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Structure and Composition of Rhinoceros Horn

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ABSTRACT

Rhinoceros horn has been used medicinally and as a talisman in many cultures and animals are slaughtered to obtain the horn. With the dwindling populations of rhinos, and the limited number and breeding success of captive rhinos, there is a critical need to learn as much as is possible about their horns to find an adequate substitute. Examination of rhino horn was made using optical microscopy, scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDS), and x-ray diffraction (XRD). The structure of the horn is unusual and consists of two separate phases, one of hair-like filaments, built around a central core in circumferential layers and the other surrounding and filling in the spaces between the filaments as a matrix. Together, these two structures make up a biological composite, structurally similar to metal, ceramic or polymer based composites. The structural morphology, the dimensions of the structures, and the chemistry of the horn are discussed. Comparisons are made between horn, hoof, and hair of rhinos and hoof and hair from horses, their nearest living relatives.

INTRODUCTION

The horn of the rhinoceros, an anatomical specialization which has evolved to its present functional form over sixty million years, has, in modern times, become the focus for rapid destruction of these animals by poachers who seek it for monetary gain. The severity of the problem is emphasized in the recently released Rhino Global Captive Action Plan, [1] which notes that of twelve subspecies of rhinoceros, composing the five surviving species, seven are considered to be *critical*, four *endangered*, and one *vulnerable*, according to the new Mace-Lande [2] method of classifying the probability of extinction. We concluded that the production of a synthetic horn facsimile, to dominate the market, could reduce, or possibly eliminate poaching pressure on these animals, and/or the mimicry of the chemistry and structure of the horn as a model for new materials could generate a greater demand for conserving rhinos. Both of these concepts require a detailed understanding of the composition and structure of rhino horn and the developmental events that produce it. This paper reports initial studies directed toward that goal.

Reference to the literature reveals some disagreement about the composition of rhinoceros horn. Ryder [3] noted that various early investigators described its basic structure as that of matted hair, coarse fibers, filaments, canals or tubules. Using light microscopy, with histological staining and swelling techniques, Ryder attempted to resolve the issue with studies of horn from the white rhinoceros (*Ceratotherium simum*). He concluded that the horn was built of closely-packed filaments composed of concentric laminae around a solid medulla and with interfilamentous material in the interstices. The same general structure was confirmed in the other four species of rhino by Earland et al. [4]. In 1963, Lynch et al. [5] reported scanning electron microscopy (SEM) studies of white rhino horn. Their results were "broadly consistent with previous studies", and emphasized the presence of flat scale-like cells which compose the layers of laminae and identified another cellular unit "associated with the outer regime of the filaments or the interfilamentous material or both". In spite of these confirming studies, some recent publications persist in describing rhino horn as consisting of "an aggregation of hollow keratin fibers, similar to hair, but lacking the outer cuticle" [6]. In this paper a variety of techniques, including light and electron microscopy, are employed to assure a clear understanding of the composition of this unique material.

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Figure 1: The top surface of rhinoceros horn which the rhino has polished to smoothness by rubbing it against harder surfaces. At this magnification (bar represents 1mm) light and dark striations can be seen on the surface along with rougher areas within the striations.

EXPERIMENTAL PROCEDURES AND RESULTS

The primary sample used in these studies was a two inch piece of horn which was broken from the tip of the horn of a captive male white rhino. It was provided for this study by the Virginia Zoological Park, for which we are appreciative. In the following discussion, this specimen was examined by several different techniques. Each technique is discussed separately with experimental results included.

Light Microscopy

Light microscopy was used to examine the surface and cross-section of the rhino horn. Visually the surface was smooth and rounded in perspective. There was a definite sheen to the top surface of the horn, which is where the animal rubs it against tree trunks, metal fence posts, and the ground. Because the horn has limited wear resistance when rubbed against harder surfaces, continuous wear will polish the surface and this is the natural state of the horn.

When examined under magnification, the surface shows parallel striations of light and darker areas and rougher areas within the striations. These features are noticeable in Figure 1 which shows the smooth surface of a section of the horn.

Figure 2 shows the point at which this section of horn was broken from the main horn. Clearly defined are hair-like filaments which project from the broken interface. The ends of these filaments come to a definite point and their diameters lessen significantly just prior to the formation of the point. Between the filaments is a separate region designated as the matrix phase. It has a fibrous texture, as well. Thinner fibers can be seen in the matrix material, as is demonstrated in Figure 2.

