KERATIN STRUCTURES OF CERATOTHERIUM

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INTRODUCTION

Keratin, in dense molecular forms, is the prevalent component of many anatomical features of mammals (extensively reviewed by Marshall et. al. 1991). In addition to being a key ingredient in the complex morphology of skin, it is the primary structural material of hair, fur, quills, vibrissae, scales, combs, hoofs, nails, claws, sheaths of some head horns, nose horns, balleen and the bill of the Platypus. The rhinoceros has four such anatomical structures, namely tail hair, ear hair, hoof and horn. Given the interest in this laboratory of developing substitutes for rhino horn, possibly from the hair or hoof trimmings of the animal, to be used in prescriptions of traditional oriental medicine, it became important to compare these structures in an effort to ascertain and expose their similarities and differences. The report of Butter et. al. (1990) presents the molecular distinctions between the three main types of mammalian keratin (hair, hoof, horn) and concludes that "Keratin is not a single substance but a complex mixture of proteins and the sulfur containing diamino acid cystine." The goal of the study reported here was to explore the microscopic differences in keratin structures of the rhinoceros. Numerous studies have been conducted on the composition of rhino horn (eg. Butler, et. al. 1990; Lynch, et. al. 1973; Groves, 1971; Ryder, 1972), but the literature is almost bereft of reports about rhino hoof or hair, possibly the only one being found in Van Orden and Daniel (1993). Horn, at all structural levels, differs somewhat between the species of rhinoceros (Butler, et. al. 1990, Groves, 1971, Earland et. al. 1962), but to evade any confusion from these differences, all of the samples used were from the white rhinoceros (Ceratotherium simum), specifically from one animal, the male at the Virginia Zoological Park - "Rufus."

METHODS AND MATERIALS

In 1990 and 1992, respectively, Rufus broke the tip of his large horn and a portion of the hoof of his right foreleg. The pieces were recovered by the keepers and given to the senior author. The keepers also cut hair samples from both the tail and the ear for this study. All samples were stored dry between the time of collection and their preparation for study.

Both types of hair were prepared in two ways. To facilitate viewing them microscopically in longitudinal array, hairs were positioned in parallel on an aluminum stub, care being given to evade overlapping, and coated with 100 Å of Au/Pd. They were photographed at different magnifications with a scanning electron microscope (Cambridge Steroscan 100). To see the internal organization, revealed by cross sections, hairs were imbedded in epoxy resin using standard histological techniques, cut in section and polished to a smooth surface. Standard metallurgical techniques for hard composite materials were used to obtain the smooth surface, namely through a series of progressively finer grit sand-paper and final polish with diamond slurry and 0.3 micron alumina as described by Van Orden & Daniel (1993). Individual hairs were measured and appropriate fields were photographed through optical light microscopes.

The denser structures, hoof and horn, were cut with a fine-toothed saw both with and across the grain striations and polished as described. The polished surfaces were studied, measured and photographed microscopically.

RESULTS

Plates A-D in Figure 1 show all structures in longitudinal position (section) and Plates E-H in Figure 2 are photographs of the cross sections.

The sections of horn confirm the compacted filamentous structure already well known from the papers referenced in the introduction. The filaments are composed of two layers surrounding an inner core or medulla, the middle one (cortex) being contained by a denser bounding layer, the cuticle. The outside diameter of the filaments ranges from 300 μm to 500 μm, averaging 420 μm; on average the cuticle is 30 μm thick, the cortex 160 μm and the medulla about 35 μm. The filaments frequently are in direct contact with each other, sometimes blending their cortex inside of a common cuticle, but are mostly separated up to about 150 μm apart and the intervening space filled with a matrix network of tiny fibers. Van Orden and Daniel (1992) used energy dispersive X-ray spectroscopy and X-ray diffraction analysis to show that both the filaments and the matrix fibers were composed of keratin. The paper of Lynch et. al. (1973) shows that the filaments are built of concentric laminae of flat cortical cells, possible of two different types.

The longitudinal sections also confirmed the preliminary observation of Van Orden and Daniel (1992) that the filaments of horn are not continuous structures that grow unimpeded from follicles to horn tip. Rather they are interrupted, elongated spindle-like filaments that appear to interdigitate and expose their bare, rounded ends at points of breakage. This fact supports the speculation that the follicles producing the filaments periodically cease production (possibly during droughts or times of starvation) and reinstate it when conditions improve or upon receipt of some unknown signal. However, if all follicles paused in their filament production at the same times, then the discontinuity would cause the horn to have one or more weak spots especially prone to breakage. To the best of the authors' knowledge this has not been observed. Furthermore, captive rhinos are well maintained and therefore not generally subject to periods of malnutrition, excess thirst or any other environmental stress: As the horn sample used here came from a captive rhino, it is therefore, most unlikely that the filament periodicity can be assigned to such events. Apoptosis, progressive cell death, of cells in the follicles followed by renewal is another possibility, but only speculative at best. The question merits further investigation.

