

VI. REFERENCES

- CARTER, M. V. (1957).—*Eutypa armeniaca* Hansf. & Carter, sp. nov., an airborne vascular pathogen of *Prunus armeniaca* L. in Southern Australia. *Aust. J. Bot.* 5, 21–35.
- CARTER, M. V., and BOLAY, A. (1972).—*Eutypa dieback* of apricot is prevalent in Switzerland. *Phytopath. Z.* 75, 187–9.
- CARTER, M. V., and MOLLER, W. J. (1967).—The quantity of inoculum required to infect apricot and other *Prunus* species with *Eutypa armeniaca*. *Aust. J. exp. Agric. Anim. Husb.* 7, 584–6.
- FRANCKI, R. I. B., and CARTER, M. V. (1970).—The serological properties of *Eutypa armeniaca* mycelium and ascospores. *Aust. J. biol. Sci.* 23, 713–16.
- GILL, H. S., and POWELL, D. (1969).—Serological relationships of physiological races A-1 to A-8 of *Phytophthora fragariae*. *Phytopathology* 59, 261–2.
- GOODING, G. V. (1966).—Preparation of macromolecular antigens from *Fomes annosus*. *Phytopathology* 56, 1310–11.
- GOODING, G. V., and POWERS, H. R. (1965).—Serological comparison of *Cronartium fusiforme*, *C. quercina* and *C. ribicola* by immunodiffusion tests. *Phytopathology* 55, 670–4.
- HOLLAND, A. A., and CHOO, Y. S. (1970).—Immunoelectrophoretic characteristics of *Ophiobolus graminis* Sacc. as an aid in classification and determination. *Antonie van Leeuwenhoek* 36, 541–8.
- LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J. (1951).—Protein measurement with the Folin phenol reagent. *J. biol. Chem.* 193, 265–75.
- MADHOSINGH, C. (1964a).—A serological comparison of three *Fusarium* species. *Can. J. Bot.* 42, 1143–6.
- MADHOSINGH, C. (1964b).—A serological comparison of isolates of *Fomes roseus* and *Fomes subroseus*. *Can. J. Bot.* 42, 1677–83.
- MADHOSINGH, C., and WALLEN, V. R. (1968).—Serological differentiation of the *Ascochyta* species on peas. *Can. J. Microbiol.* 14, 449–51.
- MANNING, W. J., RUNYAN, A. C., and MORTON, D. J. (1967).—Serological differentiation between species of *Rhizoctonia* and *Ceratobasidium*. *Phytopathology* 57, 647.
- MORTON, D. J., and DUKES, P. D. (1967).—Serological differentiation of *Pythium aphanidermatum* from *Phytophthora parasitica* var. *nicotianae* and *Phytophthora parasitica*. *Nature, Lond.* 213, 923.
- TEMPEL, A. (1957).—Serological studies on *Fusarium oxysporum* Schl. emend. Sn. et. H. *Nature, Lond.* 180, 1483.

dieback

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A SCANNING ELECTRON MICROSCOPE STUDY OF THE MORPHOLOGY OF RHINOCEROS HORN

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Abstract

The morphology of rhinoceros horn as revealed by the scanning electron microscope is reported. The results are broadly consistent with previous studies of the material. Two different-shaped cortical cell units are shown to exist. The more numerous scale-like cells form the concentric laminae which are packed around the central non-fibrous cores of fibrillar units which comprise most of the horn structure. Regions of interfibrillar material are shown to exist and it is possible that the minority cells originate from it.

I. INTRODUCTION

Microscopic studies of the morphology of rhinoceros horn (Makinson 1954; Earland *et al.* 1962; Ryder 1962) have led to the following description of the material:

- (1) The horn is composed of tubular or filamentous units 300–500 μm in diameter.
- (2) These units are closely packed and as a consequence are distorted from a cylindrical shape.
- (3) Interfilamentous horn is concentrated mainly, if not only, at the interstices rather than between the flat surfaces between the filaments.
- (4) The filaments are composed of about 40 laminae arranged concentrically around a medulla which is a "clearly demarcated solid structure that has either several small gas spaces, or one large irregular one occupying almost the whole width of the medulla" (Ryder 1962).
- (5) The laminae are sheets composed of a single layer of flat cortical cells.

We have studied the morphology of the horn of an African white rhinoceros (*Ceratotherium simus*) by scanning electron microscopy. Two instruments were used—a JSM2 and a Cambridge Stereoscan Mk.II. Both cut and sheared longitudinal and transverse surfaces were observed and also surfaces formed by snapping after immersion of the horn in liquid nitrogen. After preparation some specimens were soaked in water and then dried at 105°C. Material swollen in formic acid was also studied together with the product of disintegration in heated formic acid.