A piece of the rhino horn was cut perpendicularly to the direction of the hair-like structures. It was vacuum encapsulated into epoxy resin and polished using metallographic polishing methods. These methods involve grinding with sand paper starting at 320 grit, followed by 400, 600, and 1200 grit sand papers. It was then polished with 3 micron diamond slurry and final polished with 0.3 micron alumina until a scratch-free surface was produced for photomicroscopy. This type of preparation follows standard metallographic techniques for metal, polymer and composite

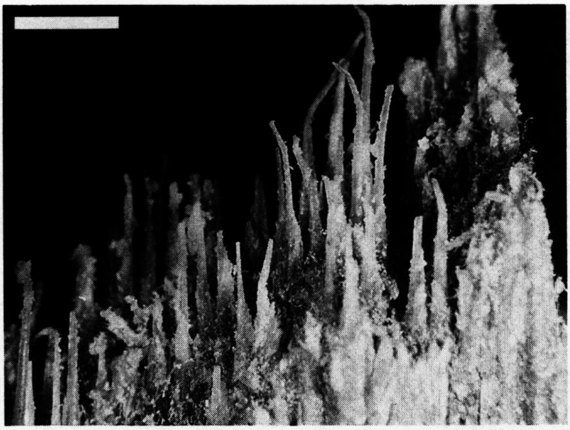

Figure 2: The broken end of the piece of rhinoceros horn with hair-like filaments projecting from the interface. Bar represents 1mm. Note that these filaments come to a point with the diameter lessening prior to the point. The fibrous texture of the matrix phase is apparent here as well.

materials, but appears to be the first time that rhino horn has been prepared and examined in this way. Figure 3 shows the result of this process. The structure of the rhino horn can clearly be seen in this cross-sectional view.

In cross-section, the filaments are seen to be built around a central core in circumferential layers and the matrix phase surrounds the hair-like structures, filling in the spaces between them. In the light micrograph, Figure 3, variations in depth can be seen from the highest point at the edge of the filaments, to the matrix phase, the lowest. This suggests differences in hardness of the phases and regions within the phases. The matrix appears to be the softest, since it polishes most easily; while the filaments are harder and polish more slowly. Some of the hair-like filaments have elongated center cores, and some appear to have developed from two separate cores. Examples of both of these features can be seen in Figure 3.

Higher magnification of one of the hair-like structures is shown in Figure 4. Smaller features which were less visible at lower magnification can be seen in this figure. The circumferential layering of the filaments, at this magnification, looks like growth rings. Some cracks can be seen within the hair-like filaments which seem to correspond to growth rings. The cracks do not extend into the matrix. There are also small dark spots at the interfaces of the feature which look like growth rings. Within the matrix phase, the filamental nature of the matrix is evident. At even higher magnification, examination of the cracks within the hair-like phase shows that there are small filaments which bridge the cracks. Some of the cracks appear to initiate at the darker regions noted in Figure 4.

Scanning Electron Microscopy (SEM) of Rhino Horn

The rhinoceros horn was examined both in cross-section and the bulk material, as received. Upon examination in the SEM, of the bulk material, most notable were the ends of the hair-like structures which extended out beyond the edges of the fracture (see Figure 2). Previous authors [5] who have examined rhinoceros horn using SEM have noted the two phases, describing them as "filaments" and "flat scale-like cells". These designate the hair-like phase and the matrix phase, specifically.

The smooth top surface was found to have a directional orientation parallel to that of the

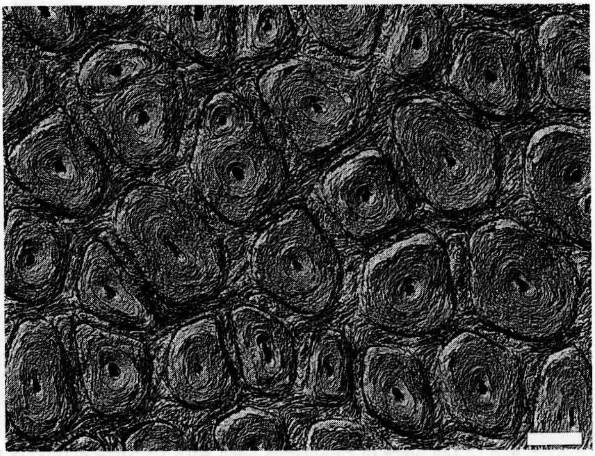

Figure 3: A cross-sectional view of the rhino horn prepared by standard metallographic techniques. Bar represents 0.2mm. In this view both the hair-like filaments and the matrix phase are apparent. The filaments are composed of circumferential layers built around a central core. The matrix phase fills in the spaces between the filaments. Differences in hardness between phases is emphasized by the polishing technique and can be seen as variations in height.

