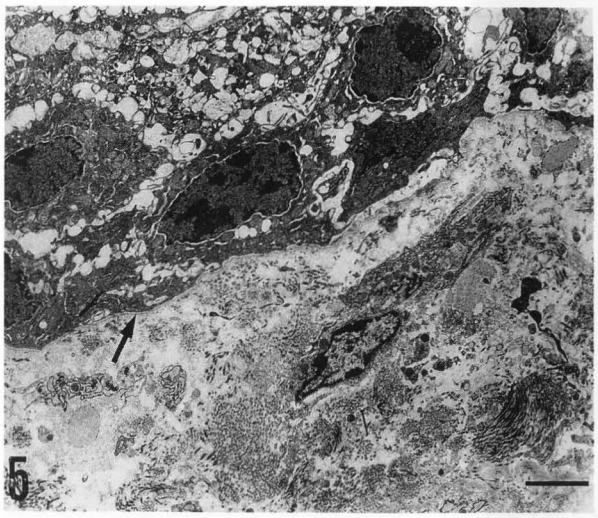

Fig. 5: Section perpendicular to the surface of the skin on an antleroma, showing basal layers of epidermis (above), basal lamina (➡), and underlying collagen with associated fibroblasts (below). Bar = 1 μm.

Discussion

It has been the objective of this research to explore the nature of antleromas at the electron microscopic level. They have been found to consist almost entirely of collagen, interspersed with fibroblasts and blood vessels, and enveloped in epidermis with velvet pelage. Neither cartilage nor bone has been observed in them.

The very occurrence of these zoological curiosities raises questions about their relation to normal antlers, their histogenesis, the physiological stimuli responsible for their initiation, and whether or not they represent examples of regeneration, scar formation, or cancer.

Antleromas are clearly antler tissue, but without the normal morphological organization. The absence of skeletal tissues in the antleromas of fallow deer raises the possibility that this could be responsible for their amorphous configuration. Yet the occurrence of spongy bone in the perukes of roe deer as reported by Olt (1927) may be taken to indicate that the lack of organization may be independent of skeletal tissues.

The histogenesis of these structures remains to be determined. There would appear to be three possible candidates: the dermis of the antler velvet; subdermal connective tissue; or periosteum. Although the dermis may be the most likely source of antleromas

Fig. 6: SEM of antleroma epidermis. The forest of hairs making up the velvet consists of many mature outgrowths as well as scattered younger ones recently differentiated from the epidermis. Bar = 0.1mm.

owing to the prevalence of collagen in both, one cannot rule out the periosteum, especially in the perukes of roe deer in which ossification has been described.

Antleromas are different from epimorphic regeneration whereby amputated appendages are replaced by new outgrowths. They are not replacements for missing parts nor do they appear to originate in association with wound epidermis the way appendage regenerates always do. Above all, they lack the morphogenetic organization of regenerating structures. Indeed, it is this autonomy from morphogenetic constraints that could be responsible for their constant growth. In this sense, antleromas bear a striking resemblance to other tumors of a benign nature. Whether or not they are cancerous is a matter of definition. They are not malignant in the invasive or metastatic sense, but they are examples of unrestricted growth.

Antleromas superficially resemble hypertrophic scars (Kischer, 1984; Kischer et al., 1982a). The amorphous configurations and collagenous compositions of both outgrowths suggests that they have much in common. There are many differences, however. Hair follicles, for example, continue to multiply as the integument expands. Scars are not known to develop hair follicles de novo. Antleromas mushroom out into nodular structures, but their constituent collagen is not organized with the internal nodular structures characteristic of hypertrophic scars. Occluded microvessels (Kischer et al., 1982b) are commonly encountered in hypertrophic scars, but are not seen in antleromas. The small blood vessels in antleromas are normal-looking with wide open lumens lined with a simple squamous endothelium (Fig. 4). Signs of cellular degeneration and tissue necrosis have not been encountered in antleromas. Nor are they known to arise from wounds the way scars are. In view of these differences, it must be concluded that antleromas and hypertrophic scars are probably not exactly the same kinds of tissues.

The physiological antecedents that stimulate antleroma development are not known. They seem not to be associated with injuries to the velvet, nor can their sites of future development be predicted. Except for a tendency to be produced basally or apically on antlers, and their frequent occurrence in clusters, their distribution is inconveniently random. Whether or not their initiation might be associated with patterns of vascularization or innervation has not been explored. Even their association with reduced levels of male sex hormones could be derivative of a more direct cause, namely, the prolonged survival of antler tissue itself in the castrated deer. Conceivably, antleromas might develop in intact deer if their antlers remained viable long enough. The fact that antlers normally live only a few months or so before dying would mask any innate propensity for tumor production. Whether antleromas are the result of castration per se or of the prolongevity of antler tissues, especially the velvet skin, are alternatives that deserve to be tested.

