Deer Antlers

Rapid Growing Calcified Tissue

A Rajaram

Deer antlers exhibit the fastest growth among all natural calcified tissues, and have attracted the attention of pathologists, endocrinologists, bioengineers, ethologists and evolutionary biologists. However, there is still much to be found out about the detailed mechanisms of antler growth, and the factors affecting the evolution of antler size. Better knowledge of antler growth may also help in a better understanding of injuries and cancers of bones.

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Antlers are bony skeletal protuberances of the skull, and consist mainly of the protein collagen and the mineral calcium hydroxyapatite ($Ca_5(PO_4)_3OH$). Antlers occur in most species of the deer family (Cervidae) and are grown and shed annually, typically only by males. Evolutionarily, horn-like structures developed in all four true ruminant families – Cervidae, Giraffidae, Antilocapridae and Bovidae – but it is not known whether they developed independently in four different forms, or whether the four forms diverged from a common ancestor which had already developed rudimentary structures. Antlers are distinct from the horns of ungulates like the bovids and antelopes. Ungulate horns are made up of the protein keratin, last throughout the animals' lifetime, and are not replaced if lost due to any reason. Unlike horns, antlers are secondary sexual characteristics, typically occurring only in males, and are functional only during the rutting (mating) season. The reindeer is the only deer species in which the females also sport antlers but these are much less impressive than those of the males. The horns of the giraffe are protuberances of the skull covered by matted hair. Two species of Indian deer that do not have antlers are the musk deer and the Indian chevrotain or mouse deer, which belong to families other than the Cervidae (*Box* 1). In these antler-less species, the canines are very well developed and

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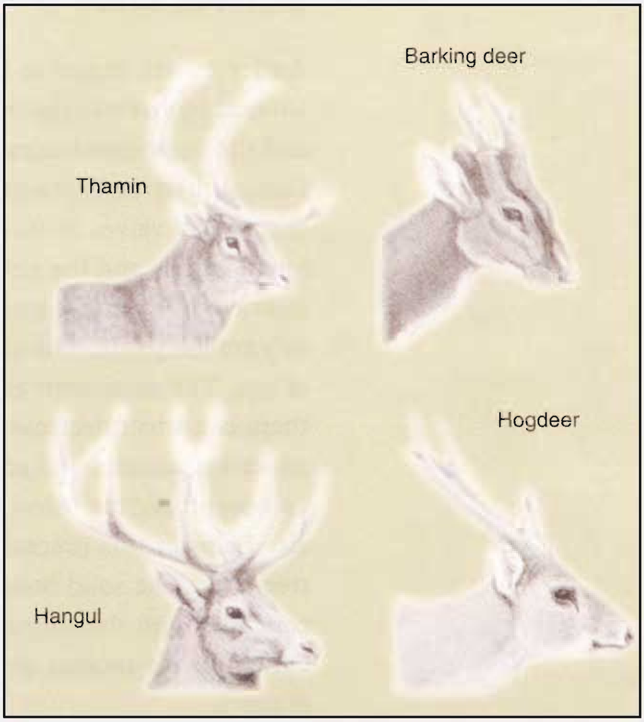
Antlers, calcification, antler biology, testosterone, Cervidae.

Box 1. Classification of Indian Deer

Four Indian deer species along with the classification of Indian deer. Three others are depicted elsewhere. Those without antlers are not depicted anywhere in the article.

The scientific names according to Prater [5] are given in brackets while the other taxonomic name is more recent and is according to Groves [7].



