THE EPIDERMIS AND ITS KERATINISATION IN THE AFRICAN ELEPHANT (*LOXODONTA AFRICANA*)

R. I. C. SPEARMAN, PH.D. F.I. BIOL.

Dermatology Department, University College Hospital Medical School, University Street, London, W.C. 1

INTRODUCTION

The family Elephantidae contains two living genera, each with a single species. The African elephant *Loxodonta africana* is found over a wide area south of the Sahara to South Africa, while the Indian elephant *Elephas maximus* occurs in most of south-east Asia. Both species inhabit savannah grass lands and tropical forests (Walker 1968).

The micro-anatomy of the skin in *Elephas maximus* was first described by Smith (1890) who mentioned regional differences in epidermal morphology and in the forms of the dermal papillae. He examined skin from the ears, trunk, body and legs as well as the nails, and noted the absence of both sweat glands and sebaceous glands and the paucity of hair follicles. More recently, Luck and Wright (1964) compared the skin of *Hippopotamus amphibius* with that of *Loxodonta africana* in finer histological detail.

In the present paper, the histology of the flank epidermis in the adult female African elephant is described and related to the localisation of certain chemical constituents of importance in the keratinisation of the stratum corneum.

MATERIAL AND METHODS

Formalin-fixed flank skin with the deep dermis dissected off was processed to paraffin wax for preparation of 7 μ thick sections on a heavy base sledge microtome, the tissue being not specially treated to soften it. Sections were stained in Ehrlich's haematoxylin and eosin and by the fluorescent staining method using congo red, titan yellow, and thioflavine t, as described by Jarrett, Spearman and Hardy (1959), and Jarrett and Spearman (1964). The latter technique showed marked differences of fluorescence staining between the *stratum corneum* in the hair follicle necks and over the intervening papules.

Disulphide bonds of cystine in keratin were shown by the peracetic acid oxidation, thioflavine t fluorescent method after ribonuclease digestion (Jarrett and Spearman 1964; Spearman 1968). Bound sulphydryl groups of cysteine in keratin and prekeratin proteins were demonstrated by the dihydroxy dinaphthyl disulphide method of Barrnett and Seligman (1952). Free cysteine was removed during histological processing. Protein-bound phospholipids were detected by the acid haematin method after removal of free lipids (Jarrett *et al.* 1959, 1965). Protein-bound calcium was demonstrated by the staining reaction with alizarin red in the metal chelation method of Cane and Spearman (1967).

The skin specimen was supplied by the Zoological Society of London.

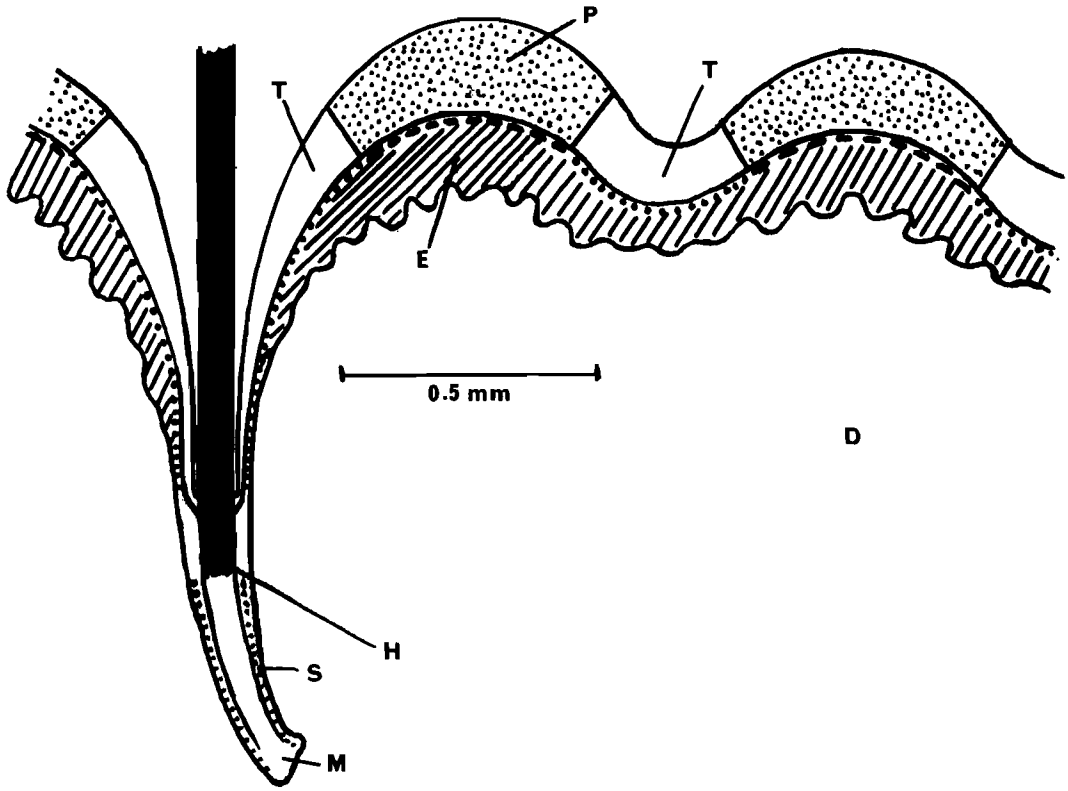


FIGURE 1
Diagrammatic line drawing of African elephant flank epidermis to show arrangement in papular (P) and trough (T) regions. The former has a weakly basophilic granular layer, shown by broken lines, and the latter a strongly basophilic granular layer, shown by dotted lines. (H) hair in anagen follicle; (M) follicle matrix; (S) outer and inner sheaths with trichohyalin in the latter; (E) unkeratinised region of the epidermis indented by deep dermal papillae; (D) dermis. The papular epidermal horny layer is stippled.