Rhinoceros' hoof does not have the heavy filamentous structure of horn, but has sparsely separated filaments in a compaction of much smaller fibers, partially aligned proximo-distally along the tip and frontal sides of each digit. These few filaments are smaller than the horn filaments, have less well defined cuticle and cortex and have no dense core as a medulla, but rather a diffuse amorphous central region about 100 μm in diameter. It is impossible to determine whether the few filaments in hoof are interrupted along their length and the entire structure is far less defined than horn. Rhino hoof resembles horse hoof (Bertram and Gosline 1987), but with fewer and larger filaments. Ryder (1962) gives 25-50 μm as the diameter of horse hoof filaments separated by 50-100 μm of matrix material: in the rhino hoof the filaments are 100-200 μm in diameter interspersed by 200-400 μm of matrix.

Tail hair possesses the same three layers as in the classical structure found in all hair (eg. Wake, 1979; Pough et. al. 1989; Marshall et. al. 1991), but aligned differently. Obviously, because each hair is a separate self-defined structure (as contrasted to the compacted form found in horn and hoof) the cuticle is, and must be, thicker and supportive. The cortex is evenly distributed throughout the central portion of the hair and running through it, seemingly at random, are dense cores (medullae) which vary in section from round to oval to semicircular and in number from one to six. Ear hair has no medulla, the cortex being the only central feature. The tail hair is the heaviest, being about half to two-thirds the diameter of horn filaments and averaging about 200 μm. (Measurements of 15 randomly selected samples ranged from 154 to 308 μm with 226 μm average.) The ear hair is smaller, less straight and averages 66 μm in diameter from 15 sample measurements.

CONCLUSIONS

Rhinoceros horn is not just compacted hair but a specialized structure of long, heavy keratin filaments bound together in a fibrous network matrix. Hoof is more amorphous with an extensive matrix infiltrated by a few filaments, far less concentrated than in horn and substituting a less compact central area for the small, dense core found in horn filaments. Ear hair has no medulla, but tail hair is structured in the typical mammalian format. Tail hair is about three to four times the size (diameter) of ear hair and about half the size of horn filaments; the internal structure of both types of hair is significantly different than that of the filaments.

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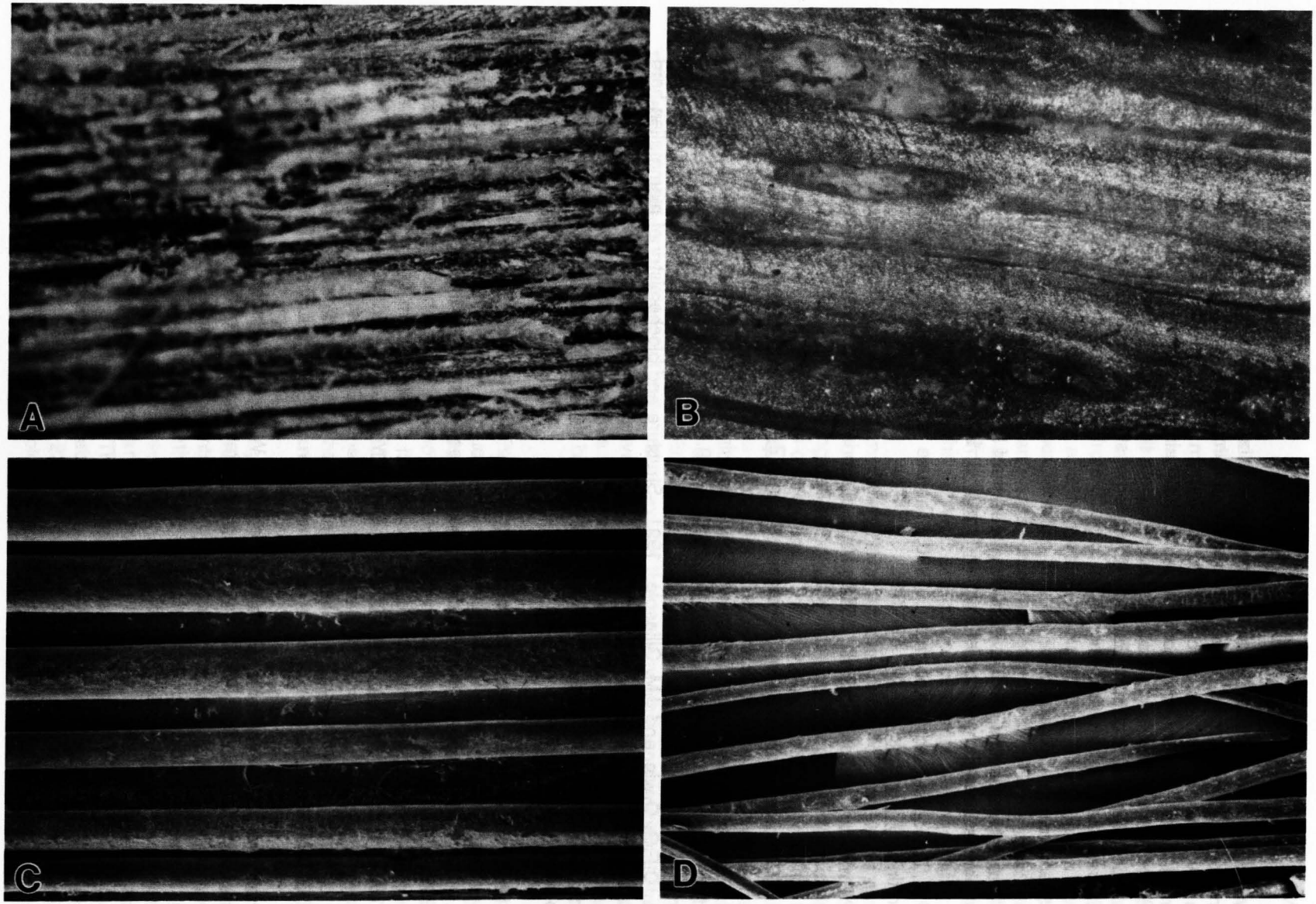