FAMILY TRAGULIDAE – no antlers; tusks in male

Genus *Moschiola*

Moschiola mimenoides (*Tragulus meminna*)

Indian chevrotain or mouse deer

FAMILY MOSCHIDAE – no antlers; tusks in male

Genus *Moschus*

Moschus moschiferus musk deer

FAMILY CERVIDAE – antlers present in male

Genus *Muntiacus*

Muntiacus muntjak barking deer

Genus *Cervus*

Cervus elaphus hanglu hangul

Genus *Recervus*

Recervus eldii (*Cervus eldii*) thamin or brow antlered deer

Recervus duvaucelii (*Cervus duvaucelii*) barasingha

Genus *Rusa*

Rusa unicolor (*Cervus unicolor*) sambhar

Genus *Axis*

Axis axis spoted deer

Genus *Hyelaphus*

Hyelaphus porcinus (*Axis porcinus*) hog deer

function as secondary sexual characteristics. Even among the antlered deer, there is a negative correlation between canine length and antler size, with the European red deer (*Cervus elaphus*), which is related to our Kashmir hangul (*Cervus elaphus hanglu*), having very small canines, whereas our small muntjac (*Muntiacus muntjak*) or barking deer has comparatively long tushes. The taxonomy of our deer is undergoing considerable rearrangement as more knowledge about species relationships – genetic and otherwise – is generated. This is a fertile field in relation to evolution and speciation and there is much to be done.

Antler Growth

Antler growth begins as a projection from the skull in a region covered by velvety skin called the pedicel. Changes in day length and the consequent release of hormones trigger antler growth. During the growth season, the projection increases daily and is covered by velvet at this stage. This covering is very rich in blood vessels and the animal can bleed profusely if there is an injury to it. Deer take great care in protecting the antlers during this growth phase. The ultimate size of the antlers is a function of age. The maximum growth occurs in the mature adult and there is a small decrease after this peak is reached. When the antler has attained full growth for the season, the blood supply gets cut off and the velvet begins to peel off in flakes. The animal also helps in this process by rubbing its antlers against rough tree bark. The solid bone gets exposed and the rutting period coincides with the animal being in hard antler. The growth of antlers in the spotted deer (chital: *Axis axis*) is represented in *Figure* 1.

Depending on the species, the antler is employed as an instrument of dominance for supremacy during the rut. Among the hangul, the males defend prime territories and guard a harem of females. In the spotted deer such extreme aggression is not seen

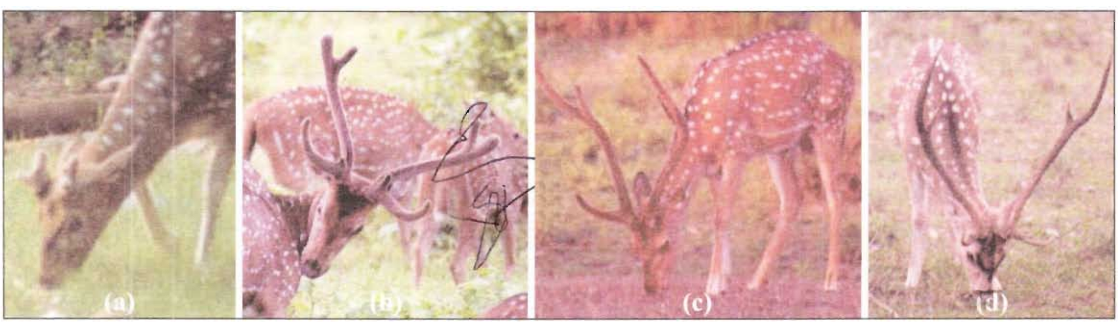


Figure 1. (a-d). The various stages of antler growth in spotted deer (chital). The growth gets completed in about three months.

but the antlers are used in pushing contests for supremacy during the short period in which the males segregate and mate with receptive females. When the rut comes to an end, a knob-like projection called the burr develops around the base of each antler above the original pedicel. The animal now has a tendency to knock the antler against hard objects and this induces the antler to fall off. There is some bleeding but this stops soon and a layer of velvety skin soon covers the pedicel area till the cycle begins again.

