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EPIDERMAL REMNANTS OF *PROTEROSUCHUS VANHOEPENI* (HTN)

by

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ABSTRACT

Epidermal remnants from *Proterosuchus vanhoepeni* are described. The significance of these findings is briefly discussed.

INTRODUCTION

There is little precise knowledge concerning the stability of epidermal filamentous proteins which make up the bulk of the protein content of the keratinized cell.

Amino acid analyses of the proteins extracted (filaments and embedding matrix) from the stratum corneum show a low percentage of sulphur amino acids, cystine and cysteine (about 2%, usually measured as cysteic acid). Thus the number of disulphide bond-forming residues is low and from this we infer that these proteins are less stable than the proteins extracted from other epidermal structures, which show much higher sulphur content, such as wool. In the latter sulphur-poor filamentous proteins are embedded in a 'stabilizing' sulphur-rich matrix. It has been proposed that the matrix surrounding the filamentous proteins in the keratinized cell of the stratum corneum functions in a similar stabilizing (cross-linking) capacity. This is yet to be proved; a controversy exists as to whether the matrix is sulphur-rich or not (Matoltsy and Matoltsy, 1970).

As the cell enters the final phases of keratinization the cell membrane is stabilized (and resists autolysis) by the addition of a new protein to the inner lamina. This is seen in electron micrographs as a thickening which partially obscures the inner lamina.

Matoltsy and Matoltsy (1966) removed the proteins from the fully keratinized cell (plantar callus) leaving a residue of stabilized cell membranes. Amino acid analysis of this residue showed a relatively high proportion of ½ cystine and proline (the latter is not compatible with α helix formation). This may be the general situation since it is also true for anuran stabilized cell membranes (A. Thornley, unpublished obs., 1971). In addition tritiated cystine injected into rats is transported to the area of the cell membrane in the cell layers immediately below the stratum corneum (Fukuyama and Epstein, 1969). This holds for the Anura as well (A. Thornley, unpublished obs., 1971).

We may therefore be justified in assuming generally that the stability of the modified cell membrane is partially due to the presence of a sulphur-rich (and proline-rich) protein.

This information gives an indication of what one might find when searching for preserved epidermal remnants. One might expect the filamentous proteins to have been destroyed by keratinolytic bacteria, while the stabilized cell membranes may remain partially intact. (The modified cell membrane fragments easily after chemical removal of the filamentous proteins).

The systematic position of *Proterosuchus vanhoepeni* (Htn) (No. C 3016, Nasionale Museum, Bloemfontein) has been examined (Cruickshank, 1971). In his paper Cruickshank mentions that epidermal 'scutes' were observed lying embedded in the matrix (Figure 1). Portions of these 'scutes' were removed, embedded in Epon 812 using standard techniques and sectioned for light and electron microscopy. The results are described here.

METHODS

The rock was suspended in 1% acetic acid in water and the fragments which gradually flaked off collected. Fragments were sorted using a dissecting microscope and the cleanest fixed (?) in 5% glutaraldehyde in 0,1M cacodylate buffer (pH 7,5) for one day. They were then transferred into fresh buffer for one day and subsequently into 1% osmic acid in 0,05M cacodylate buffer (pH 7,5) for 4 hours. After washing in 10% ethanol for one day the samples were dehydrated and embedded in Epon 812 (Luft, 1961). ½ μ sections were cut, stained with toluidine blue (Ito and Winchester, 1963) and examined with the light microscope under oil immersion. EM sections were stained with uranyl acetate and Reynolds lead citrate (Reynolds, 1963).

RESULTS

Light microscopy

A faint blue line of basophilic material was observed under oil immersion over the surface of a layer of uniform crystals. This material had been trapped between the layer of crystals and embedding rock. A further layer was found overlying and

slightly separate from the layer of crystals in two or three samples.

Electron microscopy

The layer of crystals did not show any indications of Haversian systems and therefore the samples were not from scute. The layer of crystals had the dimensions of dermis and may therefore represent the replacement of collagen by ferric (?) salts.

The nature of the material trapped between dermis (?) and rock is shown in figures 2, 3 and 4. The stabilized cell membrane of the stratum corneum appears to have been preserved and there are suggestions of filamentous proteins. The dimensions of these structures are similar to those of other vertebrates (vary from about 60 to 300 Å), but it is unwise to attach too much importance to these sort of data.

More convincing however is the morphology of the material found embedded in the colloidal layer interposed between dermis (?) and rock (Figures 5 and 6). The filamentous proteins (and matrix ?) of the epidermis appear to have been preserved intact. The dimensions of the individual filament cross-sections are within the range reported for these proteins (about 50 to 120 Å).