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II. RESULTS

At low magnification (Fig. 1) a cut transverse section clearly shows the close packing of the filamentous units. The individual filaments are distinguished by common boundary depressions. These depressions become apparent after swelling the horn in water and then drying. Filaments defined by these boundary depressions leave space in the structure for interfilamentous material. A depression also occurs at the core of the units. This non-uniform swelling of the structure suggests materials at the core and boundaries which differ from the fibrous material of the bulk of the units.

Figure 2 shows a transverse section of the central core of a filament sheared at the temperature of liquid nitrogen. The cores appear as well-defined solid structures with cross-sections similar in shape to, and roughly one-tenth the diameter of, their filaments. The core material is distinct from the surrounding material and there is some indication that it has a tendency to flake in transverse sheets. Surrounding the core, as can be seen in Figure 2, are concentric laminae of fibrous material, each about $2\ \mu\text{m}$ in thickness. The fibrous nature of the concentric laminae can be seen in a specimen formed by shearing across the grain at room temperature (Fig. 3). Examination of this type of fibrous region shows that the fracture plane cuts across the cortical cells (see below) and reveals (Fig. 4) substructural units approx. $0.1\ \mu\text{m}$ in diameter, corresponding to the macrofibrils of keratin fibres. Longitudinal cut sections which intersect the core regions reveal the material of this region to have no fibrous alignment (Fig. 5). These surfaces show cavities in the core not generally seen at the transverse surfaces. Our impression, however, is that these cavities are artifacts due to the cutting process.

By shearing the horn longitudinally after immersion in liquid nitrogen it is possible to obtain shear surfaces along the laminar interface. Region A of Figure 6 shows such a surface while region B shows a shear surface across the laminae. These exposed laminar surfaces clearly show the outline and surface appearance of flat scale-like units which constitute the laminae (see Fig. 8).

When sections a few millimetres thick are swollen by soaking in 98% formic acid there is a separation of the filaments. This separation delineates regions of material which are not part of the filaments (Fig. 7) and which as such can be described as interfilamentous material. It is not clear whether there is a sharp or gradual transition from the filament to the interfilamentous material but there seems to be some morphological difference in the materials typical of the two regions.

If the swollen material is heated while still in the formic acid it disintegrates into small cellular units of two types (Fig. 8). The more common of the two are flat scale-shaped units $50\text{--}80\ \mu\text{m}$ in maximum dimension and several micrometres thick. The other kind are thicker and more elongated, of length $40\text{--}100\ \mu\text{m}$ and $10\text{--}15\ \mu\text{m}$ in thickness. Before disintegration of the swollen material it is possible to separate the filaments from interfilamentous material. This procedure usually results in a unit smaller than the total apparent filament being obtained. When this component is disintegrated by itself there is a complete absence of the elongated cellular units and only flat scale-like units result. The dimensions of both the concentric laminae

and the flat cortical cells show that each lamina is composed of a single layer of slightly overlapping flat cortical cells. These cells are seen to peel away from the