OBSERVATIONS

Morphology and Histology

The epidermal surface is criss-crossed by flexural creases and the *stratum corneum* is completely exposed owing to the absence of a covering pelt. Nevertheless, short hairs occur at widely spaced intervals over most of the body and longer hairs are found on the tail. In addition to this gross pattern, there is a finer pattern of raised horny epidermal papules, each some 0·5 mm. in diameter, separated by narrower insunken trough regions (Fig. 1). Hair follicles are confined to these troughs and have deeply insunken necks lined by a compact continuation of the surface *stratum corneum* (Figs. 2a and b, 3a). No sweat glands, sebaceous glands or arrector pili muscles occur in *Elephas maximus* (Smith 1890) and none were found in the flank skin examined of *Loxodonta africana*.

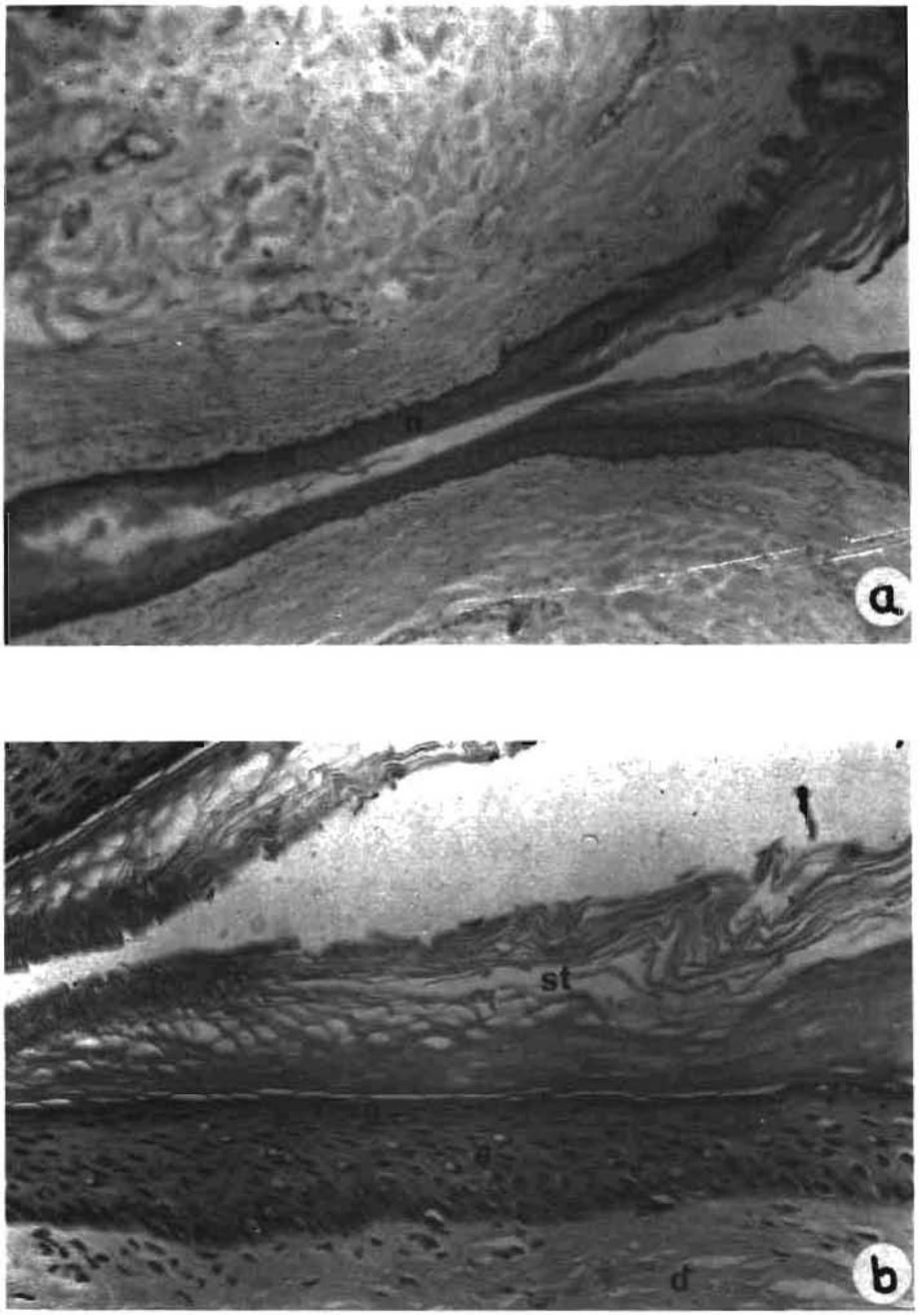


FIGURE 2

(*a*) African elephant hair follicle to show the neck region (n) and trough region (t) with a dark line; (g) the strongly basophilic granular layer. Flank skin. Haematoxylin and eosin. x 120. (*b*) African elephant hair follicle neck region with continuation of trough *stratum corneum* (st); darkly stained granular layer (g); unkeratinised region of living epidermis (e); dermis (d). Flank skin. Haematoxylin and eosin. x 620.