DISCUSSION

The modified cell membrane remnants are shown packed one layer on top of the other in Figures 3 and 4. This would be indicative of scales, which is not unexpected since *Proterosuchus* has been shown to be a reptile. All known vertebrates, except the Amphibia, have multiple layers of stratum corneum. The latter have one layer, sometimes two. There is insufficient knowledge of these structures to be able to distinguish families of reptiles.

The filamentous structures shown in electron micrograph number 6 are interesting in that they are not associated with modified cell membranes. They must therefore be part of the cell content from layers beneath the stratum corneum; that is, they have not undergone 'keratinization'.

The importance of these studies lies in the fact that they may show that not only is the keratinized cell membrane remarkably stable over millions of years but that the filamentous proteins (and matrix ?) themselves have a unique resistance to breakdown. These developments are obviously important in the evolution of vertebrates.

These observations indicate that ectodermal structures (nails, hairs, scales) may be present in rock samples which contain fossilized vertebrates. The author hopes that this paper will stimulate investigations for and ultrastructural examination of epidermal remnants in palaeontology.

ACKNOWLEDGEMENTS

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REFERENCES

CRUICKSHANK, A. R. I., (1971). The Proterosuchian Thecodonts. In: *Studies in Vertebrate Evolution*, ed. K. A. Joysey & T. S. Kemp, Oliver and Boyd, Edinburgh & London.

FUKUYAMA, K. & EPSTEIN, W. L. (1969). Sulphur-containing proteins and epidermal keratinization. *J. Cell Biol.*, 40, 830.

ITO, S. & WINCHESTER, R. J., (1963). The fine structure of gastric mucosa in the bat. *J. Cell. Biol.*, 16, 541-577.

LUFT, J. H., (1961). Improvement in epoxy resin embedding methods. *J. Biophys. biochem. Cytol.*, 9, 409.

MATOLSTY, A. G. & MATOLTSY, M. G., (1966). The membrane protein of horny cells. *J. Invest. Dermatol.*, 46, 127.

————, (1970). The chemical nature of keratinohyalin granules of the epidermis. *J. Cell. Biol.*, 47, 593-604.

REYNOLDS, E. S., (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell. Biol.*, 17, 208.

THORNLEY, A. L., (1971). Ph.D. thesis in preparation.

Fig. 1 x 4 Remnants of *Proterosuchus* embedded in mudstone. (Top left)
Fig. 2 x 36 000 Structures with the morphology of thickened membranes (tm) and filamentous proteins (Fp). Dermis (D). (Top right)
Fig. 3 x 90 000 Thickened membranes layered one on top of the other. (Arrows) (Bottom left)
Fig. 4 x 90 000 Thickened membranes layered one on top of the other. (Arrows) (Bottom right)