Function, Shape and Size of Antlers

As mentioned earlier, antlers are secondary sexual characteristics. They are not as useful in defense as are the horns of other ungulates. Antlers afford protection against predators only in the sense that they come in the way. Of course, some animals may use them in defense but in most cases the antlers afford little protection. They are mostly impressive adornments. Antlers are employed in certain threat displays without actual contact with the opponent. One such display in spotted deer is a lunge at the opponent who, if he is not alert and fails to move away quickly, ends up being badly gored. Animals subjected to this threat and wounded as a consequence, can often be seen in the rut period in the forests. During the rut, males often spar with their antlers (*Figure* 2) but in order to drive a competitor away, the animal should also be strong with a relatively greater body weight. Its position uphill or downhill may also matter. Due to the peculiar nature of the branching tines, it has sometimes happened that two animals have got permanently 'locked' in combat and have both died subsequently. The Bombay Natural History Society museum in Mumbai is said to have two such spotted deer skulls locked in this inseparable position.

Figure 2. Two evenly matched spotted deer testing their strength during the rut.

Antlers in deer are mostly symmetrical. Each one consists of a main beam forming the body of the antler along with comparatively small projections ending in sharp points called tines. The

Figure 3 (left). Sambhar – largest Indian deer with the heaviest antler.

Figure 4 (center). The endangered barasingha of Central India has the maximum number of tines for an Indian species.

Figure 5 (right). An abnormal growth of an extra long beam/tine in a spotted deer probably due to body imbalance resulting from a cancerous growth on the opposite side.

long tine nearest to the skull is called the brow-tine. In our common deer species for example the sambhar (*Figure* 3) and spotted deer, the main beam ends in two tines while there is a brow tine at the lower end. The barasingha of Kanha National Park has the maximum number of tines (*Figure* 4). Among spotted deer and other species, which have no marked breeding season, antlers in different stages of growth can be seen in all seasons but depending on the geography, there is usually a peak rutting season with a predominant number of animals being in hard antler. The antlers are less symmetrical in younger stags and tend to become more symmetrical as they come to adult age.

If either of the forelimbs of a male deer is damaged, the antler growth on the opposite side is abnormal, being of lesser size. This probably arises as a necessity for body balance. Strangely, I noticed the converse of this in a spotted deer that seemed to have a growth in its abdomen to the left (*Figure* 5) and correspondingly, the right antler had an extra beam. An injury to the antler during growth can also result in an extra tine.