The skin sample examined contained a growing (anagen) hair follicle. The germinal matrix of the follicle bulb was only slightly broader than the upper part of the follicle and showed mitotically dividing cells. The hair formative region in the lower third of the follicle contained elongated eosinophilic cells with flattened pyknotic nuclei. In the keratinised region, the hair cortical cells showed ghosts of nuclei and the keratinised cytoplasm also stained poorly with eosin. The surrounding inner root sheath contained cells with prominent nuclei and large eosin-pink-stained trichohyalin granules from the level of the bulb to that of the keratinised hair shaft. Above this, the inner sheath cells showed weakly staining nuclei up to the level of the follicle neck which was lined by the *stratum corneum*. The latter was underlain by keratohyalin granules. The fully-formed hair had a wide cortex and narrow medulla.

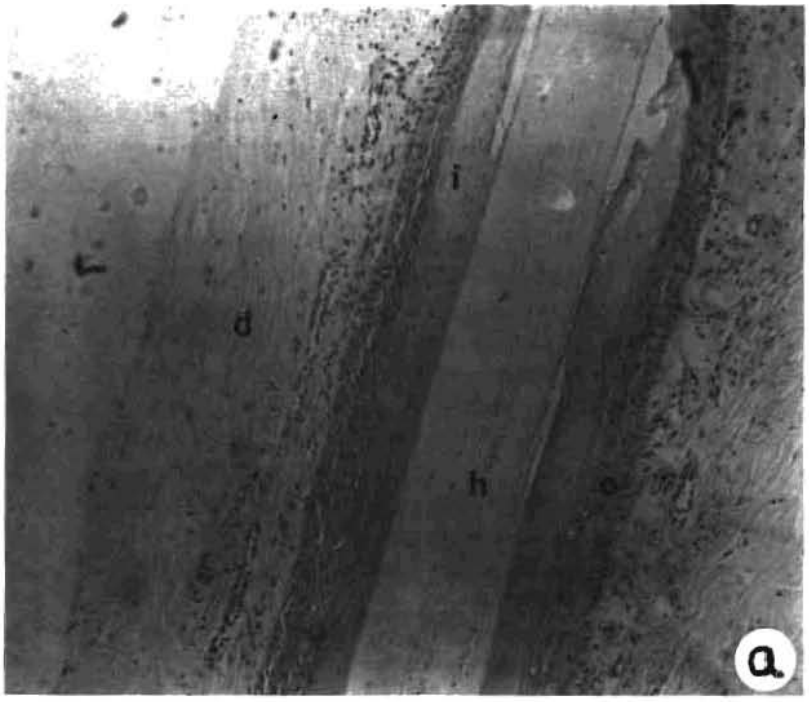
The epidermis, excluding the horny layer, was only moderately thick; 60–110 μ in the papules, slightly less in the troughs. Domed-shaped extensions of the dermis under the epidermal papules were termed by Smith (1890) in *Elephas*, primary dermal papillae, in distinction to the secondary dermal papillae shown as finger-like projections into the underside of the epidermis (Fig. 3b). In both trough and papular regions, the germinal basal layer gave rise to larger polygonal prickle cells with vacuolated nuclei containing prominent nucleoli. In the upper region of the epidermis, the cells became more eosinophilic and slightly more flattened and had only weakly haematoxylin stainable nuclei (Fig. 3b).

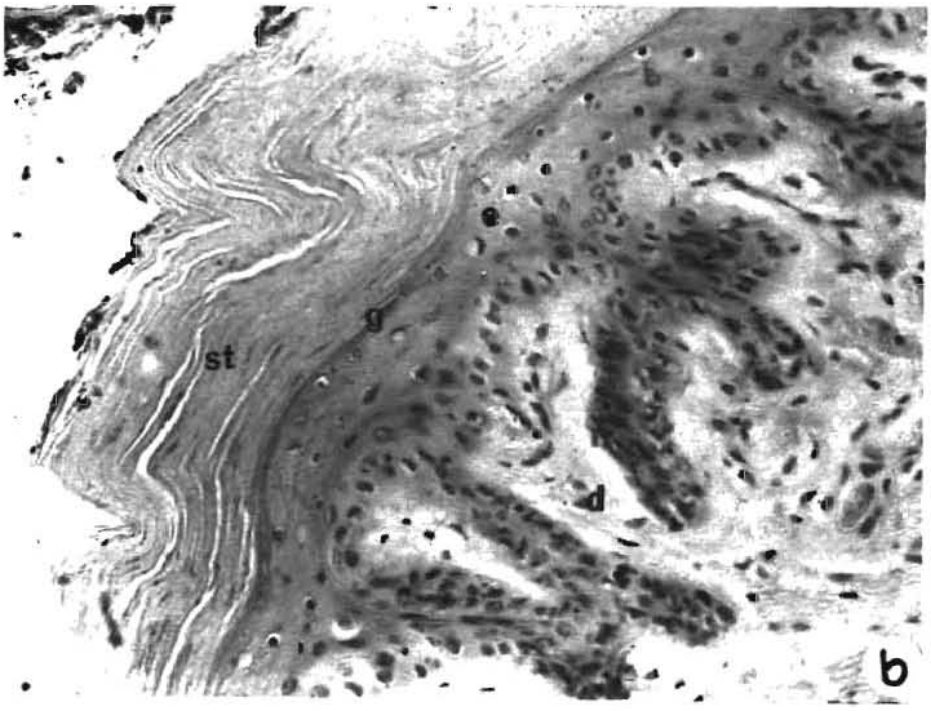
In all regions a granular layer with keratohyalin granules was developed beneath the *stratum corneum*. However, whilst in the troughs and in the necks of the follicles, keratohyalin granules were strongly staining and the granular layer was some two cells deep (Figs. 2a and b), over the papules the granules were much more weakly stained with haematoxylin and the granular layer was only one cell thick (Fig. 3b). These two types of granular layer were fairly sharply demarcated.

