

Axial Orthogonal Fiber Reinforcement in the Penis of the Nine-Banded Armadillo (*Dasypus novemcinctus*)

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ABSTRACT Examination of histological sections from flaccid and artificially erected nine-banded armadillo (*Dasypus novemcinctus*) penises confirms that the mammalian corpus cavernosum is the first known biological hydrostat reinforced by collagen fibers arranged at 0° and 90° to its long axis. The morphology of this axial orthogonal fiber array affects the mechanical behavior of mammalian penises during erection and copulation. Specifically, the axial orthogonal array gives the erect penis a reproducible shape, maximum size and resistance to tensile, compressive, and bending forces. These features are more appropriate for the mechanical regime associated with copulation than those found in structures reinforced by crossed-helical fibers, although the axial orthogonal array also gives the corpus cavernosum a tendency to fail by kinking. Crimped collagen fibers in the flaccid array as well as three-dimensional folding of the wall in the flaccid corpus cavernosum allow the structure to expand during erection. *J. Morphol.* 233:249–255, 1997. © 1997 Wiley-Liss, Inc.

Hydrostats are mechanical support systems characterized by a central volume of pressurized incompressible fluid surrounded by a membrane in tension. They are found in a wide variety of organisms, including plants, worms, mollusks, arthropods, echinoderms, and vertebrates (Wainwright et al., '76, '78; Neville, '93). The structure responsible for penile erection in mammals—the corpus cavernosum—is hydrostatic (Fig. 1). During erection, its central vascular space fills with pressurized blood, and the membrane surrounding the space, the tunica albuginea, is placed in tension as it resists the internal pressure (Purohit and Beckett, '76; Kelly, '94).

Cylindrical hydrostats are reinforced with relatively inextensible fibers that prevent aneurysms; due to geometry, wall stresses around the circumference of a cylindrical structure with a homogeneous wall are twice as great as the stresses along its length (Wainwright et al., '76). The walls of other known hydrostats are reinforced by polymeric fibers arranged in left- and right-handed helices where the fibers may be orthogonal to each other but are at an oblique angle to the long axis of the hydrostat (Wainwright et al., '76, '78; Wainwright, '88; Neville, '93). In contrast, the tunica albuginea of

dogs (Hanyu, '88) and humans (Hsu et al., '94a,b) are reported to contain collagen fibers arranged both parallel and at 90° to the penile long axis. I will refer to this organization of fibers as an axial orthogonal array.

Axial orthogonal fiber arrays have been found in biological systems that are not hydrostatically supported; examples include the body wall of the starfish *Echinaster spinulosus* (O'Neill, '89) and some insect eggshells (Neville, '93), but they have not been described in any hydrostatic system other than the mammalian corpus cavernosum. Although the ampullae of tube feet in the starfish *Luidia clathrata* and *Astropecten articulatus* are hydrostatically supported and contain collagen fibers parallel to their long axes, they have no perpendicularly arranged collagen fibers (McCurlley and Kier, '95) and are thus not reinforced by an axial orthogonal fiber array.

Published observations of fiber orientation in corpora cavernosa (Hanyu, '88; Hsu et al., '94a,b) lacked both explicit control of the plane of section in respect to the orientation of the structure's long axis and careful

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Corpus cavernosum:

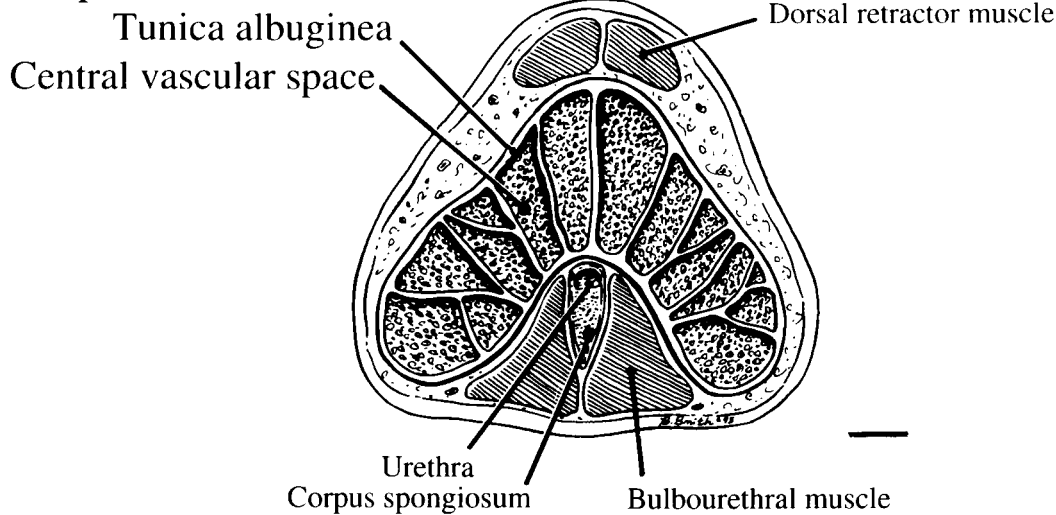


Fig. 1. Cross-section of an erect armadillo (*Dasypus novemcinctus*) penis. The corpus cavernosum is the structure responsible for penile erection and contains the vascular space and surrounding tensile membrane characteristic of hydrostats. The strands spanning the corpus cavernosum are collagenous cords; they do not divide the vascular space into smaller chambers. Scale bar = 1 mm.

measurement of collagen fiber angles. Recent studies of penile erection (see reviews by Benson, '81; Williams-Ashman, '90; Creed et al., '91; Andersson and Wagner, '95) focus on the innervation and hemodynamics of the corpus cavernosum and do not address the mechanical contribution of the tunica albuginea to erection and copulation. In this study, I attempt to determine whether the corpus cavernosum is reinforced by a true axial orthogonal fiber array and examine the mechanical consequences of this morphology on erection and copulation.

MATERIALS AND METHODS

Twelve male nine-banded armadillos (*Dasypus novemcinctus*) killed by motor vehicles on roads within 2 h before collection were collected near Tallahassee, Florida. Penial tissue distal to the ischial attachment was removed from each individual and skinned to expose the corpus cavernosum. Specimens ranged from 39.2–49.4 mm in flaccid length. Whole corpora were fixed in 10% buffered formalin (Kier, '92) six while flaccid and six after artificial inflation of their corpora to erection. A corpus cavernosum was artificially inflated by first sectioning it immediately distal to the split of its

crurae to expose its vascular space. The opening to the vascular space was tied shut with cotton thread and injected with 10% buffered formalin until fluid leaked out of the opening, indicating that the corpus was at or near maximum volume. Corpora were transferred into 70% ethanol after at least 48 h in fixative.

Pieces of tissue from these corpora were embedded in paraffin or glycol methacrylate plastic, sectioned, and examined with bright-field and polarized light microscopy. For paraffin embedding, transverse sections of the whole corpus cavernosum (approximately 1 cm thick) were cut from one flaccid and one artificially erected corpus, dehydrated in ethanol, cleared in Hemo-De (Fisher Scientific, Fair Lawn, NJ), and embedded in Ameraffin (Baxter Scientific Corporation, McGaw Park, IL). Blocks were sectioned at 10 μ m with a rotary microtome in three mutually perpendicular planes and stained for collagen and elastin with either Milligan's Trichrome or Verhoeff's elastin stain (Humason, '62).

Triangular sections of the corpus cavernosum (approximately 4 mm across their widest point) were removed from arbitrarily selected locations on the dorsal and lateral sides of five flaccid and five artificially

erected corpora. These sections included both wall tissue and vascular tissue; the fixed vascular tissue supported the wall tissue and prevented it from rolling up during preparation. Tissue was dehydrated in ethanol and embedded in glycol methacrylate plastic (Leica Instruments, Deerfield, IL). These blocks were sectioned at 4 μm with glass knives on a rotary JB4 microtome in three mutually perpendicular planes and stained with Lee's methylene blue–basic fuchsin stain (Bennett et al., '76). Because small samples of tissue had to be prepared to permit unpolymerized glycol methacrylate to infiltrate the tissue, segments of 0.5 mm thick pencil leads were inserted into the vascular tissue of four corpora to show the direction of the structure's long axis.