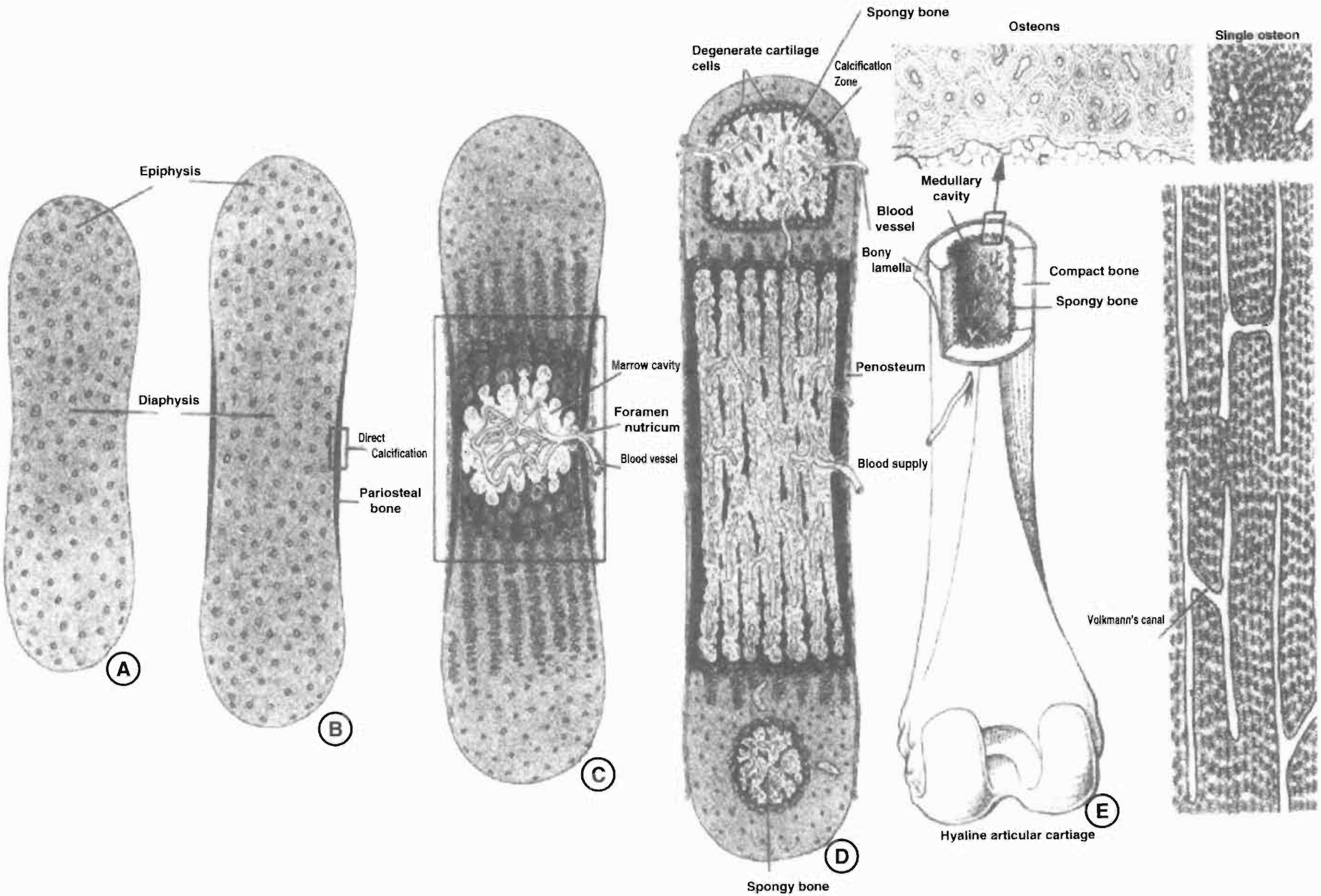
Antler Calcification

Antler being a projection from the skull (a portion of the mammalian skull has two outer membrane layers of compact bone sandwiching a porous spongy bone within), it was believed that its growth would be similar to the calcification process of mammalian skull. However, later studies indicate that antler calcification has more similarity to indirect chondral process of mineralizing long bones which arise from a cartilaginous precursor form. This process for a long bone is represented in *Box* 2. The deer antler does not have a distinct separation of cartilage and mineral even though cartilage cells are present at the periphery of the growing antler. Blood supply is present in the core region and in the mineralizing outer layer through the velvet. So antler calcification has been referred to as modified endochondral bone growth. The cartilage cells totally disappear in the mature functional antler. The structure of antler collagen, and its turnover as the antler grows and as calcification proceeds, seem to be different when compared to typical endochondral bone formation (*Box* 3). Histologically, the rapidly growing antler repre-

Box 2. Bone Growth from a Cartilage (chondral) Model – Calcification of Long Bones