The *stratum corneum* varied in thickness, averaging some 60 μ and being maximal over the papules where it was often half the total depth of the epidermis (Fig. 3b). In both the troughs and the papules the horny layer was anatomically similar and composed of moderately flattened cells containing uniformly eosinophilic pink stained cytoplasm, with complete loss of haematoxylin stainable nuclear remnants. The trough horny cells, however, stained more strongly with eosin than the papular horny cells, and the base of the horny layer stained a deep pink colour in all sites. The papular cornified cells contained clumps of melanin granules carried up from the prickle cell layer and marking the former positions of nuclei. In both regions there was extensive dorsoventral separation of the horny cells in paraffin processed tissue with a tendency to the formation of separate cell layers with wide intercellular spaces. In contrast, these flattened cells appeared much more firmly united along their

FIGURE 3

(*a*) Mid-region of African elephant anagen hair follicle with hair (h), outer sheath (o), and inner sheath (i). The inner root sheath at this level does not contain trichohyalin and it appears to grade into the neck *stratum corneum*. Dermis (d). Flank skin. Haematoxylin and eosin. x 620. (*b*) African elephant papular region to show epidermis with elongated dermal papillae (d), indented into the epidermis, and weakly staining granular layer (g). There is a thick *stratum corneum* (st). The dark superficial material in the *stratum corneum* is detritus which collects on the skin. Unkeratinised region of living epidermis (e). Flank skin. Haematoxylin and eosin. x 620.





lateral junctions. This degree of cell separation, although possibly to some extent artifactual and a result of histological processing, presumably indicates sites of weaker and stronger union between cells.

The congo red method showed the papular horny layer and hairs as blue fluorescent, and the trough horny layer, purple. Dust and other detritus tended to be trapped between the more superficial horny cells (Figs. 3b, 4a).

Histochemistry

Cytoplasmic disulphide bonds of cystine were detected only in the keratinised hair cortex and the *stratum corneum*. The hairs fluoresced strongly for cystine with a deep yellow colour under ultraviolet light. The papular *stratum corneum* fluoresced almost as strongly as the hairs, but that in the troughs and follicle necks showed much less cystine. The amount of cystine in the hairs and in the papular horny layer was sufficiently great for them to stain up yellow even under ordinary light (Fig. 4a). In contrast, the guinea-pig *stratum corneum* examined for comparison showed only a very weak cystine fluorescence, much less than that of the elephant trough horny layer. The reaction was uniform throughout the entire thickness of the elephant papular horny layer, whereas the trough horny layer showed a slightly greater fluorescence near its base.

Bound sulphydryl groups were concentrated in the papular horny layer but were found only at the base of this layer in the troughs and follicle necks (Fig. 4b). A strong reaction for bound cysteine was shown in the hair keratogenous zone within the follicle, as in mammalian hair follicles generally.

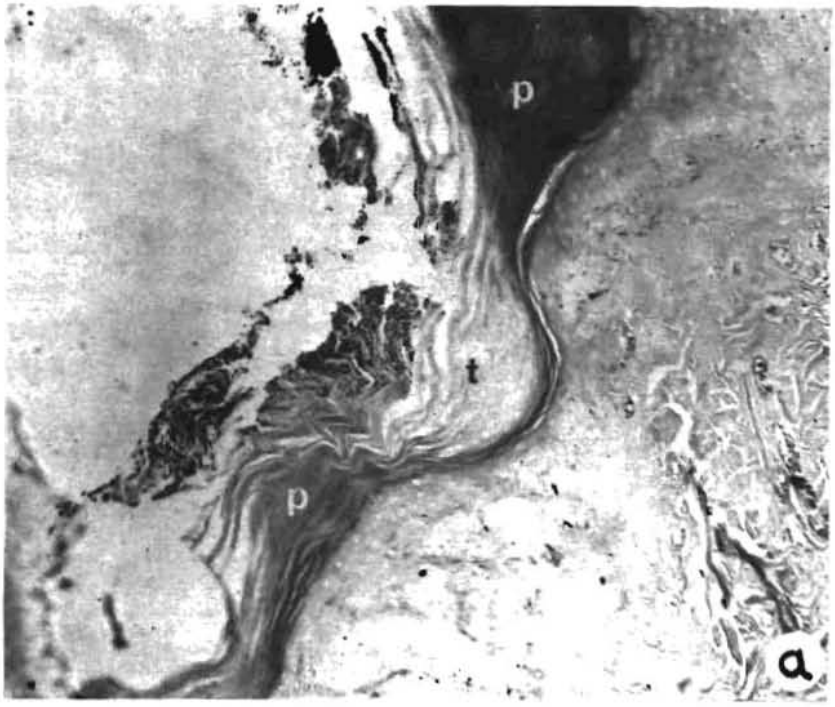
Protein-bound phospholipids showed as a uniformly dark blue reaction throughout the depth of the papular horny layer, but the trough and follicular neck horny layers were much weaker in phospholipids, except in their basal regions (Fig. 5a). The keratohyalin granules showed a punctate phospholipid reaction which was strongest in the trough regions.