Fiber angles were measured on photomicrographs of stained sections cut parallel to the outer surface of the tunica albuginea from the ten corpora embedded in glycol methacrylate. Two photomicrographs were taken of the tunica albuginea in each corpus (magnified $\times 68$), one of the outer layer and one of the inner layer, oriented so the edges of the photograph would denote the perpendicular axes of the corpus cavernosum. Two to three fiber angles were measured on a 5 in \times 7 in (12.7 cm \times 17.8 cm) print of each photograph. Fibers selected for measurement extended across the entire length of the photomicrograph. The overall direction of a collagen fiber was determined by drawing a line between two randomly selected points on the fiber. The angle of this line with respect to the long axis of the penis was measured with a protractor to a precision of $\pm 1^\circ$; measurements repeated a second time were within $\pm 2^\circ$. A pooled total of 27 measurements was taken from each layer.

Thicknesses of the collagen fiber layers in the tunica albuginea were measured with an ocular micrometer to a precision of $\pm 0.5 \mu\text{m}$ (measurements repeated a second time were within $\pm 1 \mu\text{m}$) from randomly selected transverse sections and parasagittal sections taken from all 12 armadillos. Fiber layer thicknesses were measured on two or three sections from each armadillo, for a total of fifteen measurements from flaccid tissues and twelve measurements from erect tissues.

Fiber diameters in the outer and inner layers were measured with an ocular micrometer to a precision of $\pm 0.2 \mu\text{m}$ (measurements repeated a second time were within

$\pm 0.4 \mu\text{m}$) using parasagittal sections from one flaccid corpus embedded in glycol methacrylate plastic. Fiber diameters were measured on five randomly chosen sections of each fiber layer; two fibers were measured in each section, for a total of ten measurements from each layer.

Results are expressed as means with standard error shown in parentheses.

RESULTS

Tunica albuginea morphology

Sections of *Dasypus novemcinctus* tunica albuginea stained with Milligan's Trichrome and Verhoeff's elastin stain show that the tissue is mostly composed of fibers with the staining characteristics of collagen arranged in regular parallel bundles (Fig. 2). Elastin was largely absent in sections stained with Verhoeff's elastin stain, with no more than one or two strands visible in any section.

Collagen fibers in flaccid tunica albuginea are highly crimped; most of the collagen crimp is lost in artificially erected corpora (Fig. 2). In addition, at low magnifications the flaccid tunica albuginea appears folded, with the tissue thrown into deep wrinkles perpendicular to the outer surface of the corpus cavernosum; much of the tissue folding is also lost in artificially erected corpora. The density of the collagen fibers and their angle of crimping appeared constant in serial peripheral sections of the tunica albuginea.

These data correlate well with previous studies of the tunica albuginea in humans (Goldstein et al., '82; Moreland et al., '95) and dogs (Hanyu, '88). These authors state that the tunica albuginea is made up mostly of thick bundles of type I collagen fibers and a small amount of elastin, although they do not measure the relative percentages of these fiber types in the tissue. Flaccid human and dog corpora also contain highly crimped collagen fibers and lose much of the crimp upon erection.

