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Antler Regeneration: Studies with Antler Removal, Axial Tomography, and Angiography

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Introduction

This paper includes three rather diverse approaches to the study of antler regeneration in red and fallow deer. Each study, however, shares the same goal of attempting to improve our understanding of the process of antler regeneration. All studies took place in the Southern Hemisphere, in New Zealand; this accounts for the reversed seasons.

Serial Velvet Antler Removal

If red deer stags are castrated when the antlers are hard, the antlers are cast some 10–21 days later. The antlers regrow immediately but remain in velvet. If the stag is castrated when the antlers are in velvet, then they remain so and are never cleaned or cast (Fennessy & Suttie 1985). However, if the velvet antler of the castrated stag is surgically removed, further regrowth is possible. (Suttie & Fennessy, personal observations). The aim of the present study was to remove the velvet antlers at intervals from three castrated red deer and then to measure further antler regrowth. A secondary aim was to investigate the effects of pedicle trauma and testosterone priming in restoring the growth potential of the antler.

Materials and Methods

Three young red deer (*Cervus elaphus*) stags, who had grown and cleaned their first spike antlers normally were kept indoors in pens and fed a concentrate diet to appetite. Each stag was surgically castrated under general anesthesia (Fig. 1) in either June (#3), September (#31), or December (#41) 1982.

Nine months after castration, the right velvet antler was removed under Xylazine (Rompun, Bayer Ltd) anesthesia (as was the case for all velvet removal) 2 cm above the antler/pedicle junction (APJ), and the left antler was unmanipulated. This was repeated at 3 monthly intervals (Fig. 1) on three (#41), four (#31), or five (#3) occasions. In June 1984 the left antler was removed 2 cm above the APJ,

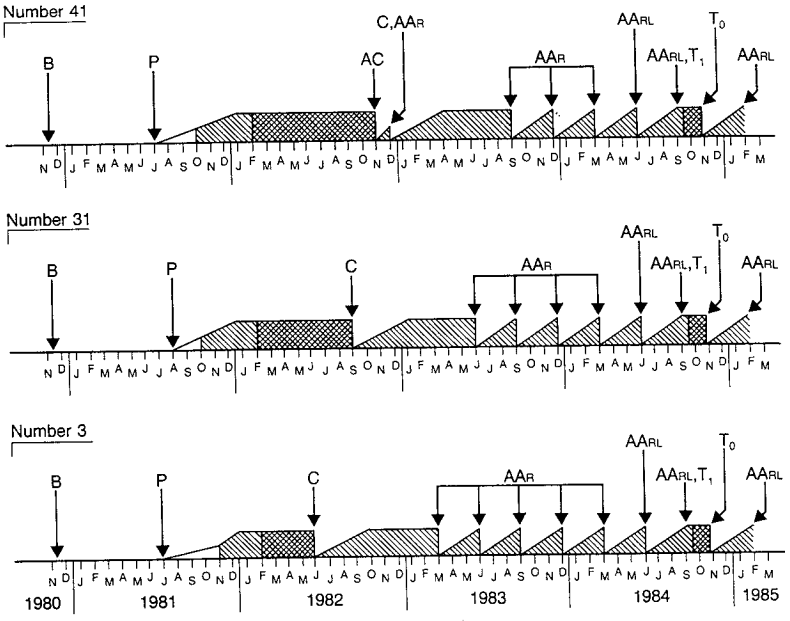


FIGURE 1. The life histories of the three stags used in this study. ▭ pedicle growth; ▨ velvet antler; ▩ hard antler. Abbreviations: B = birth, P = pedicle growth begins, AC = antler casting, C = castration, AA_R = right antler removed, AA_L = left antler removed, AA_{RL} = both antlers removed, T_1 = testosterone implant in, T_0 = testosterone implant out, followed 3 weeks later by antler casting.

and the right antler (previously serially velveted) was removed 2 cm below the APJ from each stag. In September 1984, both antlers were removed 2 cm above the APJ and a silastic testosterone implant, which previous research had shown maintained plasma testosterone levels of about 10 ng/mL and was sufficient to cause castrated stags to clean their antlers, was inserted subcutaneously. Three weeks after antler-cleaning, this implant was removed and the stags were allowed to cast their antler stubs, which had not regrown, normally. Three months after antler casting, both velvet antlers, which had regrown, were removed 2 cm above the APJ. The timing of the study is shown diagrammatically in Fig. 1.

Each velvet antler was measured with a flexible plastic tape. The portion of velvet antler removed on each occasion was weighed and the number of points was recorded.

Results

Serially Amputated (then Testosterone Treated) Right Antler

Repeated removal of the velvet antlers above the APJ at 3 month intervals led, overall, to a 42% reduction in antler growth rate, a 54% reduction in antler length, and a 72% reduction in weight (Figs. 2–4). Cutting the antler below the APJ significantly increased the degree of regeneration (antler weight and antler length) over the antlers serially amputated above the APJ (Table 1), but not to the size of the first postcastration antler. Similarly, treatment with testosterone, which stimulated cleaning, and, when the testosterone was withdrawn, casting, increased the level of regeneration in terms of both length and weight (Table 2). However, the velvet antler grown after this treatment did not exceed in size that grown immediately after castration. Both techniques were effective in stimulating regeneration. This conclusion is presented tentatively, however, as one stag seriously damaged one of his posttestosterone antlers.

The first antler grown after castration, the antlers grown after cutting below the APJ, and the antler grown after testosterone stimulation were branched; otherwise, simple spikes only were regenerated.

Testosterone Treated Left Antler

The antler grown after castration, which had remained unmanipulated for up to 2 years, was heavier than the antler removed after 9 months (739 g and 372 g, respectively, sed [standard error of the difference] = 141.3). The antler which was regenerated after the first amputation was smaller than the one grown after castration (112 g and 739 g, respectively, sed = 205 g) and had a slower growth rate (0.18 cm/day and 0.38 cm/day, respectively, sed = 0.09) (Figs. 2–4). Testosterone restored growth rate to approximately that of the first antler grown after castration (0.31 cm/day and 0.38 cm/day respectively, sed = 0.07). The testosterone-stimulated antler was significantly longer, heavier, and faster growing than the one immediately preceding it (Table 3). The antler grown after castration and the testosterone-stimulated antler were branched. The antler cut in September 1984 was a single spike.

Discussion

Repeated removal of portions of the velvet antler of castrated red deer stags leads to a reduction in growth rate. Regeneration invariably occurs but is limited in extent. This indicates that either the ability of antler cells to dedifferentiate into actively dividing cells becomes limiting or some stimulating substance is lacking. The fact that removal of the antler by cutting through the pedicle – rather than the antler itself, without any other exogenous administration of stimuli – is capable of increasing antler growth rate tends to favor a hypothesis that the potencies for regeneration of the antler and the pedicle differ.