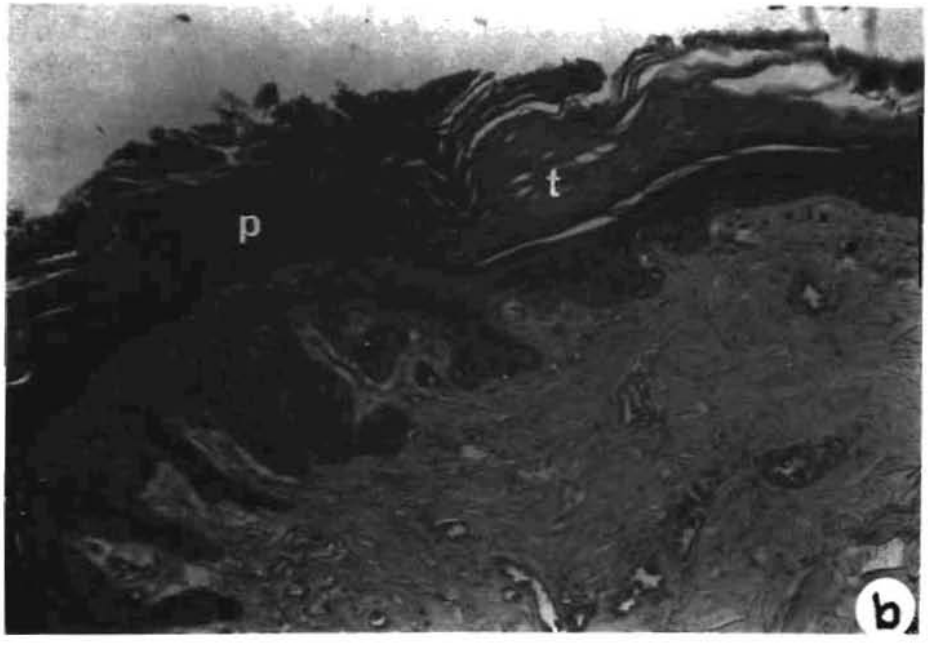
Protein-bound calcium occurred in the granules and was more concentrated in the papular than in the trough keratin layers, although the base of the trough horny layer showed a moderate reaction for bound calcium. (Fig. 5b.)

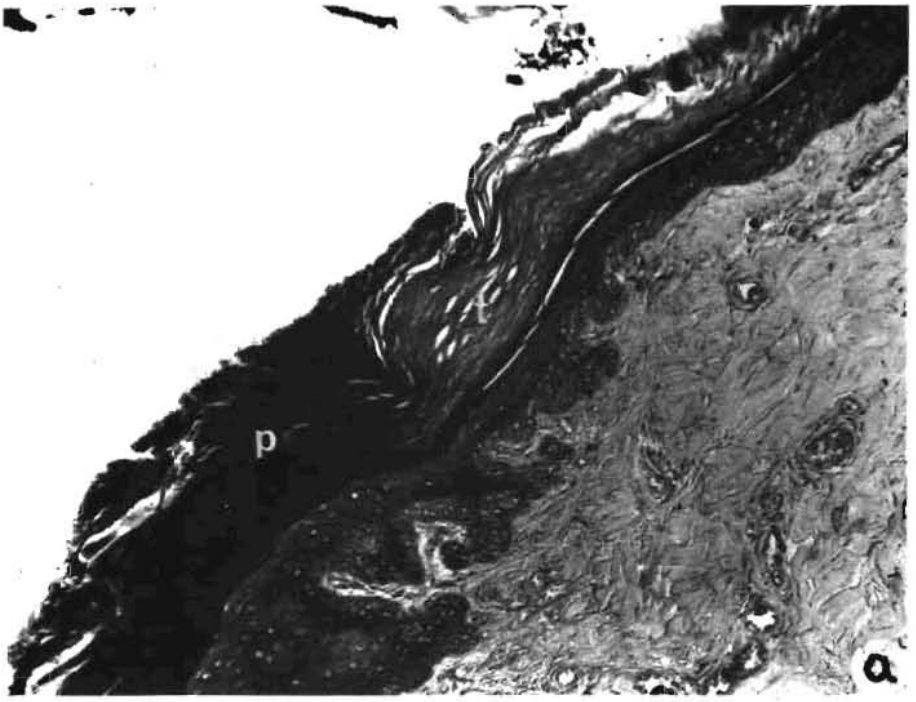
The clear histological demarcation between the trough and papular granular layers was, therefore, paralleled by sharp differences in composition of the overlying *stratum corneum* in the two regions.