Fiber arrangement about the corpus cavernosum long axis

Fibers in the armadillo tunica albuginea are arranged in two major layers (Figs. 2, 3). In the outer layer, the fibers are arranged parallel to the long axis of the penis. In the inner layer, the fibers run circumferentially around the corpus cavernosum, perpendicular to the long axis of the penis. Fibers at the transition between the outer and inner lay-

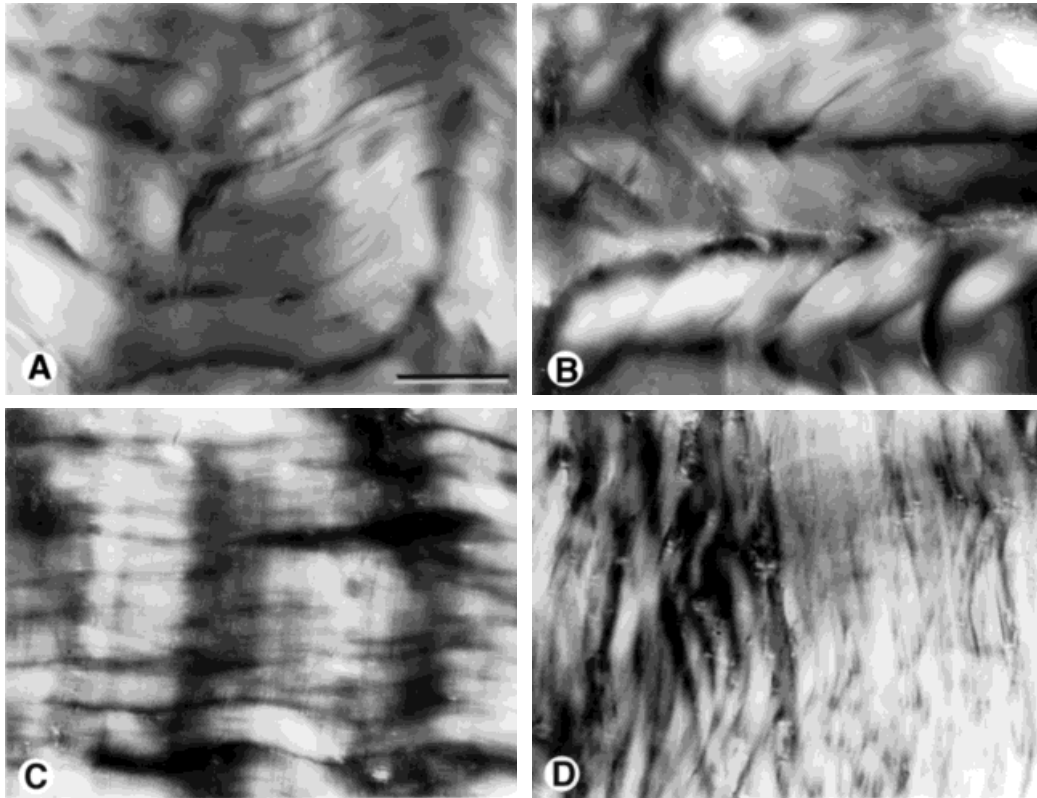


Fig. 2. Photomicrographs of parasagittal grazing sections of unstained tunica albuginea from *Dasypus novemcinctus* in glycol methacrylate. The tissue is magnified 400 times and viewed under partially polarized light. **A:** Outer layer of flaccid corpus. **B:** Inner layer of flaccid corpus. **C:** Outer layer of erect corpus. **D:** Inner layer of erect corpus. Scale bar = 0.02 mm and denotes the

direction of the long axis of the corpus cavernosum for all four photographs. Collagen fibers in the flaccid tunica albuginea are highly crimped; most of this crimp is lost in the erect tissue. Crimping of collagen fibers can be seen as alternating bright and dark bands in the direction of the collagen fibers. The small vertical lines visible in C are a sectioning artifact.

ers show a lower degree of preferred orientation; this thin layer (Table 1) contains fibers randomly arranged in a single plane.