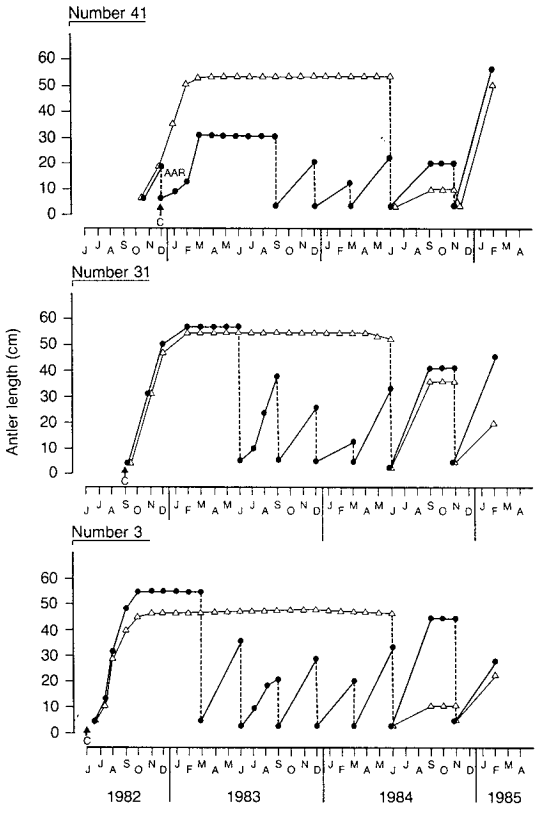


FIGURE 2. Antler length (cm) of postcastration and all subsequent antlers grown by each of the three stags. •—• serially amputated antler △—△ testosterone-treated-only antler. C indicates the time of castration. The broken lines indicate the abrupt change in antler length following amputation or antler casting.

Long-term castrated deer often show annual increases in antler size, either growing extra points or producing antleromas (Goss 1983). It is not surprising, then, that trauma to the velvet-antler caused regeneration in the present study. Lincoln (1984) carried out a similar experiment to the present study when he amputated the velvet antlers from three castrated red deer stags at irregular 4–6-month intervals for over 2 years. Regeneration was always observed but no diminution in size resulting from repeated antler removal occurred. However, the antler was removed at the top of pedicle (i.e., through the APJ) by Lincoln—not,

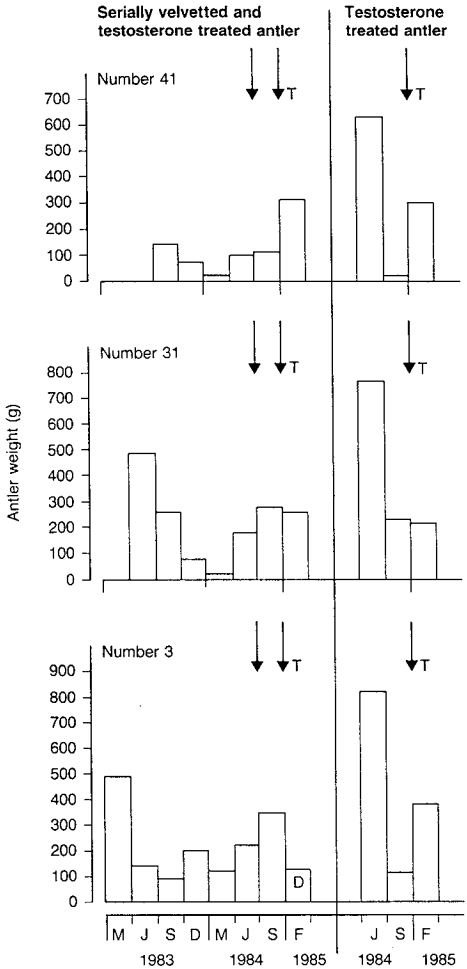


FIGURE 3. Velvet-antler weight (g) after each amputation. Weights refer to antlers removed on the 20th of each month referred to. Arrow indicates timing of antler removal below the APJ. Arrow accompanied by T indicates timing of testosterone treatment. D means antler was damaged and data were not used for the numerical analyses.

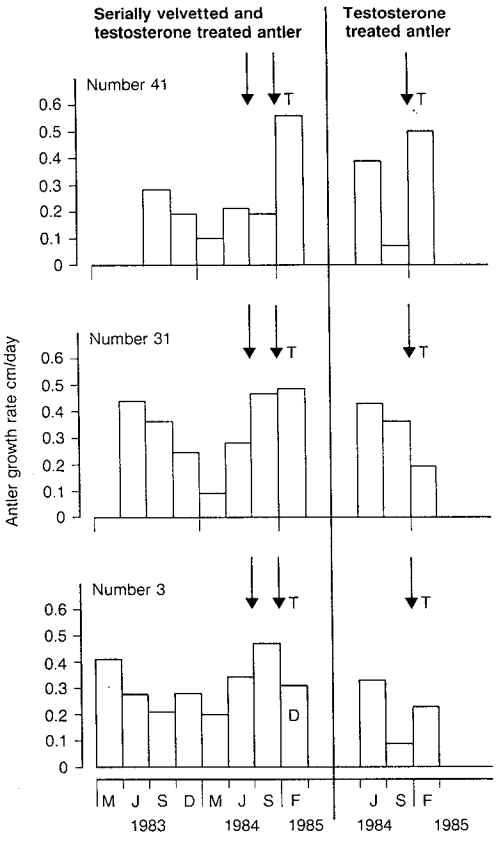


FIGURE 4. Antler growth rate (cm/day) for each stag for each antler. Remainder of legend as for Fig. 3.

as in the present study, 2 cm above or below the APJ. It seems that the regeneration capacity of the antler is restricted, compared with the pedicle, to regrowth of simple spikes or poorly organized antleromas with a low growth rate. In contrast, if the pedicle is stimulated to regenerate, growth rates are faster and branched antlers of the species-specific shape are possible. In Lincoln's 1984 study, although the antlers that regenerated did not get smaller, no branching was observed. It is considered that antler removal along the APJ is somewhat inter-

TABLE 1. Comparison of right antler growth parameters[a]

	Serially velveted antler (above APJ)		Antler after removal (2 cm below APJ)
Antler growth rate (cm/day)	0.22		0.37
sed		0.08	
Antler length (cm)	22.7		36.0
sed		6.8	
Antler weight (g)	108.3		247.7
sed		67.8	

[a] Comparison of mean of first three serially velveted antlers grown after castration with antler grown after removal below the antler/pedicle junction (APJ) (N=3). Sed = standard error of the difference.

mediate between removal above or below this line. It may well be that the antler cells are limited in their ability to dedifferentiate and grow, but the pedicle cells carry this ability.

The antler grown after testosterone stimulation had a growth rate that was either similar to that grown after cutting below the APJ or greater than the previous antler cut above the APJ. One model that was tested by this part of the study was that if pedicle fibroblasts required testosterone priming to stimulate normal antler growth in castrates, then antler growth rates of a testosterone-stimulated antler would be much higher than antlers grown without this stimulation. The antlers did indeed grow faster—but no faster than antlers grown after removal below the APJ. This seems to indicate that testosterone treatment prior to antler development brought about its stimulating effect mainly by causing trauma to the pedicle at the induced antler casting rather than a classical endocrine stimulation during growth. Casting itself would appear to be sufficient stimulus for the pedicle cells to commence regeneration.

The antler that remained intact and unmanipulated on each stag for up to 27 months after castration increased in weight but not in length over this time. The

TABLE 2. Comparison of right antler growth parameters[a]

Serially velveted antler		Antler grown after testosterone stimulation		Antler grown after cut below APJ
Antler growth rate (cm/day)	0.19	0.52		0.33
sed	0.14		0.11	
Antler length (cm)	20	51		36
sed	13.0		10.8	
Antler weight (g)	73.5	286		196
sed	67.8		127.1	

[a] Table compares serially velveted antlers, antlers grown following removal of the previous antlers below the antler/pedicle junction (APJ), and testosterone-stimulated antlers (N=2). This table is necessary, as one stag (#3) damaged his testosterone-stimulated antler and thus could not be included in the comparison.