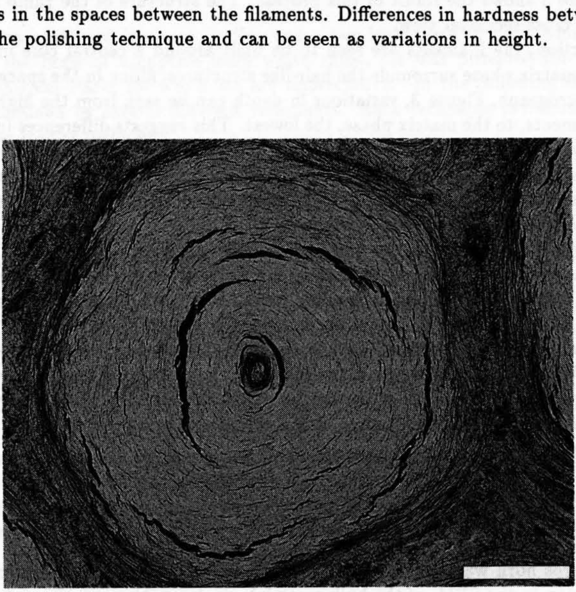

Figure 4: At this magnification (bar represents 0.1mm) the filaments appear to have a structure like growth rings. Cracks can be seen at the interfaces of the circumferential layers. The cracks are confined to the filaments and do not extend into the matrix. Small dark spots can be seen at the interfaces of the circumferential layers.

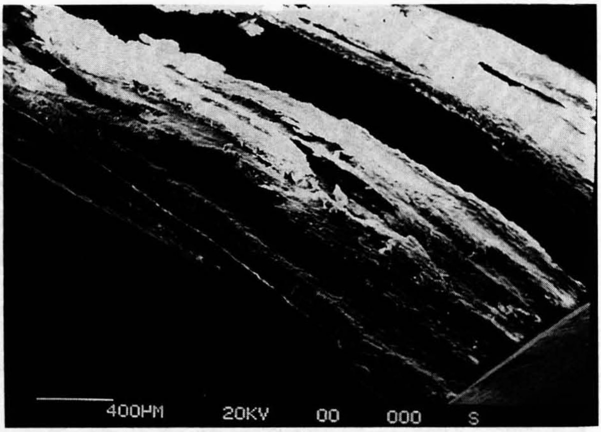

Figure 5: The top surface of the rhinoceros horn as is seen in the SEM, at 20KeV, a tilt angle of 35 degrees with a working distance of 15mm. Magnification is marked on the photo. Individual filaments cannot be noted, although there is a directional orientation to the surface which is parallel to the filament direction.

filaments, but no individual filament could be observed on the top surface. Figure 5 is a view of the surface of one portion of the horn.

Cross-section of the horn, Figure 6, showed structure similar to that demonstrated by light microscopy in Figure 3. Regions which showed black in the light micrograph are distinct and white in the SEM image and are interpreted as cracks or gaps in the horn. These cracks follow the circumference of the central core and appear to be related to the structure of the "growth rings" within the filaments. The interface between the filaments and the matrix is evident as an almost continuous white line following the contours of the edges of the filaments. In the matrix phase, the white regions are less pronounced and smaller than in the filaments. Some directionality can be noted in the white regions in the matrix, as they follow the outer contours of the filaments. The differences in contrast (white to dark) are due to charging within the SEM. Charging occurs when a less conductive area, like a gap, is next to a conductive area. The edges of holes often show charging effects in SEM photographs.