The average fiber angle in the outer layer with respect to the long axis of the corpus is 0° (0.4°), $n = 27$ measurements; the average fiber angle in the inner layer with respect to the long axis of the corpus is 90° (0.3°), $n = 27$ measurements. Student's t -tests show that the pooled values of fiber angles from the outer layer are not significantly different from 0° ($t = 1.18$, $P = 0.24 > 0.05$), and the pooled values from the inner layer are not significantly different from 90° ($t = 0.41$, $P = 0.68 > 0.05$). These data cannot detect any difference in the collagen fiber arrangement in the tunica albuginea of the nine-banded armadillo from an array where fi-

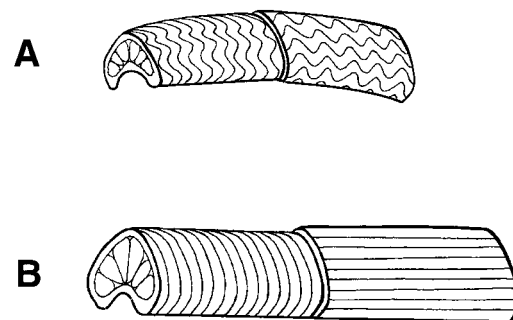


Fig. 3. Diagram of collagen fiber arrangement in *Dasypus novemcinctus* tunica albuginea. There are two layers: an outer layer with fibers at 0° to the long axis of the penis and an inner layer with fibers at 90° to the long axis. Collagen fibers in the flaccid corpus are highly crimped; most crimp is lost upon erection. **A:** Flaccid. **B:** Erect.

TABLE 1. Average thickness of layers in *Dasyopus novemcinctus tunica albuginea* from penises ranging from 39.2–49.4 mm in flaccid length and from 48.9–64.8 mm in erect length¹

	Outer layer	Inner layer	Transition layer
Flaccid (n = 15)	422 (33.9)	930 (32.7)	67 (5.6)
Erect (n = 12)	239 (26.9)	594 (53.6)	42 (4.9)
Fiber orientation to corpus long axis	Longitudinal	Circumferential	Random

¹Measurements are in micrometers, with standard error in parentheses.

bers are arranged orthogonally at 0° and 90° to the long axis of the structure.

Measurements from transverse and parasagittal sections of tunica albuginea show that in erect corpora the outer layer is 43% thinner than in flaccid corpora; the inner layer is 36% thinner in erect corpora (Table 1). Fibers in both layers have diameters of 32.8 (2.05) μm , n = 10 measurements for each layer.

DISCUSSION

This study of tunica albuginea from the nine-banded armadillo (*Dasyopus novemcinctus*) confirms observations that the mammalian corpus cavernosum is the first known hydrostatically supported biological structure reinforced by an axial orthogonal fiber array. Published observations of the tunica albuginea of dogs (Hanyu, '88) and humans (Hsu et al., '94a,b), as well as preliminary histological examination of the tunica albuginea from white-tailed deer (*Odocoileus virginianus*), maned wolf (*Chrysocyon brachyurus*), and African elephant (*Loxodonta africana*) (Kelly, unpublished observations), suggest that both the arrangement of collagen fibers orthogonal to the long axis of the corpus cavernosum and the crimping of those collagen fibers in the flaccid corpus may be characteristic of many mammalian taxa. It is thus of interest to examine the mechanical consequences of this morphology on the behavior of mammalian penises during erection and during intercourse.

*Mechanical behavior of the corpus cavernosum during erection:
The effect of folding and crimping*

Changes in penile dimensions during erection are brought about exclusively through

the macroscopic unfolding of the tissue and the straightening of crimped collagen fibers as the corpus cavernosum fills with blood. The folded tissue and crimped fibers allow the corpus cavernosum to expand simultaneously in length and width during erection; fibers in the thin transition layer may bind the highly organized outer and inner layers together and accommodate shear strains between the layers as the corpus changes its shape and size.

Tissue expansion is not a unique feature of the axial orthogonal array: structures reinforced by fibers in crossed-helices such as mammalian arteries (Fung, '81, '84), fin whale throats (Orton and Brodie, '87), and pufferfish skin (Brainerd, '94) also contain deeply folded tissue and crimped collagen fibers that extend upon erection to permit inflation. When the collagen fibers reach full extension, these tissues become stiffer due to the high tensile elastic modulus of collagen. The same mechanical behavior should be found in the tunica albuginea.