References

Blauel G (1935). Beobachtungen uber die Enstehung der Perücke beim Rehbock (Observations on the formation of the peruke in the roebuck). Endokrinolgie 15, 321-329.

Brown RD (ed.) (1983). Antler Development in Cervidae. Caesar Kelberg Wildlife Research Institute, Kingsville, TX.

Bubenik AB (1963). Rund um die Peruckengeweihe. (About the peruke antler) St. Hubertus 6, 81-85.

Bubenik AB (1966). Das Geweih (The Antler). Paul Parey, Hamburg.

Bubenik AB, Weber-Schilling C (1986). Perücken der Geweihträger und das Phänomenon des Abwerferns oberhalb der Rose (Perukes of antler bearers and the phenomenon of shedding above the base). Z. Jagdwiss 32, 158-171.

Fennessy PF, Drew KR (eds.) (1985). Biology of Deer Production. Roy Soc NZ, Wellington. Bull 22.

Goss RJ (1983). Deer Antlers: Regeneration, Function, and Evolution. Academic Press, New York.

Kischer CW (1984). Comparative ultrastructure of hypertrophic scars and keloids. Scanning Microsc 1, 423-431.

Kischer CW, Shetlar MR, Chvapil M (1982a). Hypertrophic scars and keloids: a review and new concept concerning their origin. Scanning Electron Microsc. 1982; IV: 1600-1713.

Kischer CW, Thies AC, Chvapil M (1982b). Perivascular myofibroblasts and microvascular occlusion in hypertrophic scars and keloids. Human Path 13, 819-824.

Mearns EA (1907). Mammals of the Mexican Boundary of the United States. U.S. Nat. Museum, Bull. 56.

Olt A (1927). Die Perücke der Cerviden und das Karzinom (The peruke of cervids and cancer). Tierarztliche Wochenschr 35, 131-133.

Rorig A (1907). Gestaltende Correlationen zwischen abnormer Korperkonstitution der Cerviden und Geweihbidlung derselben. (General correlations between abnormal body

constitution of cervids and their antler development). Roux Arch Entw-Mech 23, 1-150.

Wahlin T, Alm L (1989). So wächst ein Perückengehörn (How the peruke horn grows). Die Pirsch 41 (13), 43-45.

Discussion with Reviewers

C.W. Kischer: Do you know whether or not these nodules grow, after castration, in the absence of an invasive incident? Could it be possible that the nodules developed after contusions, lacerations or a similar type of occurrence, which might have been overlooked? If not, what mechanism do you believe might be invoked to account for the excessive collagen synthesis?
Author: It cannot categorically be ruled out that these nodules might be initiated by "invasive incidents", but careful inspection of close-up photographs where nodules have later originated has revealed no evidence to this effect. Nor have nodules developed from integumental wounds intentionally inflicted to test such a hypothesis. This question was a major incentive for undertaking this research in the first place, but I am still at a loss to explain the proximate etiology of antleromas.

J.M. Suttie: Can you experimentally induce antleromas in deer of other species or in fallow deer?
Author: Unfortunately, I have thus far been unsuccessful in inducing antleromas by experimental interventions other than castration.

J.M. Suttie: Antleromas, although they have been reported in two of the major sub-families of Cervidae, are not known in all species. In particular they are unknown in red deer. Why do you think this is so?
Author: I have observed castrated sika deer (a close relative of the red deer) over many years, but have never seen outgrowths such as those produced by fallow or roe deer. Their antlers become thickened, and may exhibit pearlation, but do not give rise to tumorlike nodules. As for other species, very little is known. Clearly, we need a comparative survey of how different species of deer react to castration.

C.W. Kischer: Do you see any evidence by electron microscopy of degenerating microvessels or degenerating fibroblasts? Would such an observation vary with age of the nodules, post-castration?
Author: I have seen no evidence of degenerating microvessels or fibroblasts. Inasmuch as biopsy specimens were taken from nodules less than two years old, it is possible that subsequent studies on older ones might show such changes.

C.W. Kischer: What is the ultimate fate of these nodules?
Author: Time will tell. Thus far, they simply continue to grow. In the roe deer, perukes have been reported to have become necrotic in places, rendering them vulnerable to infections, and even invasion by maggots.