The initial skeleton in mammals is composed of hyaline cartilage and contains primitive chondral cells (A). (See *Figure* on p.56) The mid-diaphysis calcifies at the outer surface directly in the form of a collar called periosteum. This process is controlled genetically and expresses at about seven weeks in the human embryo and is the only step that has similarity to direct membrane calcification of the kind that occurs in the skull. All subsequent steps occur by the endochondral process where chondroblasts and their degradation sites are replaced by osteoblasts laying down calcified matrix. The calcified periosteum gives structural support to the developing hyaline tissue (B). Primary calcification begins in the centre and chondroblasts get stacked into longitudinal assemblies (C). Blood vessels penetrate the calcified perichondrium at the foramen nutricum. The chondroblasts are replaced by chondroclasts at the center. They then destroy the cartilage and this region later develops, extends and becomes the marrow. The end epiphyses of the long bones remain cartilaginous throughout. The columnar chondroblastic region gets calcified. The calcifying front in the antler bone has similarities to this, albeit over a small region. Cavities subsequently develop in the epiphysial regions also (D) by the activation of penetrating blood vessels, which bring with them mesenchymal cells which differentiate into osteoblasts. The matrix vesicles after chondrocyte activity may also become sites of mineralization. Blood vessel connections are established from the marrow to the cavities developed in the epiphyses. At these ends, the calcified matrix is laid down as trabecular spongy bone with density optimized in the direction of mechanical stress (not shown). Ultimately in mature bone the compact bone tissue of the diaphysis is as shown in E. The long bone has a hollow marrow surrounded by a thin spongy bone layer and the mechanical stress is borne by the compact bone that has the structure shown in F (cross-section) and G (longitudinal section). The cross-section of a single osteon is shown in the top corner. The cavities follow the direction of blood vessels. The individual units are represented as osteons that are directed mainly along the long bone while in antler compact bone, they occur in all directions in conformity with the blood supply from the velvet. In mammalian long bones, as time goes on, the primary osteons get resorbed and are relaid which results in secondary osteons. The latter can be recognized by their clear boundary.

Box 2. continued...



Adapted from [4]

sents certain neoplasms (cancers) of bone like Ewing's sarcoma, a form of primary bone cancer in young people, on account of which its study has attracted attention. However, an understanding with respect to normal or pathological bone disease is not yet within sight.

Collagen and Calcification

Collagen is the predominant protein in bone and antler and is a ubiquitous component of most tissues, occurring to the extent of 90% in ligaments and tendons. The primary sequence in all collagens can be represented as $(\text{Gly-X-Y})_n$ where Gly represents the amino acid glycine, n is around 300, and X and Y represent the 19 other amino acids, although proline and hydroxyproline account for nearly 25% of these (for a discussion on collagen structure and properties see *Resonance*, Vol. 6, No.10, pp. 38-47, 2001). At the secondary level of organization these thousand and odd peptides twist to form a left handed helix with a pitch of around 0.9 nm, represented as an α chain (*Box* 3). In the collagen molecule (at the tertiary level), three α chains are held together by inter-chain hydrogen bonds resulting in a right handed super-helix of pitch around 3 nm. At the terminal regions there are non-helical regions that do not have the typical sequence. These regions are however involved in stabilization of collagen molecules via intra- and inter-molecular linkages at higher