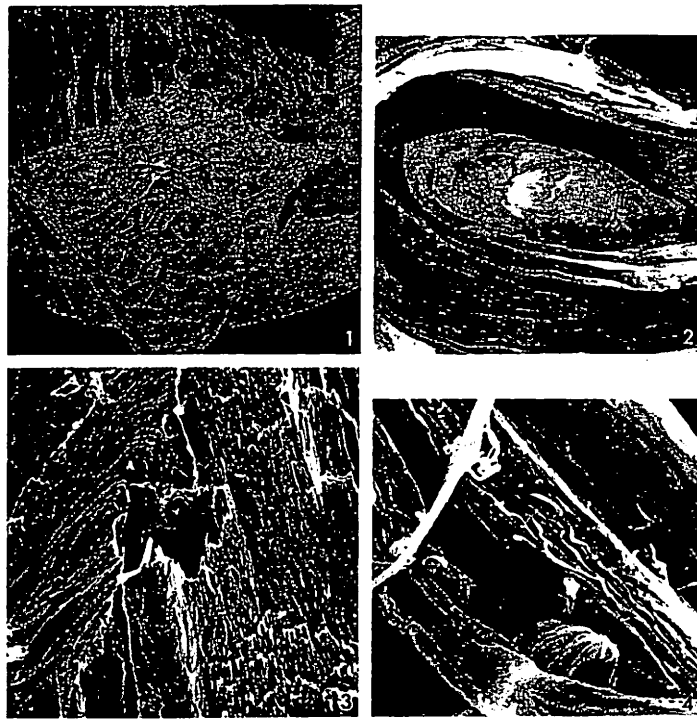


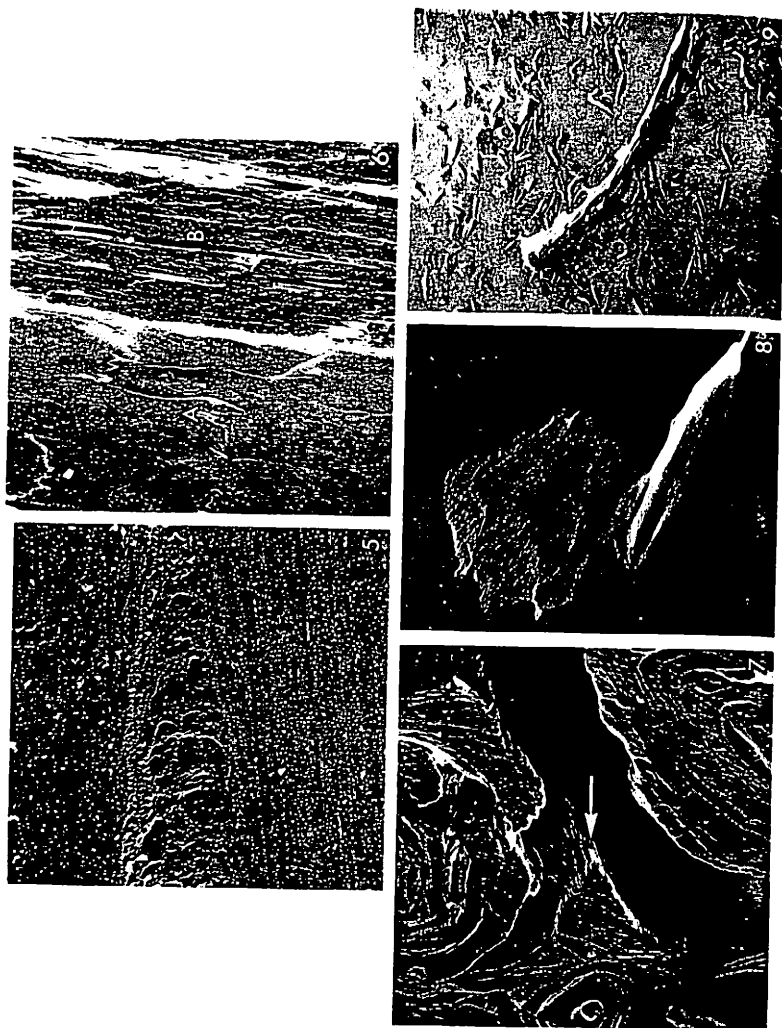
Fig. 1.—Transversely cut section of rhinoceros horn previously swollen in water. Stereoscan Mk. II. $\times 12.5$.

Fig. 2.—Transverse section formed by snapping at liquid nitrogen temperature, showing the central region of a single filament. JSM2. $\times 625$.

Fig. 3.—Transverse section formed by shearing at room temperature. Stereoscan Mk. II. $\times 125$.

Fig. 4.—Strap-like elements revealed by tearing across the grain. A substructure of macrofibrils can also be seen. JSM2. $\times 5000$.

cylindrically shaped fibrils until only the central core remains (Fig. 9). The residual material after separation has an increased proportion of the elongated units further supporting the idea that these units are confined to the interfilamentous material or the outer regions of the filaments or both.



III. CONCLUSIONS

This study supports a morphological model of rhinoceros horn broadly consistent with that described in the Introduction in which (1) close-packed filaments 300–500 μm in diameter comprise almost all of the structure; (2) the filaments consist of a solid non-fibrous core about one-tenth the radial extent of the filament and around which are layered concentric laminae composed of flat scale-like cells, 50–80 μm in extent and several micrometres thick; (3) there exists a material which is not associated with the filaments, which surrounds most of the filaments, but is mainly clumped in interstitial regions common to three or more filaments; (4) there exists a cellular unit different from and fewer in number than the flat scale-like cells which comprise the inner laminae of the filaments. These cells are thicker and more elongated and are associated with the outer regions of the filaments or the interfilamentous material or both.

IV. REFERENCES

- EARLAND, C., BLAKEY, P. L., and STILL, J. G. P. (1962).—*Nature, Lond.* 196, 1287.
 MAKINSON, K. (1954).—*Aust. J. biol. Sci.* 7, 336.
 RYDER, M. L. (1962).—*Nature, Lond.* 193, 1199.

Fig. 5.—Longitudinally cut section of filament revealing core region. JMS2. $\times 400$.

Fig. 6.—Longitudinal section across a single filament formed by shearing at liquid nitrogen temperature. Shows shear plane between the laminac (A) and across the laminac (B). JSM2. $\times 530$.

Fig. 7.—Rhinoceros horn swollen in 98% formic acid. Separate region of interfilamentous material is indicated by arrow. JSM2. $\times 120$.

Fig. 8.—Cellular units of two types which result from the disintegration of rhinoceros horn on heating in 98% formic acid. JSM2. $\times 660$.

Fig. 9.—Decomposition products of filaments in 98% formic acid exclusive of interfilamentous material and some of the outer laminae of the filaments. Scale-like cells are seen to peel progressively from the concentric laminae of the filaments. JSM2. $\times 100$.