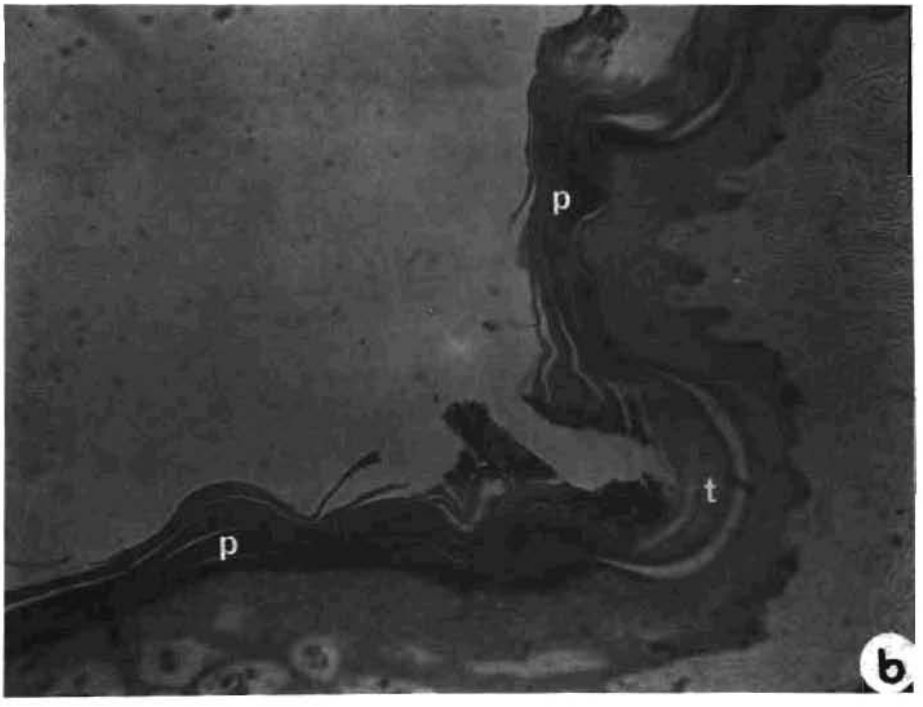
FIGURE 4

(*a*) African elephant skin to show distribution of (cystine) disulphide bonds in keratin. Gray colouration, yellow in original by ordinary light and also strongly fluorescent yellow. The papular stratum corneum (p) showed more S-S than the trough horny layer (t) which only had a strong reaction towards its base. The underlying epidermis was negative. Note the detritus above the horny layer, opaque and unstained in the preparation. Flank skin. Peracetic acid oxidation method photographed by ordinary light. x 210. (*b*) African elephant skin to show protein-bound (cysteine) sulphydryl groups. The papular horny layer (p) was purple and strongly reactive, while the trough horny layer (t) contained little protein-bound (SH) groups and was a weak pink colour, except at its base which stained strongly for SH. The underlying epidermis shows only a weak reaction, except in the transitional keratogenous zone which had more SH. Flank skin. DDD method. x 210.









DISCUSSION

Finger-like dermal papillae similar to those first shown in the Indian elephant by Smith (1890) have also been described in the hippopotamus by Luck and Wright (1964), in the white rhinoceros by Cave and Allbrook (1959), in the Sirenia by Dosch (1915), and in Cetacea by Sokolov (1960), Spearman (1966), and Giacometti (1967). In human skin, and that of most hairy mammals, dermal papillae of this type occur only in abnormal states of proliferation when the epidermis is said to show acanthosis (Lever 1961). In human psoriatic lesions, acanthosis is associated with loss of a granular layer and a change in cornification with the production of nucleated parakeratotic cells. The more extensive undulated basal layer in acanthotic epidermis provides a wide surface area for epidermal proliferation, and cell turnover rate has been shown to be considerably more rapid in psoriasis than in normal human epidermis (Rothberg *et al.* 1961). The same is probably true of the normally acanthotic epidermis seen in these pachydermatous mammals.

The thick *stratum corneum* in the African elephant is composed of cells filled with structural cytoplasmic constituents, unlike arrangements in the guinea-pig and many other hairy mammals in which the interiors of the keratinocytes are largely lysed to soluble breakdown products only precipitated by certain histological fixatives, and in which the horny cells are virtually keratinised shells (Spearman and Riley 1967). In this respect the elephant resembles the hippopotamus and manatee, both in the morphology of the horny layer and in its bound phospholipid content (Spearman 1966). Flesch and Esoda (1964) have emphasised the degree of chemical similarity of this type of horny layer to that developed in abnormal human parakeratosis (epidermal keratinisation with retention of shrunken, darkly stained remnants of nuclei), the chief difference being the absence of basophilic nuclear remnants in these pachydermatous animals, with the exception of the Cetacea which have nucleated horny cells (Sokolov 1960, Spearman 1966). The retention of perinuclear melanin granules in the elephant papular stratum corneum gives a superficial impression of parakeratosis.