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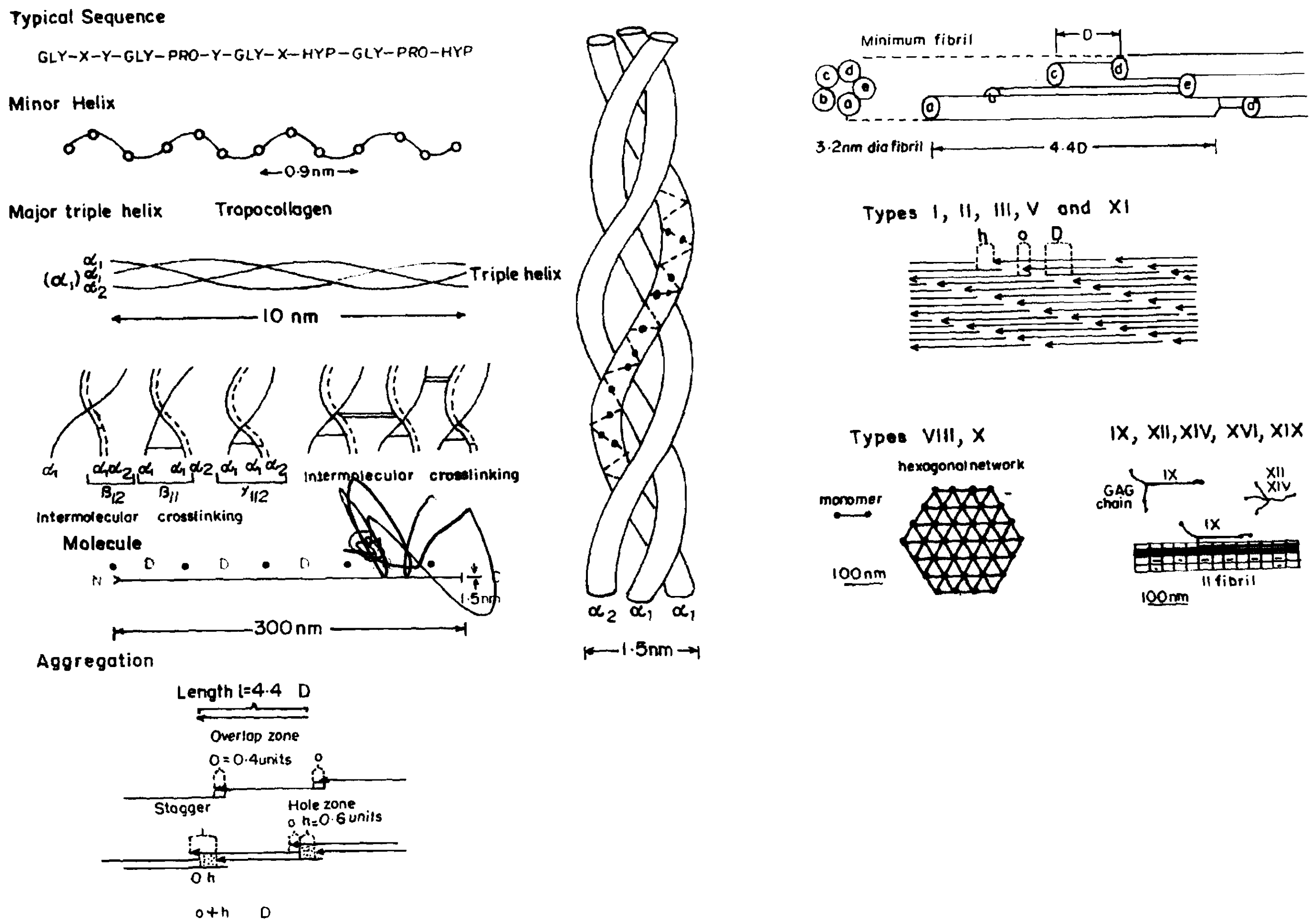
Box 3. Collagen Structure and Calcification

The length of the collagen molecule (see *figure* overleaf) is about 300 nm (for convenience 4.4 D where D is the 64-68 nm periodicity seen in the transmission electron microscope). Molecules aggregate laterally and longitudinally to form fibrils of indeterminate length. Lateral aggregation of adjacent molecules has a stagger equivalent to 0.4 D while the lag along a length results in a gap or hole of 0.6D. At least five molecules are to be arranged in this way in a pentafibril order to get the characteristic D periodicity. Usually in tissues, the collagen fibre is composed of hundreds of molecules arranged laterally and longitudinally. The mineralization process gets initiated in the hole (gap) region and once it is started, mineral deposition occurs all over. The rare collagen Types VIII, IX, X, XII, XIV, XVI and XIX form fibrillar structures different from those that aggregate with the 64-68 nm periodicity.

Box 3. continued...