In general, the SEM photographs provided useful comparisons with those taken using light microscopy. The SEM also allowed some of the features in the interior of the cross-section to be imaged directly and emphasized some of the structural aspects of the cross-section to be demonstrated. The SEM provided the imaging capabilities while the energy dispersive x-ray spectroscopy (EDS) system allowed composition to be examined.

Energy Dispersive X-ray Spectroscopy

EDS was performed on the samples which were prepared for the SEM. Both the surface of the horn and polished cross-sections were examined. For analysis of the exterior surfaces, a layer of gold-palladium metal was sputtered onto the surface to enhance the conductivity of the surface and eliminate "charging" of the sample, which is caused when a surface charge builds up (due to the electron bombardment) and then discharges suddenly. This adversely affects the elemental analysis, since the specimen may actually move when the discharge occurs. Since neither gold nor palladium were present in the horn itself, the coating did not affect the compositional analysis.

The results of the EDS analysis of the surface of the horn showed that the horn was composed

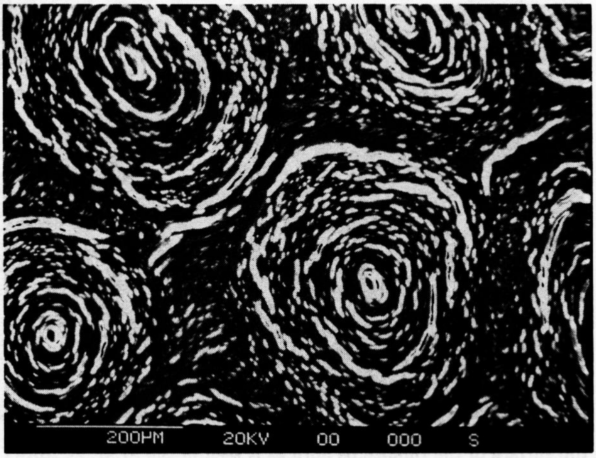

Figure 6: Scanning electron micrograph of the polished cross-section of rhino horn taken at 20KeV at a tilt angle of 35 degrees with a working distance of 15mm. Magnification is marked on the photo. The filaments are apparent with their outer edges defined by an almost continuous white line at a distance from the central core extending around the circumference of the filaments. The matrix can be seen between the filaments. Small cracks or separations are also apparent as smaller white regions within the filaments.

of carbon, oxygen, nitrogen and sulfur. Significant amounts of silicon (Si), calcium (Ca), and phosphorous (P) were also found, most of which can be attributed to the soil in the enclosure where the rhino is kept. This was demonstrated by examining the chemical content of the soil and by comparing the surface composition with the composition in the interior of the horn. The Si, Ca and P were found in higher amounts on the surface of the horn than in the interior. Also present in surprisingly high concentration on the surface was iron (Fe), although some Fe was also found in the cross-section. The question of the existence of the Fe was resolved when the rhino keeper informed us that the animal from which the horn sample was obtained regularly rubs his horn against the iron fence posts in his enclosure.

In cross-section, both phases of the horn were examined. Figure 7 is the EDS spectrum of the hair-like filament and Figure 8 is the spectrum for the matrix. The filaments were found to be significantly higher in S than the matrix phase, although both contain S. Further study is required to draw any specific conclusions that might relate the composition to the structure.

X-ray Diffraction Analysis

Samples of the horn were powdered and examined using x-ray diffraction (XRD). An x-ray tube which provided copper x-rays was used for this analysis. The sample of powdered horn was mounted in a low background holder made of bakelite. A broad scan was done from 0 to 180 degrees in 2Θ. The broad scan provided information on the peaks of interest for this sample. Following this initial run, a region from 20 degrees to 90 degrees was rescanned slowly (over about 8 hours) to concentrate the x-ray counts from the small sample size.