TABLE 3. Comparison between the left antler grown before and after testosterone stimulation (N=3)

	Before testosterone treatment		After testosterone treatment
Antler growth rate (cm/day)	0.19		0.31
sed		0.09	
Antler length (cm)	19		31
sed		8.9	
Antler weight (g)	112		296
sed		76	

Sed = standard error of difference.

base of the antler became thicker, and progressive bone deposition had probably also taken place.

It is not strictly possible to compare weight or length of the antler grown after castration with subsequent antlers. They were allowed to grow for only 3 months, whereas those grown immediately after castration were allowed to grow freely; in fact, growth took about 4 months. However, growth rates do make for valid comparisons. Neither antler removal below the APJ nor testosterone stimulation consistently increased antler growth rate over that of the first antler grown after castration. Thus, about 0.5 cm/day appears to be maximal for castrated red stags, despite treatment. Similar-aged intact stags would be expected to average about 0.7 cm/day (Suttie & Fennessy, unpublished observations). Why the discrepancy? Presumably, this is not due to lack of steroid priming. Stags treated with medroxyprogesterone acetate, which effectively inhibits luteinizing hormone (LH) release, and thus testosterone, both before and during antler development, grew antlers of similar or only slightly smaller size than controls (Muir et al. 1982; Suttie & Fennessy, unpublished observations). This seems to indicate that lack of testosterone before and during antler growth may not be directly responsible. It is known, however, that in sheep testosterone is necessary for normal pulsatile release of growth hormone (GH) (Davis et al. 1977). As GH results in insulin-like growth factor 1 (IGF1) release and IGF1 has been shown to correlate positively with antler growth (Suttie et al. 1985a), it may be that these castrates have less GH or an abnormal GH release pattern. In support of this hypothesis, castrated red deer stags indeed have lower IGF1 levels than controls (Suttie, unpublished), and testosterone administration stimulates IGF1 secretion in intact red deer stags (Suttie et al. 1985b). A further possibility is that a non-LH-dependent testicular factor is responsible for maintaining antler size at a maximal rate. Although testosterone has been located immunohistologically in the velvet-antler (Bubenik, G. et al. 1974), its role in antler regeneration (as opposed to mineralization) is not clear.

The present study has underlined the importance of the pedicle in controlling the extent of regeneration. Regeneration from the velvet-antler is of limited

extent, but near normal regeneration is possible from the pedicle—probably independent of direct stimulation from testosterone.

Computerized Axial Tomography

Computerized axial tomography (CAT) is unlike conventional radiography. In CAT, screens and films are not used to detect x-rays, nor do x-rays penetrate parts of the body outside the section being examined. Instead, an x-ray tube which is aligned precisely with an electronic detector, 180° opposite to it, moves around the subject and measures x-ray transmission through the transverse section many thousands of times. This information is sent to a computer which calculates an array of numbers (called CT numbers) from which it reconstructs a picture. The picture can look superficially like a radiograph and is an image of the structure being examined in a transverse section. Sections of the body can be examined every 2 millimeters to build up anatomical pictures that would not be possible using conventional radiography. Preliminary results of a CAT scan study of fallow deer, (*Dama dama*) antlers are presented in this section.

Methods

Three fallow bucks were slaughtered in either April 1983 (hard antler), November 1983 (velvet-antler, 4 weeks after antler casting) or in January 1984 (velvet-antler, 12 weeks after antler casting). The heads were removed and, within 1 hour, were scanned using a Technicare Deltascan 2020 (Ohio Nuclear Inc.). Scans were made at 120 kV, 50 mA, with 2 mm between sections. The scanner produces images of 512 × 512 pixels, in a square matrix.

Analysis

Although the scanner produces an accurate image of the tissue being examined, it can also print out the actual CT number (an index of density) of any area of the CT scan. This area can be from 1 pixel to 2.6×10^5 pixels, which is from the limit of resolution to the total area of the scan. The area can be set precisely by the operator. For the present analysis the area used was a window containing 53 pixels (0.13 cm^2), because this was the largest area that gave high sensitivity. This window was moved over the scan of the antler or pedicle in rows, and the CT number of each area was recorded. Because the tissue being examined is heterogeneous within each area, estimates of error tell more about the accuracy of positioning of the window than about the variability of the CT numbers of the tissue itself. Nevertheless, typical standard deviations of a range of mean CT numbers are presented in Table 4. This should enable interpretation of CT numbers presented in the following figures.

TABLE 4. Range of CT numbers with typical standard deviations associated with them

CT number[a]	Typical standard deviation
100	15
300	20
500	25
700	50
900	80
1,100	80
1,300	80

[a] Water as a CT number of 0, the densest bone has a CT number of around 4,000, and air has a CT number of −1,000.

Results

A CT scan of the whole head of the buck in hard antler (antlers sawn off above the pedicle) is shown in Fig. 5. The CT numbers at the edge of the antler and pedicle were uniformly high, indicative of compact bone; indeed, the bone was as dense as the braincase. The CT numbers from the center of the antler and pedicle were lower, indicative of cancellous bone. The CT numbers from around the APJ were about 200 units higher, indicating that the bone was slightly denser at the junction between living and dead bone.

Four weeks after antler casting (Fig. 6), the CAT scan reveals that appreciable demineralization of the pedicle must have taken place (as CT numbers in lines E and F were 587-891, having fallen from 960-1371 (lines E-J, Fig. 5)). There was little evidence of compact bone on the outer edge of the pedicle with a cancellous core, as CT numbers at the edge were similar to those of the core. The CT numbers at the tip of the antler (lines A-B), which represents the actual growing point, were low, indicative of a high water content, probably due to the blood capillary network found there and the presence of cartilage. The CT numbers were higher below the tip, where the CT scan becomes markedly darker (line C). This is probably due to calcifying cartilage, which was beginning to differentiate into a more dense outer edge with a cancellous core, as the CT numbers at the edge are higher than those of the core. However, the numbers are too low to be indicative of bone. The CT number of the brain, −51, is indicative of tissue less dense than water (i.e., mainly lipid). The dermis/epidermis of the antler and pedicle appear to be of similar tissue density.

In January (Fig. 7) the pedicle, although still of lower density than the braincase, appears to have begun to redifferentiate into a hard compact outer edge (lines E_b and $_f$, F_b and $_f$, and G_b and $_f$) and a cancellous core (lines $E_{c\text{-}e}$, $F_{c\text{-}e}$, and $G_{c\text{-}e}$). The top of this section of the antler (lines A and B), which was about 40 cm from the growing tip, has high CT numbers, indicative of bone formation. Although CT numbers of the lateral edge (C_g, D_f, and E_f) were higher than the

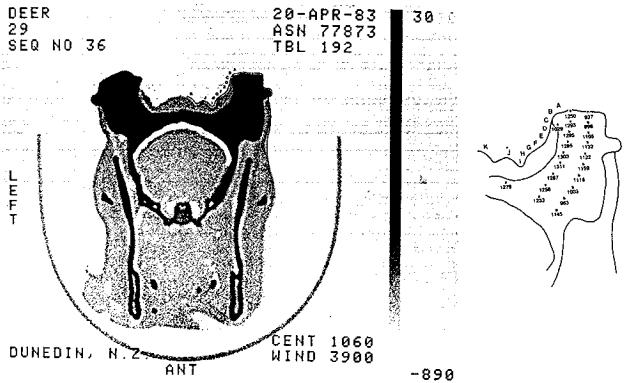


FIGURE 5. CAT scan of the head of a fallow buck (#29) on April 20, 1983, when the buck was in hard antler. The original tomograph has been printed as a negative for illustrative purposes. ASN refers to the serial number of the scan, TBL refers to the position of the head relative to the scanning gantry, and Seq No refers to the position of this scan in the sequence taken on his head. The vertical scale on the right, from 3,010 to −890, refers to the CT numbers that can be visualized in the scan. CT numbers are based on a scale of −1,000 for air, 0 for water, and +4,000 for the densest bone. All tissues have a number which is between +4,000 and −1,000, depending on density. Wind refers to the window width, which at 3,900 is very wide, indicating that almost all tissues should be seen. Cent refers to the center of this scale, 1,060 is a number that gives good detail of hard tissue (a lower number would give better detail of soft tissue). The small numbers on the overlay are the CT values for tissue density at that position. Each line of CT numbers, where appropriate, is labeled with a capital letter and each CT number within each line with a small letter.

core, the medial edge was of similar density to the core (compare $C_{b \text{ and } c}$ and $D_{a \text{ and } b}$ with $C_{d\text{-}f}$ and $D_{c\text{-}e}$).