Human parakeratotic cells appear more coherent than in normal skin (Brody 1962) and they slough in larger flakes. This also appears to be true of the elephant *stratum corneum* first described by Leeuwenhoek (1712). He showed that sloughed pieces of the horny layer adhered very closely to the moulted hairs, but whether or not there is a direct continuity of the *stratum corneum* with the hairs, as in certain seals (Ling 1965, Spearman 1968), requires examination of the skin at various stages of the hair cycle, which has not been undertaken. There is a slight indication in *Loxodonta africana* that such continuity may occur through the inner sheath and *stratum corneum* (Fig. 3a). The deeply insunken follicle necks in the

FIGURE 5

(*a*) African elephant skin to show protein-bound phospholipid. The papular horny layer (p) was a dense blue-black colour, rich in phospholipid. The trough horny layer (t) was rich in phospholipid in the basal region, but elsewhere showed only a very weak greyish-blue reaction. The underlying epidermis showed no appreciable reaction and was coloured yellow in the original. Flank skin. Acid haematin method. x 210. (*b*) African elephant skin to show protein-bound calcium. The papular horny layer (p) was stained a deep orange and showed more calcium than the trough horny layer (t) which was pink in the original with a stronger reaction in its basal region. The underlying epidermis showed little reaction. Flank skin. Alizarin red chelation method. x 210.

elephant (Fig. 1, Fig. 2a) are reminiscent of the pinnipedia (Montagna and Harrison 1957, Ling 1965, Spearman 1968).

The internal structure of the elephant (anagen) hair follicle was similar to that of other mammals. However, the paucity of hairs and apparent complete absence of all skin glands and arrector pili muscles in both species of elephant, as shown by Smith in 1890 and reported here, for *Loxodonta africana*, suggests that there has been a retrogressive evolutionary reduction of all the epidermal appendages as compared with the much more hairy extinct proboscidea which inhabited colder regions. Understandably, a thick pelt would be disadvantageous to so bulky a tropical mammal wherein basal heat loss is a problem, but it is difficult to see why sweat glands, useful in temperature regulation, should also have vanished. Elephants can, however, greatly increase basal heat loss when required by flapping their large ears which are well supplied with dermal blood vessels (Smith 1890). In contrast, both the white rhinoceros (Cave and Allbrook 1959) and the hippopotamus (Luck and Wright 1964) possess apocrine sweat glands and the rhinoceros hair follicles have associated sebaceous glands. Sweat and sebum normally help to keep the *stratum corneum* moist and consequently flexible (Kligman 1963). The elephant, unlike the rhinoceros, has no internal provision for such lubrication, and probably for this reason elephants in captivity need to have their skin regularly hosed with water to keep them in condition. Wild elephants bathe regularly if they have access to water or, if not, they often coat their backs with mud (Walker 1968), which possibly helps to prevent the horny layer from becoming unduly dry and brittle. Elephants both in captivity and in the wild have the habit of showering themselves with sand and gravel which may act as an abrasive in the shedding of the hard keratinised flakes, and which probably accounts for the detritus seen in histological sections of the *stratum corneum* by Luck and Wright (1964) and by the present writer.

There is a close association in both ontogeny and phylogeny, between hair follicle development and keratinisation of the surrounding *stratum corneum* which in mammals, in contrast to lower vertebrates, is normally formed over a granular layer containing prominent basophilic keratohyalin granules in the cytoplasm (Spearman 1964).

This association is particularly well shown in the scaly tails of rodents where an epidermal granular layer is restricted to the hinge areas around the hair follicles, and the horny scales undergo a different type of cornification (Jarrett and Spearman 1964). The elephant develops more prominent keratohyalin granules around the hair follicles and a very much more weakly stainable granular layer associated with a chemically different type of horny layer over the intervening papules. The papular *stratum corneum* shows a chemical similarity to that of rodent tail scales since both are rich in protein-bound substances and the keratin has a high cystine content. The trough *stratum corneum*, although it shows less cytolytic change, resembles the rodent hinge horny layer in retaining few bound substances and in having less cystine (Jarrett and Spearman 1964, Spearman 1966).

Probably, therefore, the higher content of bound substances in the elephant papular *stratum corneum* is associated with the weaker *stratum granulosum* development, which is possibly itself associated with a numerical reduction in hair follicle development (Spearman 1964).

The elephant papular *stratum corneum* is fairly uniformly constructed throughout its

depth, except for the thin basal region which shows certain differences reminiscent of the compact human *stratum corneum conjunctum* (Szakall 1958).

The proverbial toughness of the elephant hide is mainly a property of the dermal connective tissue, the living epidermis, even in the papules, being not much thicker than in normal human skin. The *stratum corneum* is, however, extremely thick and in the absence of an effective pelt provides additional protection against impinging objects.

The skin of *Loxodonta africana*, as found in the present investigation and shown by Luck and Wright (1964), and that of *Elephas maximus* (Smith 1890) appear remarkably similar in structure despite wider differences in the general anatomy of the two genera.

SUMMARY

1. The African elephant flank epidermis is divided into raised papular regions 0·5 mm. in diameter, showing a weakly basophilic granular layer, separated by narrower trough zones having a much more prominent granular layer.

2. Finger-like dermal papillae indent the dermo-epidermal junction and are particularly numerous in the papular regions.

3. Hair follicles are widely spaced apart and confined to the trough zones. They show a normal mammalian follicle structure. The hairs have a broad cortex and narrow medulla. Arrector pili muscles are absent.

4. The thick stratum corneum in both the papular and trough regions is composed in paraffin processed tissue of eosin-stained, solidly constructed, flattened cells without basophilic nuclear remnants and with dorso-ventral intercellular spaces. Tinctorial differences in staining with eosin and with congo red sharply demarcate the horny layers in two regions.