The x-ray data were examined using a computerized peak search program with a database which included organic products. Peaks from the analysis most closely matched those for keratin, but were not an exact match. The inexactness of the match might be due to several factors. For instance, some contamination of the horn material with soil may have occurred, but, it is more

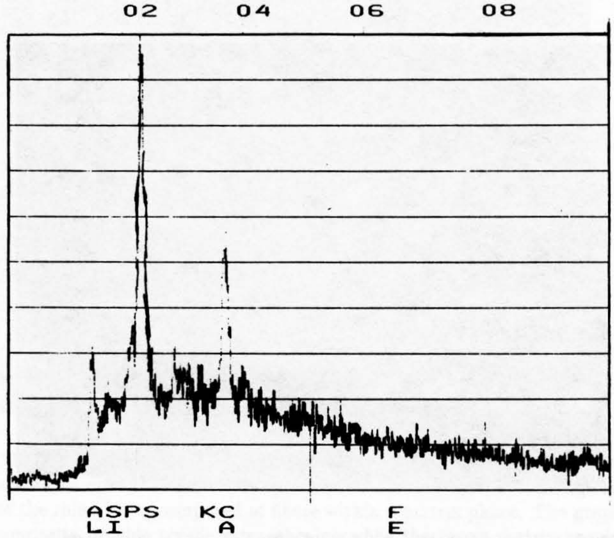

Figure 7: Energy dispersive x-ray spectrum for the filament portion of the rhino horn taken using the spot analysis capability with a spot size of a few microns, at 20KeV with a beryllium window detector. Compare this spectrum with the following spectrum.

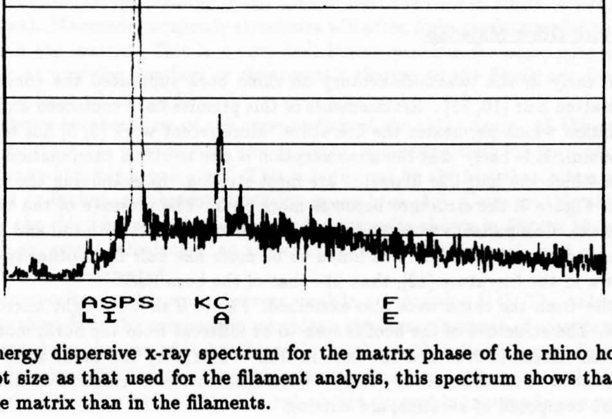

Figure 8: Energy dispersive x-ray spectrum for the matrix phase of the rhino horn. Taken at the same spot size as that used for the filament analysis, this spectrum shows that less sulfur is present in the matrix than in the filaments.

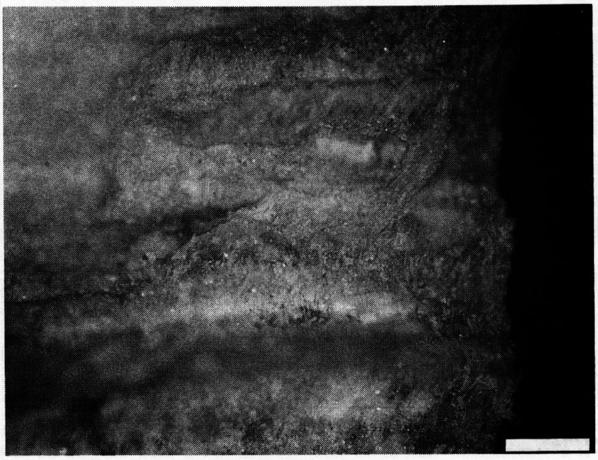

Figure 9: Light micrograph of rhinoceros hoof. Bar represents 1mm. The structure appears different from that of the rhino horn. Note the apparent lack of filaments within the hoof structure, which is closer to hooves of horses than to the rhino horn.

likely that the keratin from the horn is different from that included in the peak search program. Several investigators [7, 8] have reported differences in keratin based biological materials from different species. Keratin from rhino horns may also be of a different type [9].

Comparisons can be made between the elemental composition found by EDS and the composition determined by XRD. In the XRD analysis, the structure was found to be composed of keratin. In the EDS analysis, sulfur was found to be present in both the filament phase and the matrix. Keratin contains disulfide bonding, which would clearly account for the sulfur content. The elements found by EDS are those from which keratin is formed. The two techniques yield complementary results on the composition of the horn.

Comparisons with Other Materials

Work from early in the twentieth century on rhino horn supported the concept that it is composed of matted hair [10, 11] . Restatements of this premise have continued and are the base for the information which permeates the literature. More recent work [3, 5] has not thoroughly disputed this claim. It is likely that the misconception is due to visual examinations such as that in Figure 2, in which the hair-like filaments are most striking. In examining the cross-sections, such as that of Figure 3, the structure becomes more clear. The presence of the two phases are instantly apparent. Comparisons were made of the hair-like filaments with tail and ear hairs from the rhino. The structure of the hair was found to be more like hair from other species, such as horses, as shown in the literature [12], than like that of the horn itself.