The development of the trey tine is depicted in Fig. 8. The CT numbers of a representative sample of the scans are also shown. The sequence from 24-42 begins from above the trey tine to below it. Thus, sequence 24 is only of the main beam, 25 also has the very tip of the trey tine, 32 and 33 have the junction of the trey tine with the main beam, and the remainder illustrate the united tine and antler. The main beam above the trey tine, sequence 24, has a hard bony outer edge (e.g., line A) with a cancellous core ($B_{d \text{ and } e}$, $C_{c \text{ and } d}$). Indeed, this varies little throughout sequence 24-42. The tip of the trey tine appearing in sequence 25 was characterized by very low CT numbers, but 2 mm below this (sequence 27), higher numbers, indicative of cartilage, were present in the core (A_b and $B_{b \text{ and } c}$). In

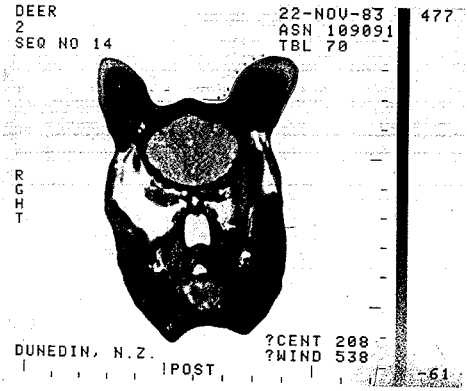


FIGURE 6. CAT scan of a different buck (#2) on November 22, 1983. Legend as for Fig. 5. This window at 538 with a center of 208 is designed to give better soft tissue detail. Note sinuses below the right antler.

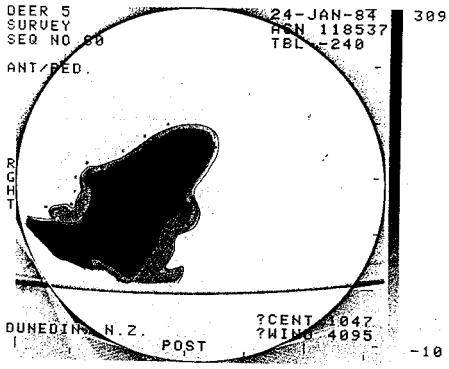
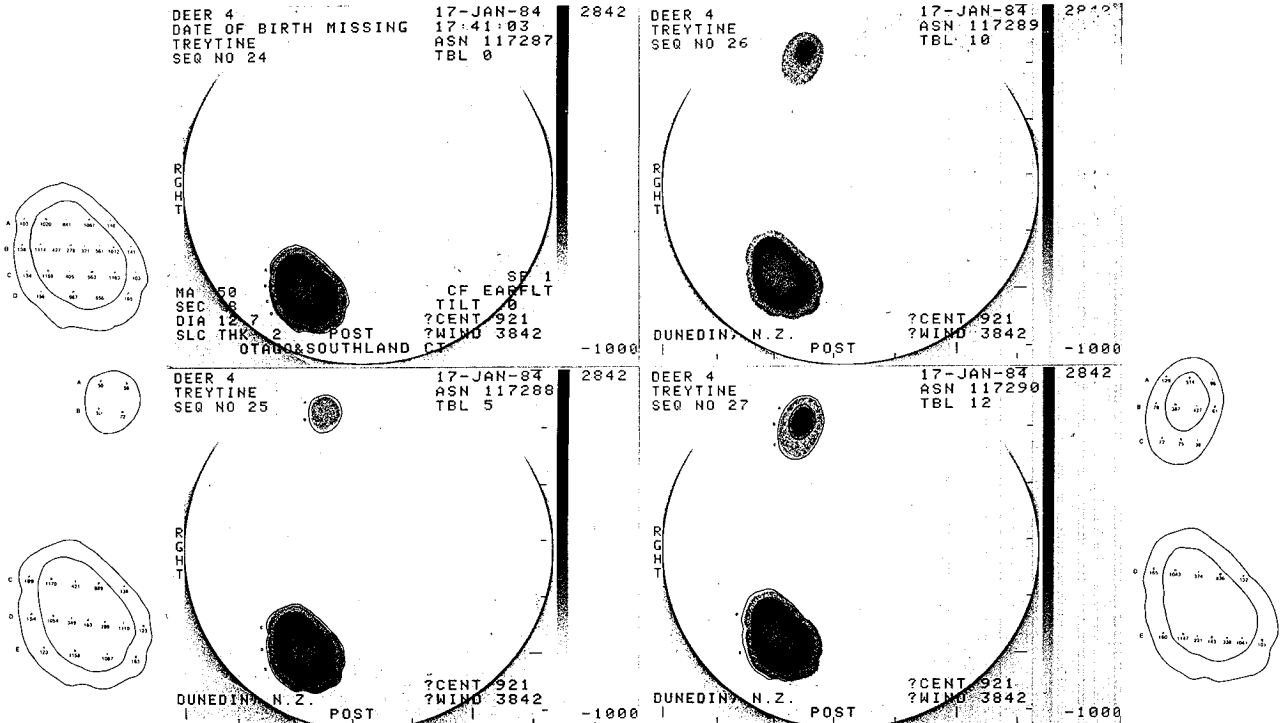


FIGURE 7. CAT scan of the antler base and pedicle from a buck on January 17, 1984. Legend as for Fig. 5. Note the very wide window.