R.D. Brown: How do "antleromas" of castrate deer differ morphologically from a) velvet growing antlers of intact deer, and b) injuries to antlers of intact deer?
Author: Antleromas differ primarily from normal velvet antlers in that they lack the organized morphogenesis of the latter. Like the tips of growing antlers, they consist of fibroblasts and collagen with an abundant blood supply. They also give rise to hair follicles *de novo*. Otherwise, they fail to undergo chondrogenesis or endochondral osteogenesis. Injuries to velvet antlers of intact deer tend to heal normally, although the ultimate morphology of such antlers may be adversely affected. Mechanical trauma to the growing antlers of either castrated or intact deer are not known to produce outgrowths resembling antleromas.

J.M. Suttie: Why are the outgrowths not organized into typical antler patterns of tines and, in the case of the fallow deer, palmation?
Author: One wonders if there might not be a causal relationship between the absence of morphogenesis and the unrestricted growth of antleromas. Do antleromas develop because they lack the constraints of morphogenetic regulation? Do normal antlers not become tumorous because they are morphologically inhibited from doing so? It is worth recalling the case of a castrated white-tailed deer protected from freezing for 9 winters in a row [Wislocki GB, Aub JC, Waldo CM (1947). The effects of gonadectomy and the administration of testosterone propionate on the growth of antlers in male and female deer. Endocrinology 40, 202-224]. Instead of growing amorphous masses of tissue from its antlers, it sprouted numerous branches, eventually giving rise to a large cluster of antler points. Yet in other white-tailed deer, masses of nodules may be produced. Why this species reacts to castration in these two different ways we do not know.

B. Forslind: Do you know specifically

what androgens are missing in the castrated deer? Have you tried stopping "outgrowths" in the castrated animals by administering androgens?
Author: The specific androgens that are missing in the castrated fallow deer have not been identified. However, exogenous administration of testosterone promotes solid ossification of the antlers followed by ischemic necrosis of the velvet, including the antleromas.

B. Forslind: Do female fallow deer carry antlers? If so what happens to their antlers if the animals are subject to higher-than-normal doses of estrogens?
Author: Females of this species do not grow antlers. If they did, I suspect estrogen would cause them to ossify and to shed their velvet. In the male sika deer, if estrogen is administered while in velvet the antlers undergo a precocious maturation, just as they do following treatment with testosterone [Goss RJ (1968). Inhibition of growth and shedding of antlers by sex hormones. Nature 220 (5162), 83-85.]

R.D. Brown: How do "antleromas" of castrate deer differ morphologically from velvet antlers of female caribou (Rangifer) (the only other cervids which grow antlers in the absence of testosterone)?
Author: Caribou and reindeer cows do grow antlers in the absence of testosterone, but their antlers appear to be normal structures like those of the bulls except that they tend to be smaller. Whether or not they would develop antleromas if protected from their annual demise (induced either hormonally or by freezing) is an interesting problem still awaiting investigation.

R.D. Brown: How do "antleromas" of castrate deer differ morphologically from human osteosarcomas?
Author: Osteosarcomas are mesenchymal cell tumors typically undergoing direct osteoid or bone formation, and sometimes chondrogenesis. They are hard, invasive neoplasms. Although they develop in association with bone, as do osteosarcomas, antleromas in fallow deer exhibit none of the above skeletal differentiations. They are not invasive, and form only on the surface of the body beneath the epidermis.

J.M. Suttie: "Antleroma" suggests a similarity with cancer or a tumor. Could the author expand on this similarity?
Author: The suffix "-oma" means tumor, not necessarily cancer per se. The similarity with other tumors derives from the amorphous shapes of antleromas and their tendency for unlimited growth. They exhibit a morphological autonomy that may reflect an underlying physiological independence not unlike comparable attributes in some cancers.

B. Forslind: Please provide more information on the tonicity of your fixative, buffers, etc. The TEM micrograph gives the impression of a tissue subject to hypertonic buffer, e.g., Fig. 4.
Author: Karnovsky's fixative (Karnovsky, MJ, A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy, J. Cell Biol. 27, 137a-138a, 1965) has been around for many years, and its basic formula is well-known to be hypertonic (around 1100 mosmols). I do not think that Fig. 4 shows the effects of hypertonic fixation. Close observation of the organelles in the photo shows no effects of hypertonicity. The rapidity of aldehyde fixation obviates effects from the hypertonicity of Karnovsky's fixative.