Hoof samples from the rhino were also examined. Figure 9 shows a light micrograph of the sample of hoof. The structure of the hoof is seen to be different from the horn, more like hooves of other animals. Horse hooves and the keratin from which they are made have an extensive literature associated with their study [7, 8]. Differences between rhino hoof and horn, even though both are composed of keratins, are striking.

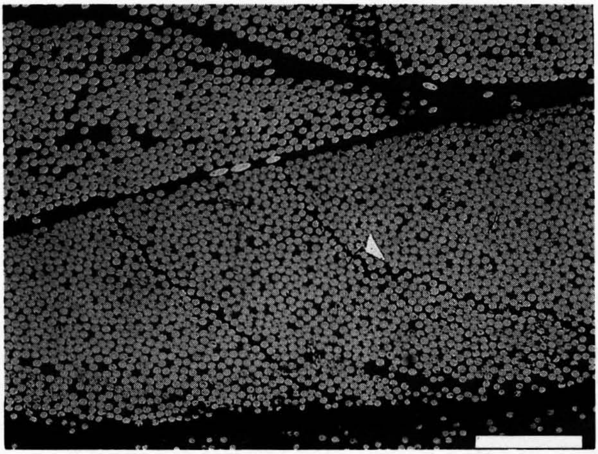

Figure 10: Light micrograph of a polymer matrix composite material composed of graphite fibers encapsulated within an epoxy matrix. Bar repreesents 0.1mm. Note the striking resemblance to the structure of the rhino horn, composed of fibers within a matrix phase. The graphite fibers in the polymer composite provide tensile strengthening while the epoxy matrix provides ductility. The arrow marks a crack within the matrix. Note that it runs between the graphite fibers; no fibers are broken.

Comparisons to Manmade Composites

Figure 10 shows a light micrograph of a polymer matrix composite with graphite fibers within the polymer matrix. It bears a striking resemblance to the structure of the rhino horn. The graphite fibers are, of course, much smaller in diameter than the filaments in the rhino horn, but the overall nature is quite similar. Graphite reinforced polymer matrix composites are usually not monodirectional such as the rhino horn, but consist of layers of oriented fibers alternating directions by 60 or 90 degrees. This provides the composite material with structural strength in at least two directions. It also provides some torsional stability to the composite. The rhino horn appears to suffer from neither lack of torsional strength nor from debonding along the interface between the filaments (except possibly in old horn or near the base of horns, where the matrix is no longer present). Manmade composite structures will often form cracks parallel to the interface of the fiber with the matrix. This is a very well known problem for composite materials and techniques such as applying coatings or electrostatic charges to the fibers are done to try and optimize the interfaces which are formed.

Figure 11 shows another view of the cross-section of the rhino horn. At the arrow, a crack had formed. The crack has filled in with new material, suggesting that the horn may be a living, growing structure which can repair itself. This is borne out by the fact that, when a rhinoceros's horn is removed or broken, a substantial portion grows back [13]. Contrast this ability to repair itself shown by the rhino horn, with the manmade composite shown in Figure 10, where a crack is marked with an arrow. Obviously, no material has filled in this crack, and no self-repairing mechanism can be demonstrated.

Manmade composites are known to be very weak in compression [14]. The natural composite structure of the rhino horn, by comparison provides a significant amount of compressive strength, as is demonstrated by the use of it by rhinos in battles with other rhinos. Seldom does the horn break under these conditions. The rhino horn appears to provide a good combination of compressive and torsional strength, which is not always present in manmade composites.