FIGURE 8. Sequence of CAT scans from above the trey tine to below it in deer #4 on January 17, 1984. The main beam is in the lower left of each scan. When it appears, in sequence #25, the trey tine is in the upper central part of the scan. Legend as for Fig. 5.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RIGHT
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CF EARFLT
TILT 0
?CENT 921
?WIND 3842
-1000
POST
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SEQ NO 26
17-JAN-84
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TBL 10
2842
RIGHT
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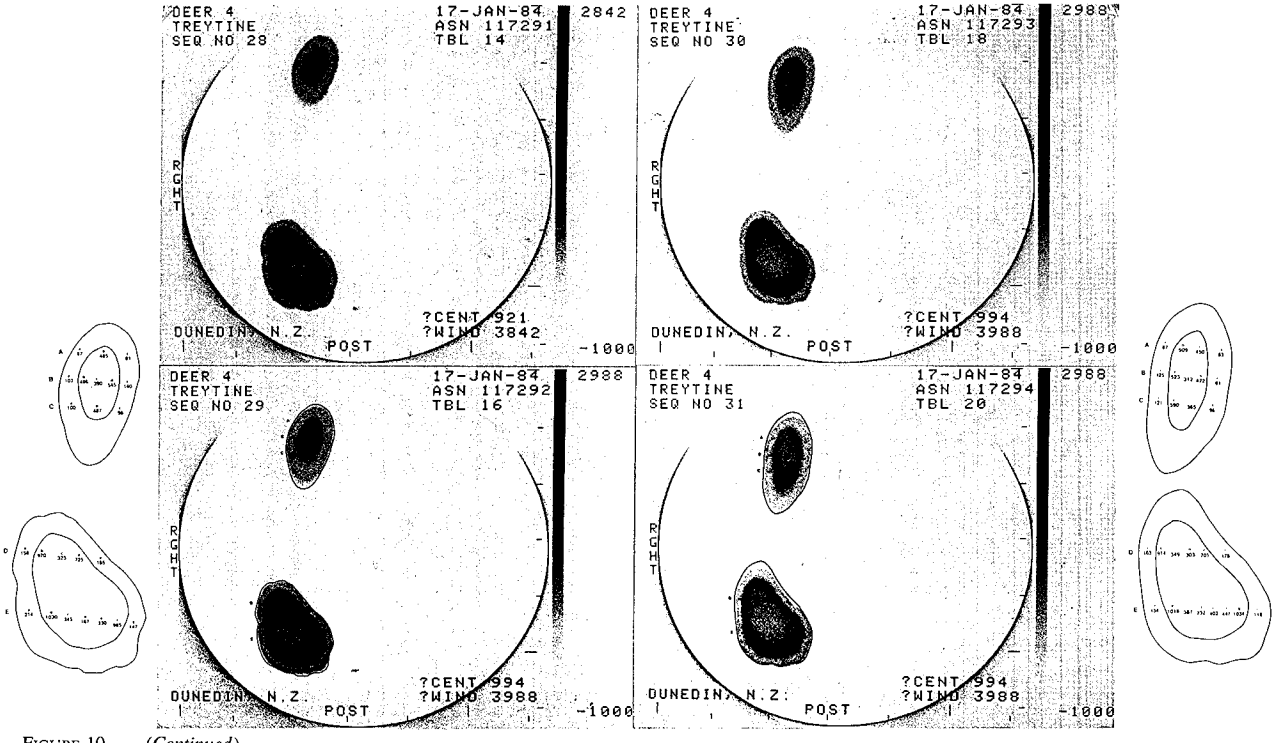
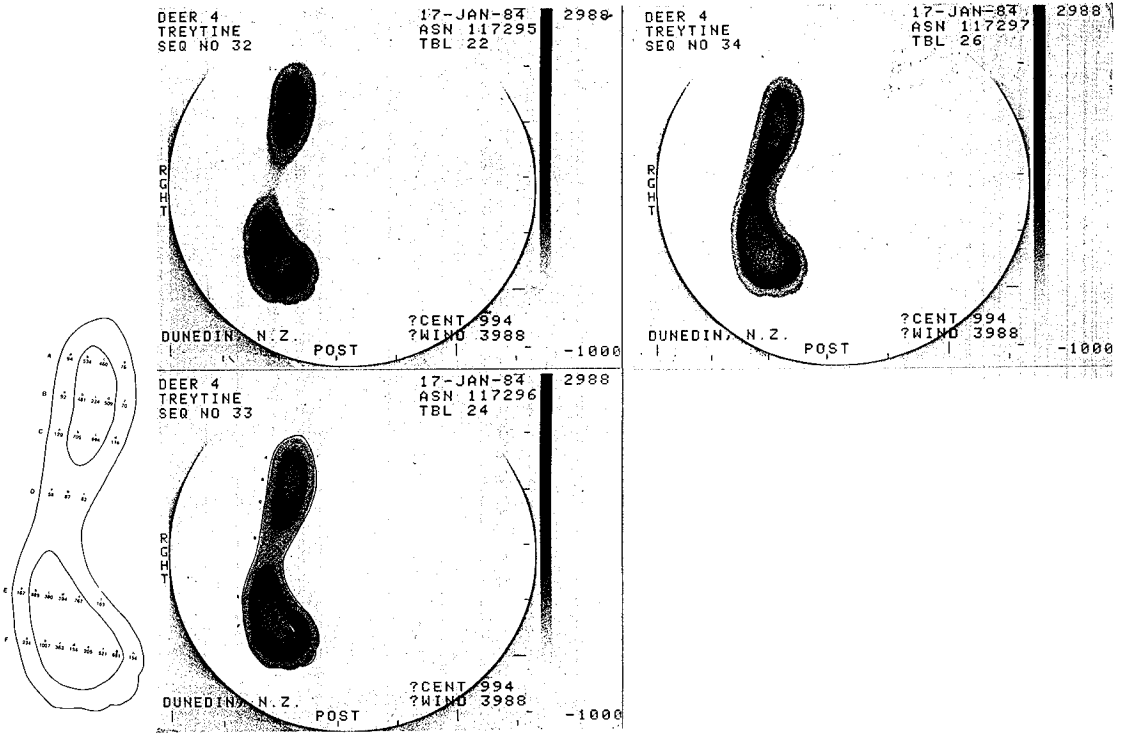


FIGURE 10. (*Continued*)



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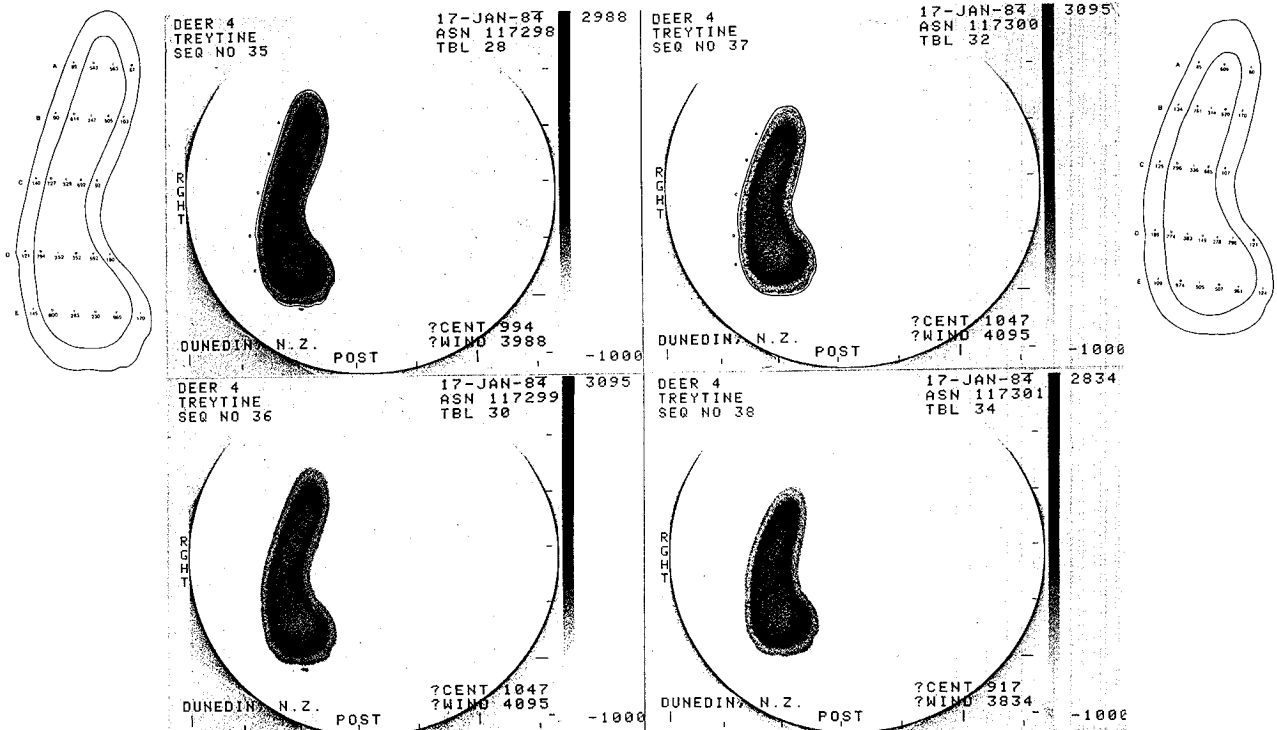