Gly- glycine residue, X, Y – other amino acid residues.

levels of aggregation of the collagen molecule. Many of the crosslinks formed are also implicated in the initiation of the mineralizing process. For Type I collagen, which is the most common, two identical α chains (α1) combine with a third α chain of different amino acid sequence and composition (α2) and is represented as $(\alpha 1)_2\alpha 2$. Type II collagen, present in cartilage, has all three α chains identical but different from those of Type I. Type III, which has a still different α chain, forms thinner fibres than Type I and is found in skin. Some collagens like basement membrane collagen (Type IV) do not form fibers but form a meshwork aggregate. At present 19 types of collagen have been described. Some minor ones are associated with other more common types. In the mammalian growth plate of long bones, collagen Type II is predominant in the cartilage matrix while at the calcifying front it is associated with collagens IX and XI. When chondrocytes are resorbed, osteoblasts synthesize and lay down Type I and mature compact bone has all Type II replaced by Type I. In growing antler bone however, during all stages of growth, Type I is predominant even though Type II is present in small quantities throughout. Hardened antler has Type I alone. Type X that is present at the interface between bone and cartilage in calcifying long bone, and is manufactured by the calcifying cartilage zone cells, can also be detected in young antler, along with Type XI. There is however no conclusive evidence for the presence of Type IX. The minor collagens like Types IX, X and XI mentioned here occur in too low quantities that they cannot be extracted and purified. However, they can be detected with antibody staining methods by what is called Western blotting. This involves raising antibodies to the purified collagens prepared from other animals in a suitable animal model. Preparations of collagen from antler bone are separated by gel-electrophoresis. The collagen, thus separated, are transferred to nitrocellulose/PVDF membrane. Then they are reacted with the primary antibodies that have been prepared from the highly purified collagens. Subsequently, a secondary antibody tagged with a chromophore or a fluorophore is made to react with the first antigen-antibody complex so that the collagen can be detected and identified. It is possible that among the rarer types, the antibodies raised against one type can cross react with another collagen type from a different source. This results in some ambiguities in understanding the calcification process in antler. An improvement in resolving these involve culturing cells from the antler and probing the mRNA sequence and cDNA sequence using techniques in molecular biology, but studies on antler with these probes have been limited.

Antler growth and mineralization also involve several growth factors. Even in normal calcification, apart from collagen and growth factors, the enzyme alkaline phosphatase, matrix vesicles, the non-collagenous proteins osteocalcin, osteopontin, and sialoproteins are also involved and the biochemistry and their interrelationships are still being worked out.

Structure and Properties of Antler Bone

Antler has an inner porous structure similar to spongy bone surrounded by a very solid compact bone, as in mammalian long bone. This composition extends throughout the antler. The structure of the compact bone is shown in *Figure* 6. The Haversian or osteonal structure in antler consists only of primary osteons. In adult mammalian long bone, where resorption and subsequent reconstruction of the osteons take place, we normally get secondary osteons as well. These are distinctly notable due to a well-marked boundary. Secondary osteons do not occur in antler bone since the growth is fast – about three to four months after which there is no blood supply and no reconstruction. The chemical composition and physical properties of antler bone are shown in *Tables* 1 and 2 respectively. The organic content, especially the collagen, is also distinctly higher in antler compact bone (*Table* 1). This confers on it more extensibility and higher work to fracture (*Table* 2); in essence, antler bone yields more before breaking, which is probably a functional adaptation. The primary osteons of antler compact bone

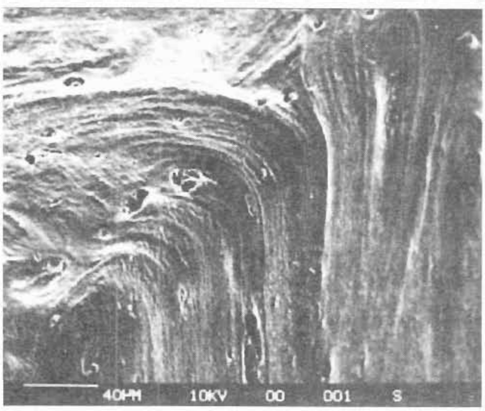


Figure 6. Scanning Electron micrograph of metallurgically polished orthogonal surfaces of compact bone from spotted deer. The osteons are in various directions.