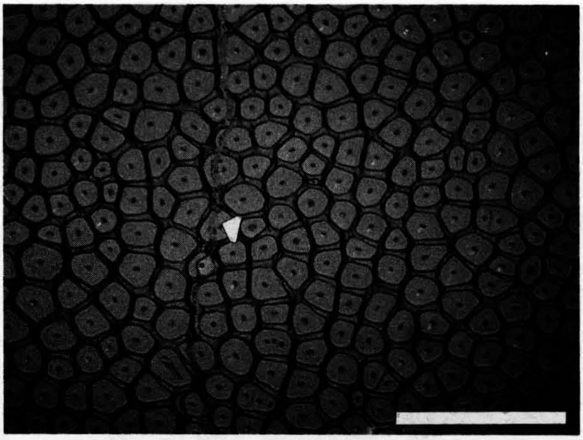

Figure 11: This light micrograph of rhinoceros horn, shown in cross-section, shows the site of a previous crack which has been repaired. Bar represents 1mm. The arrow denotes the crack which has filled in with new material, demonstrating the self-repairing mechanism of the rhino horn

Structure and Composition

The rhino horn has evolved to its present form and evolution has optimized it for the uses made of it by the animal. The structure of the horn, as a composite material, provides some of the same advantages that manmade composites do. The fibers provide greater tensile strength than does the matrix, while the matrix provides greater ductility than the fibers. There is a need to demonstrate that this is the same for the rhino horn. It appears to be the case from the uses made of the horn. The rhino cows use their horns to protect calves from attack. Male rhinos use it in territorial disputes and to drive off interlopers, and all rhinos use their horns for digging in the ground. Other uses that the rhino may make of the horn have been speculated upon. Berger and others are seeking to examine these suggestions in dehorning studies which are being undertaken in rhino populations in Africa [15].

There are a number of lessons which may be possible to learn from studying the rhino horn. Materials science could benefit from information about the interfaces between phases in the horns which are clearly not as weak as they are in manmade composite materials. By studying the mechanical properties of the rhino horn, it may become apparent how the properties of each phase have been optimized for the uses. Since rhino horn has excellent compressive strength, it may be possible to improve the compressive strength of composite materials based on information derived from the study of these horns.

Mimicking the composition and structure of rhino horn may lead to the development of a synthetic material which would serve as a substitute for rhino horn. As was mentioned in the introduction, previous attempts to substitute other types of horn or bone for rhino horn have not been successful with the cultures that use it pharmaceutically. However, no substitute has been tried which is chemically the same as rhino horn. Recently, several authors have suggested that an attempt be made to produce and distribute a synthetic rhino horn [6, 16]. If such a substitute could be made, produced inexpensively, and be accepted, it would provide the possibility for eliminating some of the demand for horn culturally and thereby eliminating some of the poaching pressures on the animal populations. This could impact significantly on their survival and possibly remove them from the threshold of extinction. Detailed information about the chemistry and structure of rhinoceros horn could also be useful in forensic inspection where it may be necessary to confirm the material origin of questionable artifacts.

SUMMARY

Initial experimental analysis was made of rhinoceros horn. Light microscopy of the cross-section of the horn showed it to be composite in structure with two phases present. This work appears to be the first to prepare rhinoceros horn in a manner similar to metal or composite samples. Preparation of the sample in this way allows much detailed information to be available using both light and electron microscopy. One of the two phases present in the composite material of the horn is hair-like in structure and probably accounts for the misconception that rhino horn is composed of matted hair. The hair-like filaments are surrounded by a continuous or matrix phase which is space filling and has some structure within it. The filaments have a central core and circumferential markings similar to growth rings. Cracks in the filaments follow the growth rings, but do not extend into the matrix phase.

Surfaces and cross-sections of the rhino horn were examined using SEM and EDS. The SEM revealed the internal structure of both phases in even greater detail. EDS analysis showed that there were significant differences in the composition of the two phases, specifically in sulfur content. Existence of a surprizing, substantial iron peak in the spectra was concluded to be due to contamination from the soil and the animal rubbing the horn on the iron posts of its enclosure.

X-ray diffraction analysis showed that the overall composition of the horn is, indeed, keratin, but a different type of keratin from that used for the standard in the x-ray diffraction database. A comparison of the x-ray diffraction data with the EDS data presents evidence that the sulfur content is consistent with the disulfide bonds found in all structures formed from keratin.

With the chemical and molecular composition of the rhino horn identified, the possibility of producing a synthetic horn material was discussed. A low cost, chemically equivalent substitute, used instead of natural horn for medicinal purposes, might help alleviate the pressure on the rhino population due to poaching. We are investigating this possibility.

The structure of the rhino horn offers some unique prospects for study and may provide insight into the special combination of strength and ductility along with the unique properties of self repair and biodegradability which are exhibited by rhino horn. Further work to examine the specifics of the mechanical properties of rhino horn is planned.

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