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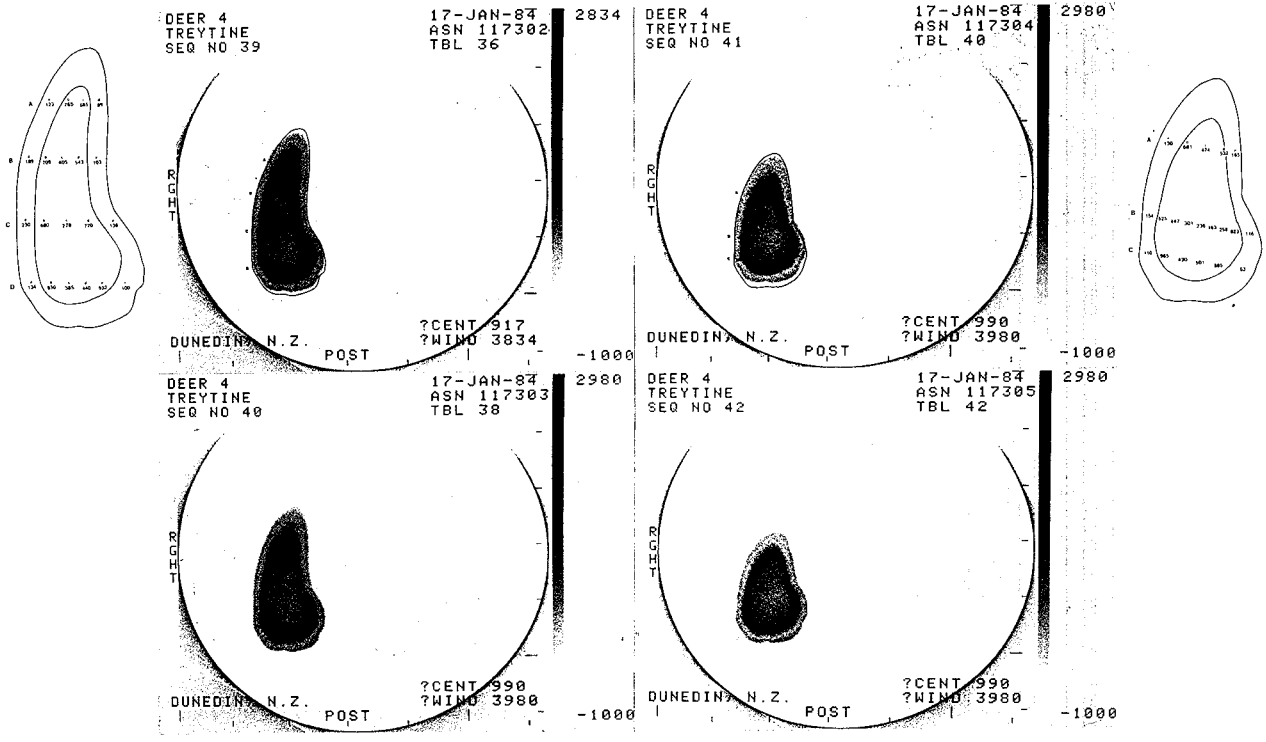


FIGURE 10. (*Continued*)

sequence 29, both main beam and tine have a hard outer edge with a soft core, although CT numbers of the tine were less than the main beam of the antler particularly for the outer edge of bone (compare sequence 31 line B with line E). Curiously, B_c was higher than the E_d; this may mean that the cancellous cavity of the tine was more poorly developed than the main beam. In sequence 33 the tine and antler are joined with an area of low CT numbers between the cores (line D). CT numbers of the tine bone were less than the antler bone. In sequence 35-37 the cores of the tine and antler were joined, although the antler bone was denser than the tine bone.

In sequence 39 the tine is almost completely merged with the main beam although CT numbers of the ventral (tine) edge, i.e., line A has lower density than the dorsal (main beam) edge.

Discussion

Essentially the CAT scans support the findings of Banks and Newbrey (1983) on the developing antler, based on light microscopy. The degree of differentiation from undifferentiated cells, to cartilage, to calcified cartilage, to bone occurs in a sequence from the tip of the antler.

The CT scan of the buck in hard antler (Fig. 5) revealed that both antler and pedicle are composed of very hard bone, but the hard bone of the pedicle was much less dense during early velvet-antler growth (Fig. 6). It is known that blood draining the antler flows down through the cancellous bone (Suttie et al 1985c), and it is reasonable that the core of the pedicle develops the capacity to allow this. The CT numbers in Fig. 6, line C, are higher than line D, particularly in the core. This is interesting, as CT numbers usually increase proximally—or, put another way, increase with stage of development. This apparent anomaly can be interpreted as the calcifying cartilage having higher density than the developing cancellous bone below it.

The scans of the developing trey tine reveal that the stages of differentiation of the tine are not the same as the main beam at the same relative position; the tine differentiates more slowly. The compact bone at the junction between the main beam and tine is relatively weaker, at least during development, than the posterior part of the antler. Clearly, CAT offers important possibilities for the study of the developing antler.

Cranial Angiography

Although the gross anatomy of the blood supply to the growing and regenerating antler has received some study (Waldo et al. 1949; Adams 1979; Suttie et al. 1985c), the relevance of the pattern of blood supply for antler growth has not received any attention. The aim of this study was to use the technique of cranial angiography to trace the pattern of development of the arterial supply during

development of the first spike antler and the second branched antler. An attempt is made to relate pattern to function.

Materials and Methods

These are given fully in Suttie et al (1985c). Briefly, 3-month-old red deer stags were fitted with a carotid prosthesis to permit easy, repeated cannulation of the carotid artery (Suttie et al. 1986). At intervals from 3–25 months of age, under general anesthesia, 15–20 mL of 76% radiopaque contrast medium (Urograffin, Schering AG Berlin) was injected manually via the cannula as fast as possible (5–10 seconds). Simultaneously, radiographs were taken of the head and/or antlers. A rapid film changer was used which permitted up to four radiographs per second for 15 seconds to be taken, using XRP1 (Agfa) blue sensitive film.