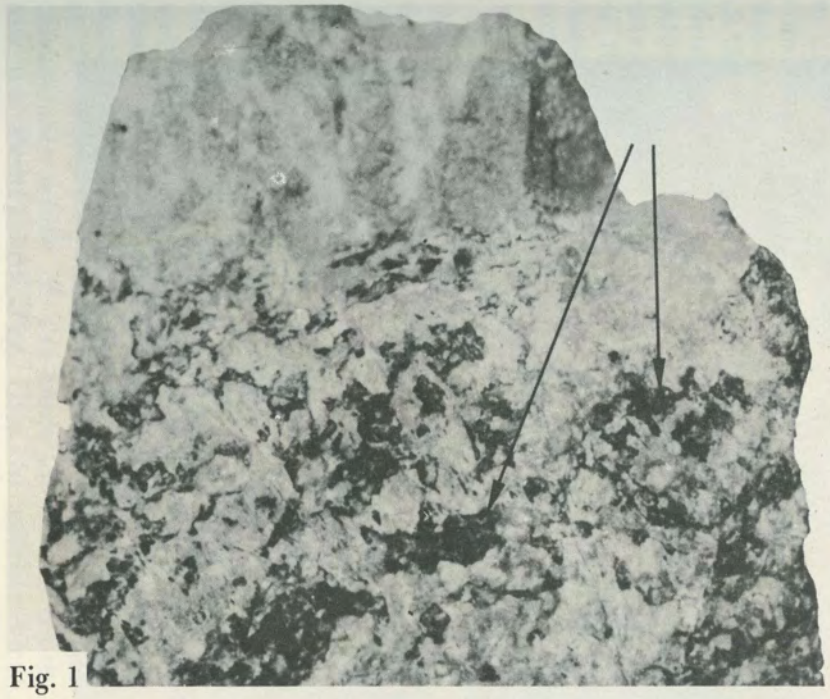

Fig. 1

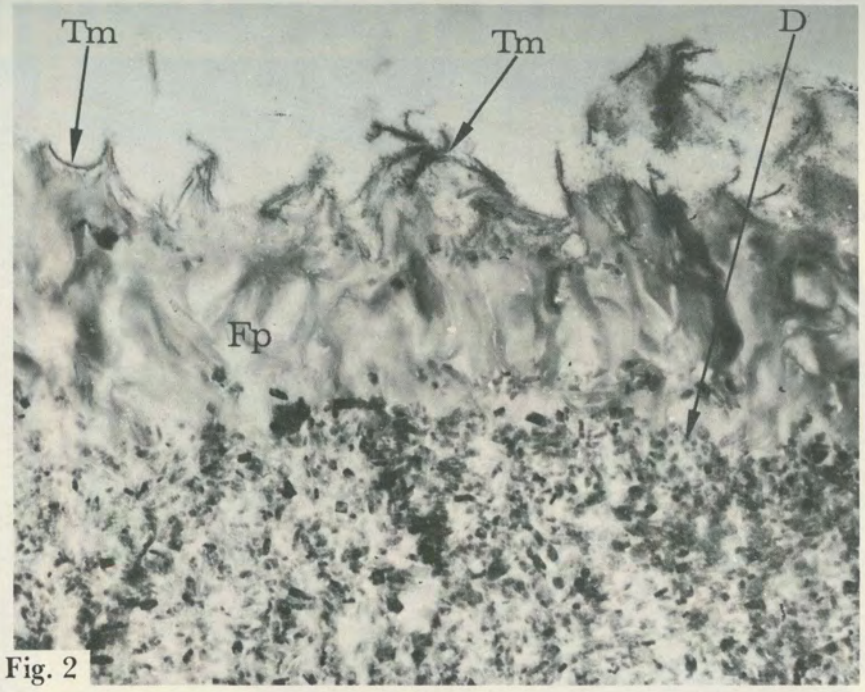

Fig. 2

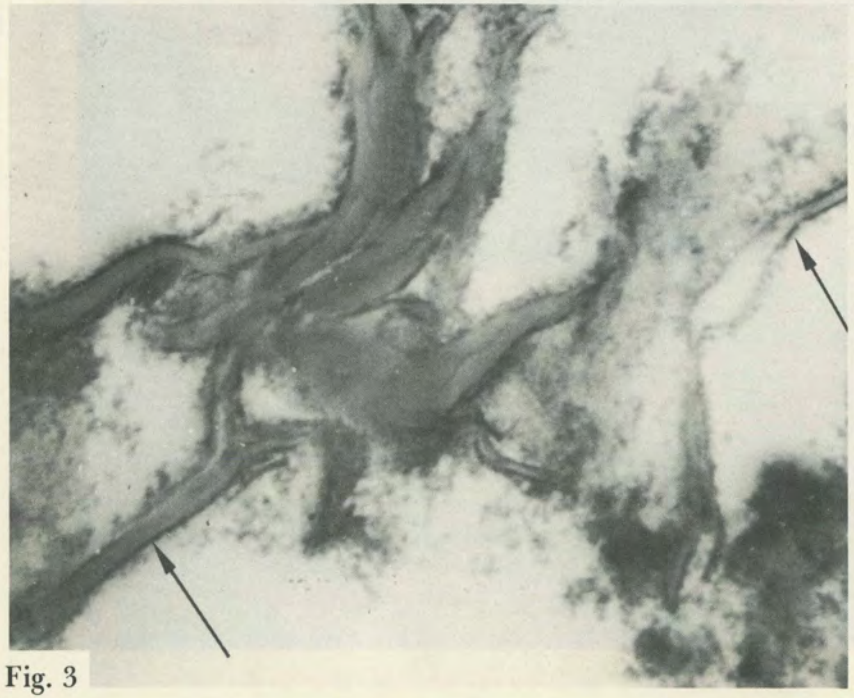

Fig. 3