Figure 1. Longitudinal views of: A. Horn section at 10x. B. Hoof section at 50x. C. Tail hair intact at 36x. D. Ear hair intact at 50x. A and B are light micrographs; C and D are scanning electron micrographs.

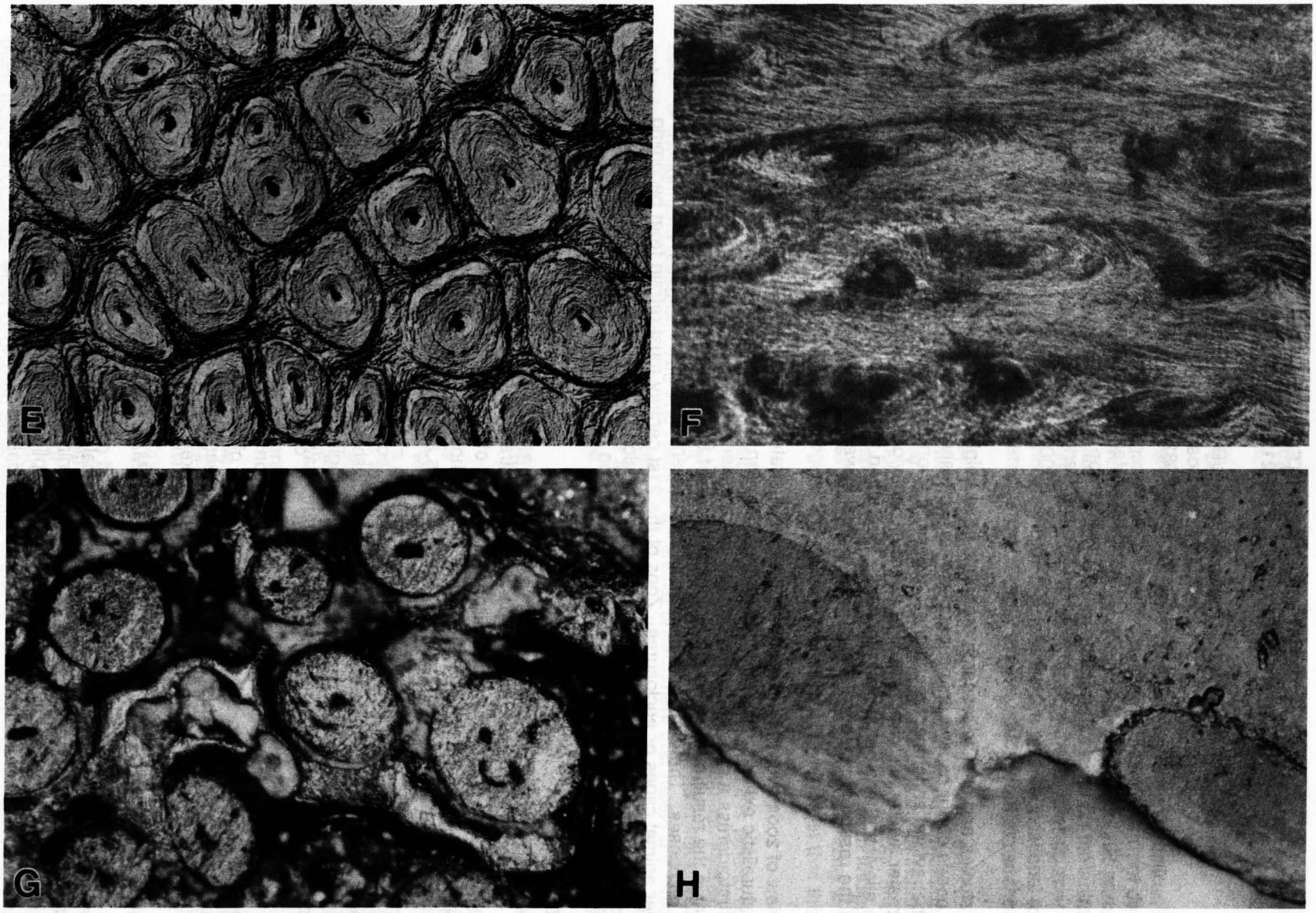

Figure 2. Light micrographs of cross-sections of: E. Horn at 50x. F. Hoof at 100x. G. Tail hair at 100x. H. Ear hair at 350x.