Sample	Collagen	Phosphorus (P)	P/Nitrogen	Ash
Antler	29.1 ± 0.9	9.56 ± 0.21	1.64	55 ± 1.2
Bovine femur	23.6 ± 1.0	10.11 ± 0.26	2.29	66.6

Table 1. Chemical composition of compact bone from antler and bovine femur, expressed as per cent dry weight.

are also in all directions (*Figure* 6), whereas in mammalian long bones like the femur, they are oriented more along the length enabling it to bear the tensile and compressive forces acting on it. The degree of calcification of the organic matrix in antler is also less as reflected in the ratio of phosphorus to nitrogen – the rapid growth results in weaker bonding between mineral and collagen.

Testosterone and Antlers

A distinct correlation has been found with respect to serum testosterone and antler growth. Antler growth gets initiated by increasing levels of serum testosterone and this is also linked to day-length in the temperate regions. The level of testosterone remains high till antler growth is complete as also when the antler is functional. After the rut, the testosterone concentration diminishes and as it reaches the lowest value, antlers are cast off. Even though daylight is not of much consequence in the tropics, in the Kashmir hangul, with the onset of summer, the deer migrate to higher altitudes for breeding. The aggression actually develops in the rut season after antler growth is complete. Testosterone is also linked to decreased immune system function and subsequent higher parasite load.

Deer in hard antler during the rut are aggressive animals. Odd cases of zoo-keepers being gored by the 'gentle' deer in hard antler are known. In the far-east and the west, these observations have led to a market in dried antler velvet as an aphrodisiac. Many countries also commercially farm highly fecund species

Table 2. Physical properties of antler bone compared to bovine bone.

Sample (in normal functional state)	Dry density (g/mL)	Tensile strength (MPa)	Work to fracture (J/m^3 x 10^{-5})
Dry antler bone	1.86 ± 0.016	188 ± 12	13.5 ± 1.2
Bovine femur (wet)	1.94 to 2.04	99.2 ± 4.7	4.0 ± 0.4

like the spotted deer for skin and meat and the velvet may be an additional marketable item. Females, castrated males and hummels (or hornless males) can in fact, be induced to develop antler-like protuberances if injected with testosterone.

The structure and properties of antlers have been well studied, but our understanding of the biochemical and physiological mechanisms involved in antler growth is still unclear.

Utilization of Fallen Antlers

In nature, the fallen antlers of deer are a source of calcium to many animals. Porcupines are known to gnaw at antlers. It is speculated that calcium is required for quill growth and indeed, detectable levels of the element are found in the cortex region of quills though other hair keratins do not normally have any calcium. Gnawed antlers in fact, are an indication of the presence of porcupines around. Deer themselves are sometimes reported to chew on fallen antlers and perhaps other animals do the same for their calcium requirements. Fallen antlers constitute a minor forest produce that earlier used to be auctioned by forest departments. Antler bone is a constituent of many siddha and ayurvedic formulations, especially those used in relation to chest diseases. As in other areas of native medicine, much however, needs to be done for scientific validation of the described uses.

Conclusions

The structure and properties of antlers have been well studied, but our understanding of the biochemical and physiological mechanisms involved in antler growth is still unclear. Some of the as yet unanswered questions are:

1. How has the antler mechanism evolved? ie. How does it commit suicide by withdrawing blood supply, cast off the antler and re-grow it the next year?

2. How does the scalp transform to velvet skin as the first antlers develop?

3. When the antler is cast off, how and why do pedicel stumps give rise to antler buds instead of scar tissue?

4. How is it that tropical deer generate antlers any time of the

year whereas antler growth is linked to day-length in the temperate zones?

5. How does calcification occur at such a fast pace in antlers?

6. What is the connection between collagen type(s) present and mineralization at various stages of antler growth?

7. How does environmental quality affect antler evolution apart from sexual selection?

An understanding of the various mechanisms involved in antler growth is worth pursuing for its own sake, and also for the possible benefits in understanding bone pathologies.

Suggested Reading

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(Many of the above articles, especially post 1995, are freely downloadable from the Internet)

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