But the axial orthogonal fiber array gives the tunica albuginea a mechanism for stopping its shape change once it approaches its maximum volume. Hydrostats reinforced by crossed-helical arrays of fibers can still change their dimensions when their fibers are fully extended by changing the angle the fibers make with the long axis of the structure (Clark and Cowey, '58; Wainwright, '88). For example, a short and fat crossed-helical hydrostat with a fiber angle close to 90° can increase its internal volume and become longer and thinner because its reinforcing fibers reorient, reducing their fiber angle toward 55° (Clark and Cowey, '58). In contrast, the parallel and perpendicular arrangement of collagen fibers to the long axis of the corpus cavernosum means that once the fibers are fully extended any further increases in intracavernosal volume will place them in tension. The axial orthogonal array will therefore prevent further changes in the length and girth of the corpus cavernosum, giving it a reproducible maximum erect size.

*Mechanical behavior of the corpus cavernosum during copulation:
The effect of an axial orthogonal array*

The mechanical behavior of a hydrostat is directly affected by the arrangement of the fibers in its wall. Most pressurized organs and organisms are reinforced by a crossed-helical fiber array, where fibers are wrapped

in left- and right-handed helices around the long axis of the structure. Crossed-helical fiber reinforcement permits constant-volume hydrostatic bodies to lengthen and shorten easily, to bend smoothly without kinking, and to resist torsional stresses. If crossed-helical hydrostats at constant volume change their dimensions, their fiber angle reorients to accommodate forces in the tissue (Clark and Cowey, '58; Aspden, '86). When one of these structures elongates and becomes longer and thinner, its fiber angle decreases; when it contracts and becomes shorter and fatter, its fiber angle increases (Clark and Cowey, '58). These structures can bend easily because they contain no collagen fibers that are oriented parallel to the direction of the bending force.

The helical arrangement of fibers makes sense for structures that are flexible. But penises need to remain rigid during intercourse, resisting external forces that could change the shape of the penis and prevent the male from achieving intromission. Once the penis is erect, the axial orthogonal fiber array helps the corpus cavernosum resist external forces.

The axial orthogonal array gives the erect corpus cavernosum the ability to resist tensile and compressive forces. Because venous outflow is restricted through the corpus cavernosum when it is erect (Hanyu et al., '87; Andersson and Wagner, '95), it remains at constant volume throughout copulation. So forces on the end of the erect penis that would tend to telescope its length and increase its girth are directly opposed by the high tensile modulus of the circumferential collagen fibers. Similarly, compressive forces from the vulvar musculature that would tend to extend the erect penis are directly opposed by the longitudinal collagen fibers. Therefore, the array gives the erect corpus cavernosum a fixed shape.

The axial orthogonal fiber array is also the collagen fiber arrangement that gives maximum flexural stiffness (Koehl et al., '95). In an erect corpus cavernosum, bending forces put the longitudinal collagen fibers on one side of the array directly into tension. Bending experiments with inflatable models supported by fiber arrays have shown that cylinders supported by axial orthogonal arrays have a higher flexural stiffness than those supported by crossed-helical arrays (Koehl et al., '95). However, collagen fibers do not resist compressive forces well, so large bend-

ing forces will tend to make the structure fail on the side under compression, giving the penis a sharp kink. Penile kinking has been observed in copulating dogs (Grandage, '72) and rhinoceros (Fish, personal communication).

CONCLUSIONS

The corpus cavernosum of the nine-banded armadillo is a hydrostatic structure reinforced by collagen fibers arranged in an array orthogonal to the penile long axis. This feature should give the corpus cavernosum properties more appropriate to the mechanical regime associated with copulation than those found in structures reinforced by crossed-helical fibers. Specifically, it should allow the erect corpus cavernosum to resist tensile, compressive, and bending forces during copulation, although this type of reinforcement will also make the corpus cavernosum susceptible to failure by kinking. Three-dimensional folds and crimped collagen in the flaccid tissue allow the corpus cavernosum to expand during erection; the axial orthogonal array stops the expansion at a repeatable maximum size.

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