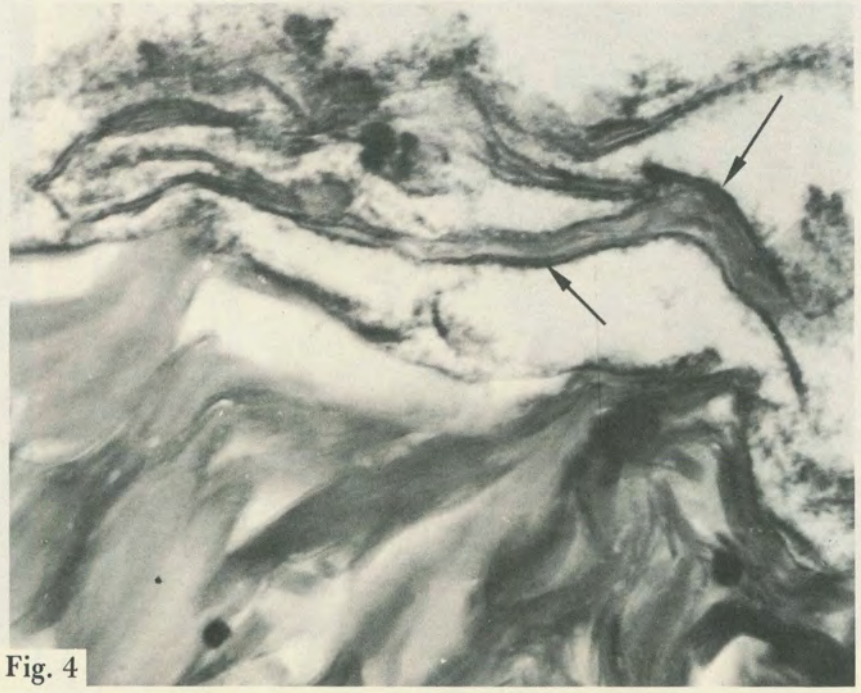

Fig. 4

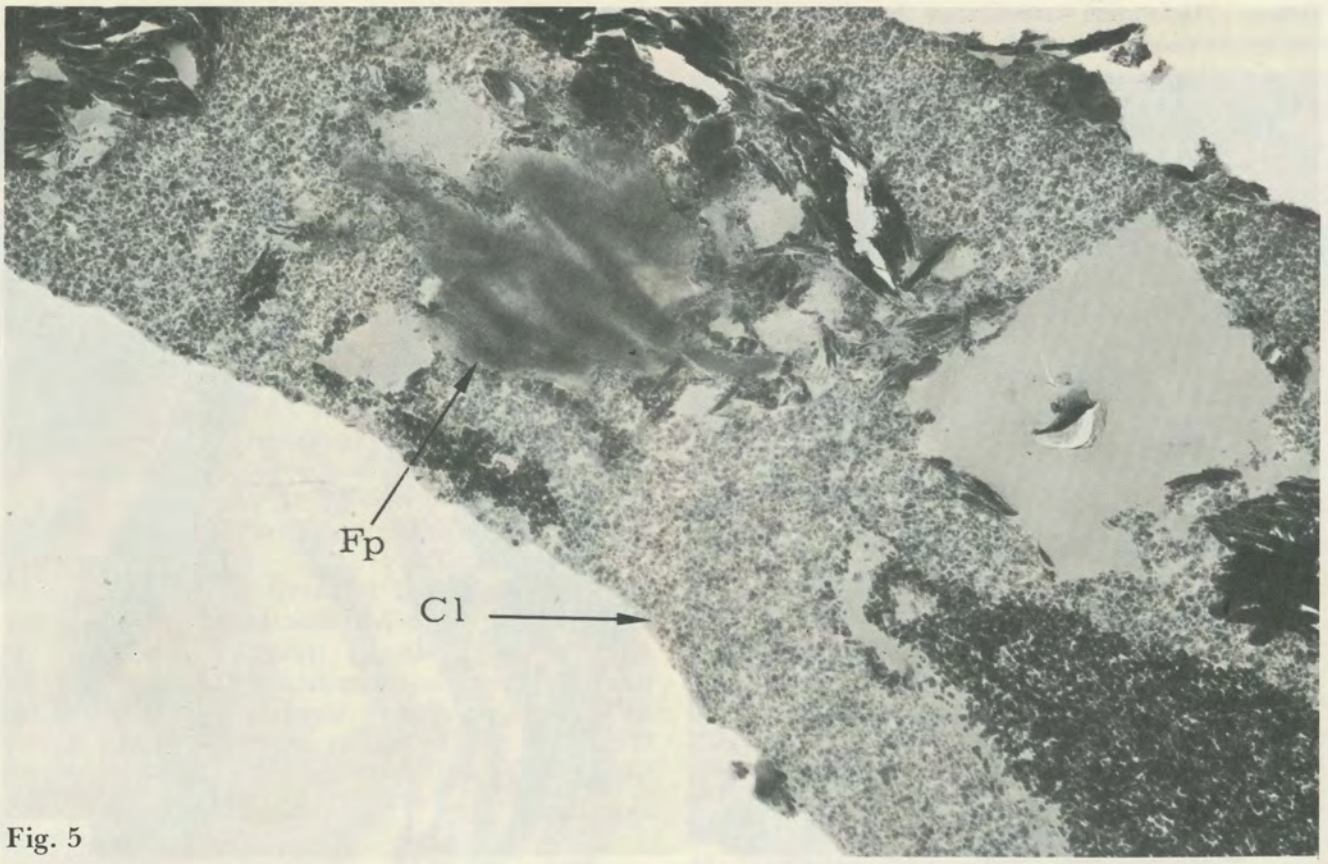

Fig. 5

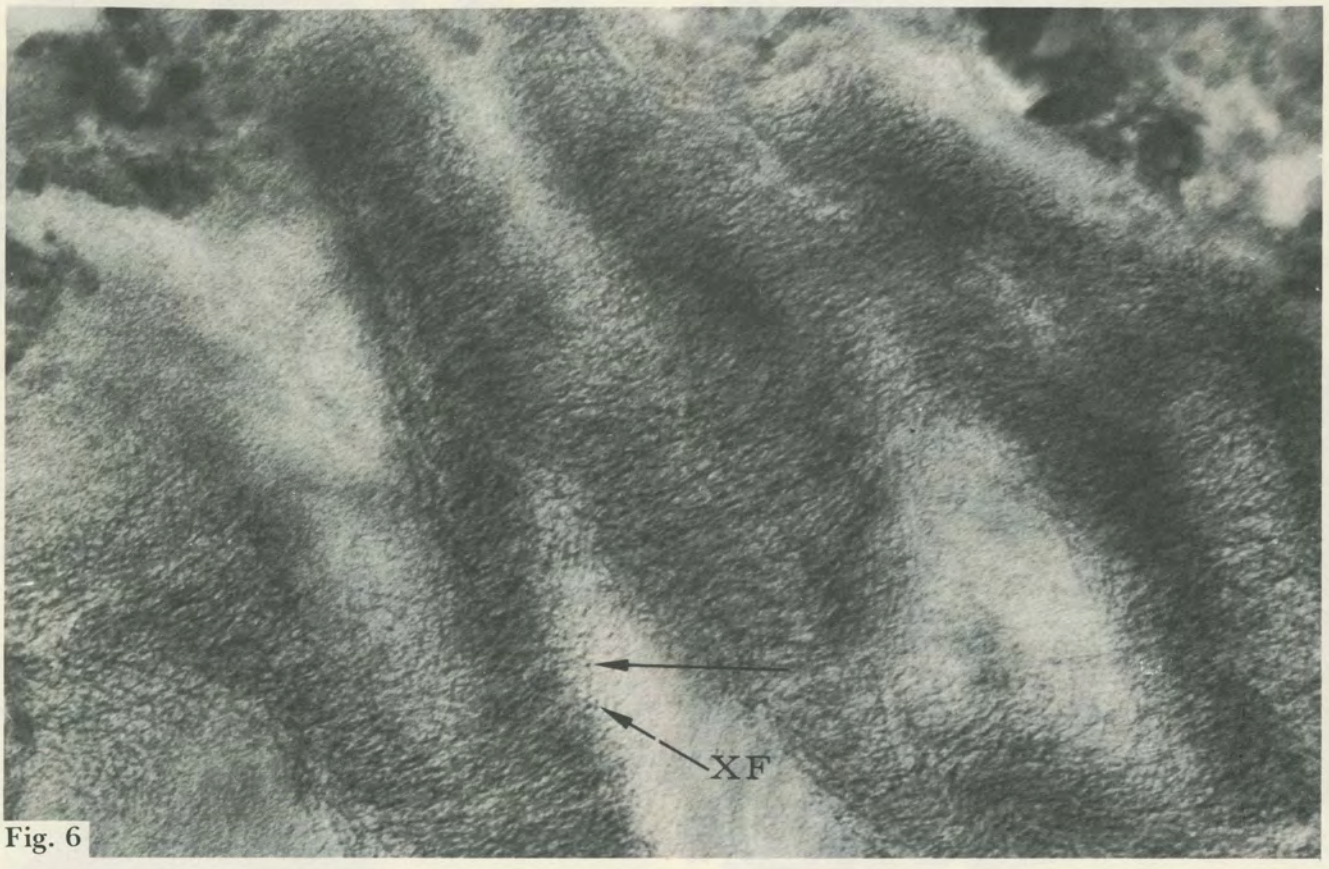

Fig. 6

Fig. 5 x 24 000 Filamentous proteins (Fp) lying within a colloidal layer (Cl). (Top)
Fig. 6 x 83 000 Filamentous proteins. Arrows indicate filaments in cross section (XF). (Bottom)