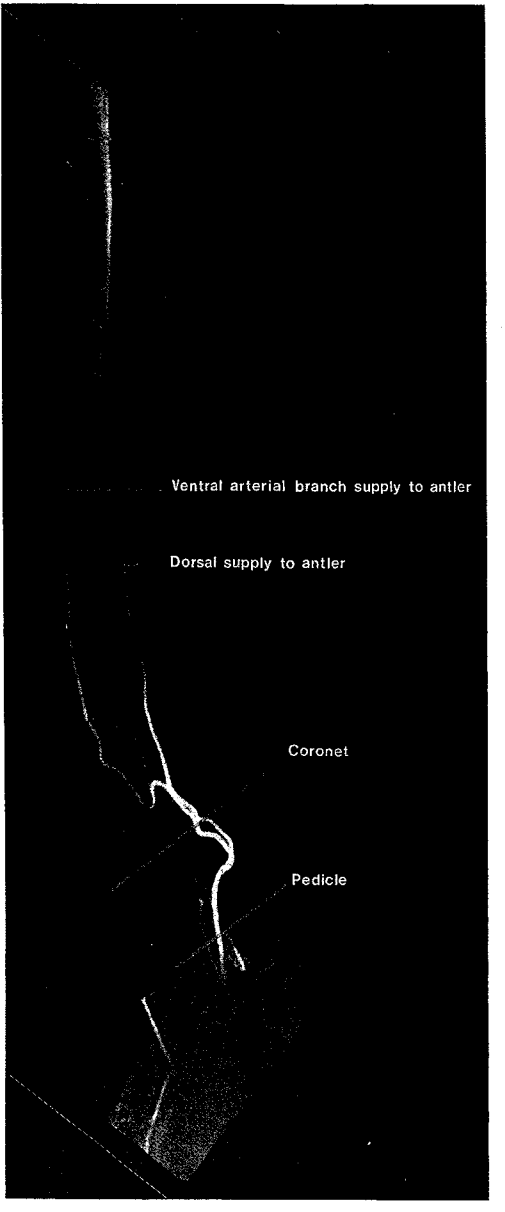
Results

The antler/pedicle is supplied by the lateral and medial branches of the superficial temporal artery (STA) which arises directly from the carotid artery. Only one branch of the lateral STA supplies the antler (Fig. 9), the remainder supplying only the pedicle or only advancing a short distance up the antler. The medial branch of the STA supplies only the pedicle. The branch of the STA supplying the antler branches itself at the APJ, one branch continuing along the posterior aspect of the antler while the other crosses to the anterior aspect of the antler, before continuing upwards. After the antler is clean of velvet, the STA no longer supplies the antler (Fig. 10). The pedicle continues to be supplied by branches of the artery, however. The branched nature of the pedicle arteries is evident from Fig. 11.

After antler casting, when the growth of the second branched antler begins, it is clear from Fig. 11 that from each branch of the artery in the pedicle, a vessel grows into the new antler. In contrast to the spike antler, the branched antler has several major arteries. The first branch of the STA at the zygomatic arch yields an artery that supplies the posterior aspect of the antler and an artery that supplies the anterior aspect and the brow, bey, and trey tines. The next branch, at the level of the base of the pedicle, supplies the brow and bey tines, and the trey tine and the anterior main beam above the trey tine, respectively. Thus, the arteries supplying these highly evolutionally conserved tines are divided prior to antler growth, and not even in the antler. In contrast (Fig. 12), the arteries supplying the terminal fork branch just before the terminal fork of the antler.

Discussion

Some caution is prudent in attempting to assess the wider implication of the foregoing results, because they are largely based on angiography of the antlers and head of only one stag. However, with this in mind, the antler is supplied by


Ventral arterial branch supply to antler
Dorsal supply to antler
Coronet
Pedicle

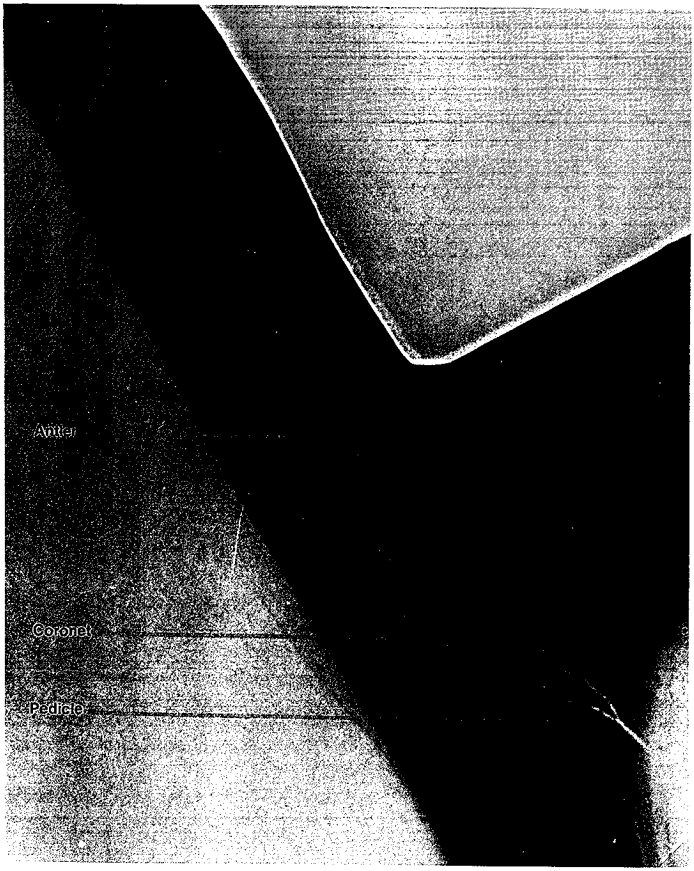


FIGURE 10. Angiograph taken on February 25, 1983, 10 seconds after injection of contrast medium. Antler growth was complete and the antler was clean of velvet; consequently, no arteries supply the antler. Note the branched pattern of arteries in the pedicle.

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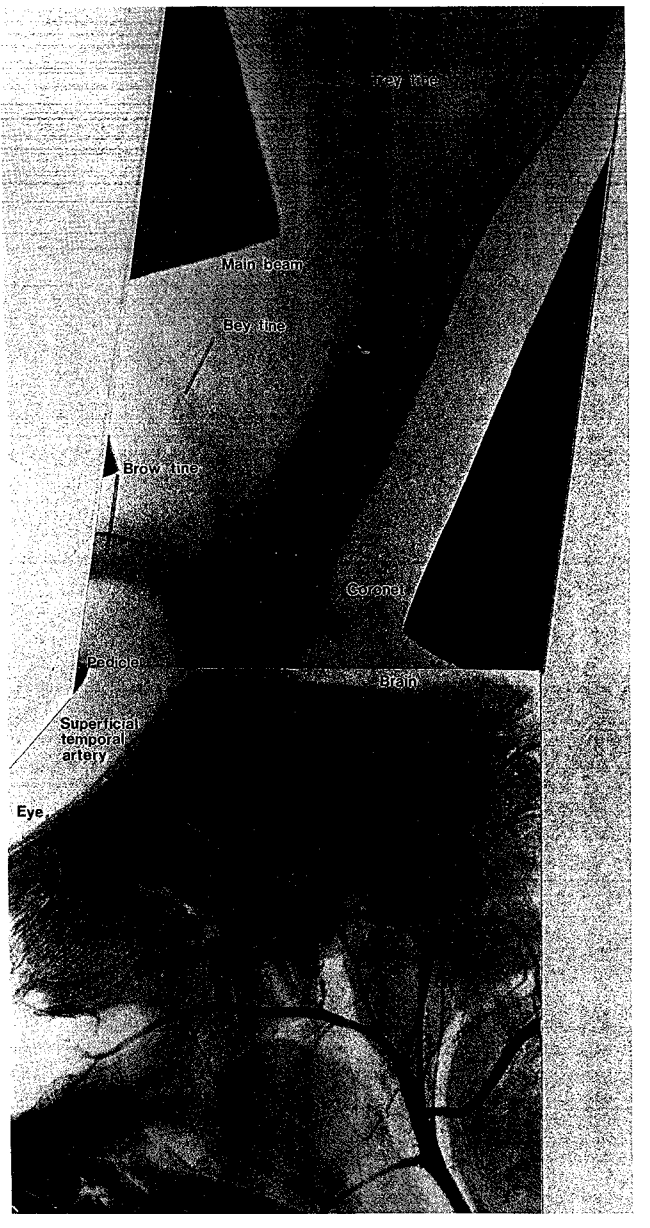
FIGURE 9. Angiograph taken on November 19, 1982. Midspike antler development 10 secs after injection of contrast medium. One major artery from the pedicle (a branch of the lateral STA, to the right in the figure) branches as it flows round the coronet. One branch supplies the ventral and one the dorsal aspects of the antler. The medial branch of the STA (to the left of the pedicle in the figure) supplies only the pedicle and does not enter the antler.