5. The papular horny layer is much richer in the keratin-bound substances (cystine, cysteine, phospholipids and calcium) than the trough horny layer. It is suggested that these differences are associated with a numerical reduction of hair follicles in *Loxodonta*.

6. Sweat glands and sebaceous glands were not found in the specimen of skin examined.

ACKNOWLEDGMENTS

I wish to thank Dr. A. Jarrett for his helpful criticism, and the Medical Research Council for supporting this project.

REFERENCES

BARRNETT, R. J. and SELIGMAN, A. M. 1952. Histochemical demonstration of protein bound sulphydryl groups. *Science*, 116: 323-327.

BRODY, I. 1962. The ultrastructure of the horny layer in normal and psoriatic epidermis as revealed by electron microscopy. *J. Invest. Derm.* 39: 518-528.

CANE, A. K. and SPEARMAN, R. I. C. 1967. A histochemical study of keratinisation in the domestic fowl (*Gallus gallus*). *J. Zool. Lond.* 153: 337-352.

CAVE, A. J. E. and ALLBROOK, D. B. 1959. The skin and nuchal eminence of the white rhinoceros. *Proc. zool. Soc. Lond.* 132: 99-107.

DOSCH, F. 1915. Bau und Entwicklung des Integuments der Sirenen. *Jena Z. Naturw.* 53: 805-854.

FLESCH, P. and ESODA, E. C. J. 1964. Chemical anomalies in pathological horny layers. In: *The Epidermis*. Edit. MONTAGNA, W. and LOBITZ, W. C. Academic Press, New York. pp. 539-550.

JARRETT, A. and SPEARMAN, R. I. C. 1964. *Histochemistry of the Skin: Psoriasis.* Eng. Univ. Press, London.

JARRETT, A., SPEARMAN, R. I. C. and HARDY, J. A. 1959. The histochemistry of keratinisation. *Brit. J. Derm.* 71: 277-295.

JARRETT, A., SPEARMAN, R. I. C., RILEY, P. A. and CANE, A. K. 1965. The distribution of epidermal phospholipids and their relation to alkaline phosphatase activity in the granular layer. *J. invest. Derm.* 44: 311-319.

GIACOMETTI, L. 1967. The skin of the whale (*Balaenoptera physalis*). *Anat. Rec.* 159: 69-76.

KLIGMAN, A. M. 1963. The uses of sebum. In: *Advances in Biology of Skin 4. The Sebaceous Glands.* Edit. MONTAGNA, W., ELLIS, R. A. and SILVER, A. F. Pergamon Press, Oxford. pp. 110-124.

LEEUWENHOEK, A. VAN. 1712. Some remarks and microscopical observations upon the contexture of the skin of elephants. *Phil. Trans. Roy. Soc.* 27: 518-522.

LEVER, W. F. 1961. *Histopathology of the skin.* 3rd Edit. Lipponcott, Philadelphia.

LING, J. K. 1965. Hair growth and moulting in the southern Elephant seal (Mirounga leonina L). In: *Biology of the Skin and Hair Growth.* Edit. LYNE, A. G. and SHORT, B. F. pp. 525-544. Angus and Robertson, Sydney.

LUCK, C. P. and WRIGHT, P. G. 1964. Aspects of the anatomy of the skin of the hippopotamus. *Quart. J. exp. Physiol.* 49: 1-14.

MONTAGNA, W. and HARRISON, R. J. 1957. Specialisations in the skin of the seal *Phoca vitulina*. *Am. J. Anat.* 100: 81-114.

ROTHBERG, S., CROUNCE, R. G. and LEE, J. L. 1961. Glycine-C^{14} incorporation into the proteins of normal stratum corneum and the abnormal stratum corneum of psoriasis. *J. invest. Derm.* 37: 497-505.

SMITH, F. 1890. The histology of the skin of the elephant. *J. Anat. Phys.* 24, new series 4: 493-503.

SOKOLOV, W. 1960. Some similarities and dissimilarities in the structure of the skin among members of the sub-orders Odontoceti and Mystacoceti, cetacea. *Nature Lond.* 185: 745-747.

SPEARMAN, R. I. C. 1964. The evolution of mammalian keratinised structures. In: *The mammalian epidermis and its derivatives.* Symp. No. 12 Zool. Soc. Lond. Edit. EBLING, F. J. Acad. Press, London. pp. 67-81.

SPEARMAN, R. I. C. 1966. The keratinisation of epidermal scales, feathers and hairs. *Biol. Rev.* 41: 59-96.

SPEARMAN, R. I. C. 1968. A histochemical examination of the epidermis of the southern Elephant seal (*Mirounga leonina* L) during the telogen stage of hair growth. *Aus. J. Zool.* 16: 17-26.

SPEARMAN, R. I. C. and RILEY, P. A. 1967. The effect of osmium fixation on the histological appearance of the normal epidermal horny layers of Man and the guinea-pig. *Brit. J. Derm.* 79: 31-42.

SZAKALL, A. 1958. Experimentelle Daten zur Klarung der Function der Wasserbarriere in der Epidermis des lebenden Menschen. *Berufsdermatosen* 6:171-192.

WALKER, E. P. 1968. *Mammals of the World.* Vol. 2. John Hopkins, Baltimore.