Trey tine
Main beam
Bey tine
Brow tine
Coronet
Pedicle
Brain
Superficial temporal artery
Eye

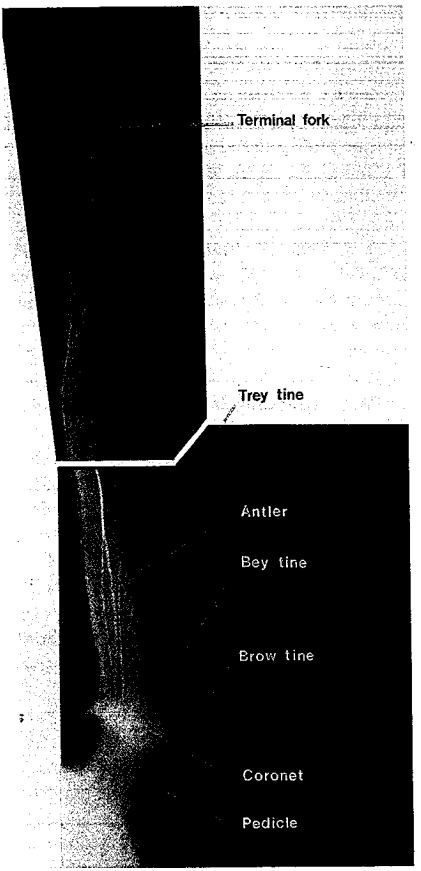


FIGURE 12. Composite angiograph of the antler taken on the same day as Fig. 11 on the same stag. The arteries supplying the terminal fork of the antler branch before the antler branches.

◄

FIGURE 11. Subtractions of angiographs taken in November 1983, when the second branched antler was growing in velvet. The major branches of the artery (the dark lines), to the lower half of the antler, have divided before they reach the antler.

branches of the lateral STA. The spike antler is supplied by one branch, whereas the branched antler is supplied by several. Moreover, the arteries destined to supply the brow, bey, and trey tines are already divided prior to antler growth. Does this have any consequence for theories of antler regeneration?

Antlers invariably branch into two forks during growth. In early growth, the first branch between the brow tine and the main beam is often evident before the previous antler scar is completely healed, say, 2 weeks after antler casting. It is conceivable that such early antler branching requires arteries that are already branched, to invade the tissue, possibly in response to angiogenic factors. As all of the early tines branch from the ventral aspect of the antler, is not surprising that the more ventral branches of the artery supply them. In cases where brow tines fail to grow or are very small, one may ask, has the tine failed to branch, or has the blood supply been insufficient to promote growth? This is rather a chicken and egg situation which can best be answered by asking a further question—namely, do skull and pedicle arteries carry positional information for the antler? Clearly they branch regularly and the number of major branches (four) equals the number of points prior to the terminal fork (where in any case the antler artery branches). Branching is likely to occur because two growth centers diverge; for the antler, the growth centers are likely to be blocks of fibroblasts or generally undifferentiated mesenchymal cells. These mesenchymal cells may secrete angiogenic growth factors, thus drawing arteries which have already branched—however, the fact remains that the "right" number of branched arteries is available for the species-specific branching pattern. It seems the pattern of artery branching could well influence antler branching. If fewer branches are available, then fewer points could be grown. Perhaps if these arteries are artificially stimulated, then more points could be possible. The size of the arteries supplying each tine, by determining blood flow, might partly control tine size by limiting nutrient flow to the developing cells. However, this spatial correlation need not necessarily imply a temporal priority of arterial branching before antler branching. In view of the prevalence of collateral circulation, or the tendency for arteries to enlarge commensurate with the peripheral fields vascularized by them, it is possible that the bud of a tine might be supplied by small arterioles, and as the tine elongates one or more of the arterioles might increase in diameter accordingly. Further work must be done to resolve whether there is indeed a functional relationship between arterial and antler branching; visualization of an already enlarged artery prior to tine formation would provide evidence of such a relationship.

General Discussion

For a general discussion on antler regeneration, a distinction must be made between (1) whether regeneration will occur or not, and (2) the extent of species-specific nature of the regeneration.

Likelihood of Regeneration

Goss (1961), Lincoln (1984), and the present study have shown that antler regeneration is very hard to prevent by merely repeatedly removing portions of the growing antler. That is, regeneration of some sort will tend to occur despite repeated antler or pedicle amputations.

Pattern of Regeneration

The pattern or extent of regeneration after antler amputation or modification is different. Repeated removal of portions of the growing antler in castrated red deer results in slowing of growth rate and unbranched regeneration. Stimulation of the pedicle—by physical trauma or testosterone—induced antler casting in castrates and restored growth rate and shape, but the ultimate size was smaller than would have been expected in an intact stag. This lends support for an argument that (1) the pedicle holds information for the normal species-specific antler pattern, and this can be expressed by trauma, (2) although the velvet and the pedicle both have the capacity to regenerate, this property is more strongly held by the pedicle, and (3) other trophic factors influence antler size. It is not within the scope of this discussion to consider the identity of trophic factors that influence antler size, but they are probably endocrine, paracrine, autocrine, or neurocrine in origin.

The influence of repeated antler removal on the nerve and blood supply to the antler tissue is also relevant. It could just be that the normally "deciduous" antler nerves and arteries have little capacity to regenerate, but those nerves and arteries in the pedicle have a high capacity to regenerate. Reduced regeneration from the amputated antler might then be due to lack of nutrients and/or peripheral or central neural stimulation, rather than indicating a functional disparity in regenerative capacity between antlers and pedicles. However, the healing process typically requires the involvement of both blood vessels and nerves and, as the antler must heal prior to regeneration, it seems that there is ample opportunity for artery/nerve repair or replacement. It is also conceivable that arteries and nerves carry trophic or positional information, and this capacity is lost on differentiation from pedicle to antler arteries or nerves.

A consensus of the above might be that pedicle cells whether bone, nerve or blood are fundamentally different from the same cells in the antler: The cells of the pedicle carry full capacity to regenerate; those of the antler do not. Whether the positional information for the antler is solely vested in the pedicle cells or is centrally located (or both) is, as yet, unknown. It is tempting to reason that, as the regenerative capacity of the antler and the pedicle differ, a local mechanism is responsible for communicating positional information: a central mechanism would have been expected to stimulate antlers and pedicles equally.

The pattern of arterial branching in the pedicle certainly provides many blood vessels to supply the new antler—it is intriguing that even before antler growth

the number of major branches of the artery matches the number of forthcoming major early antler branches—despite the fact that the stag in this study had never grown a branched antler before. Whether this effect is due to chance, whether the arteries do carry some positional information, or whether the arteries supply blocks of tissue in the pedicle with positional information must remain speculative. What is clear is that the arteries that supply the major tines are present and do not need to branch in the antler; this is in contrast to the arteries supplying the terminal fork.

In conclusion, antler regeneration is a highly conserved mechanism, although the actual mechanisms governing antler size and shape are more plastic.