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NOTES:
Histological changes in the epidermis of snakes during the sloughing cycle

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(Accepted 13 October 1964)

(With 3 plates and 8 figures in the text)

The histological changes which occur in the epidermis of snakes during the sloughing cycle have been studied. The results are based mainly on material obtained from *Elaphe taeniura* and are supported by observations on material from nine other genera. The sloughing cycle is divided into six stages. The regions of the epidermis and the histological changes occurring in each stage are described. Each region is named and the nomenclature applied is homologized as far as possible with the terminology used by earlier workers. It is suggested that the liberation of the old, outer, keratinized epidermal generation at sloughing is effected by the destruction of a single layer of living cells by the possible proteolytic action of immigrant eosinophil granulocytes.

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Introduction

Periodic skin-shedding or "sloughing" is a very well-known characteristic of snakes and lizards, and references to the process can be found in nearly all books on reptiles. Klauber, for example, in his monograph on rattlesnakes (1956) discussed the behaviour of snakes during sloughing, the frequency of sloughing and the properties of the shed slough. It is surprising, therefore, that the histological changes associated with the process are still not well understood.

Maurer (1892, 1895) was the first to suggest that periodic changes in the epidermis are associated with sloughing and his observations were extended by Schmidt (1914) who described the histological features in greater detail. In recent years, Bechtel (1957) and Goslar (1958) have described further microscopic and histochemical changes in the

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epidermis during the sloughing cycle, while Zimmermann & Pope (1948) discussed the relationship between skin-shedding and the growth of the rattle in rattlesnakes. Rudall's (1947) identification of two distinct molecular types of keratin in the skin of snakes is of relevance to the present study in interpreting the various layers described.

None of these authors, however, gave a complete account of the sloughing process, and the terminologies they used for the various skin layers are different and confusing. The present investigation is intended to provide a systematic account of the changing histological events throughout the sloughing cycle as a preliminary to an investigation of the endocrinological factors involved.

**Materials and methods**

This study is based mainly upon daily biopsy samples taken from the anterior lateral scales of the colubrid snake *Elaphe taeniura* Cope (Striped Racer). The animals were kept at room temperature (approximately 30°C). Skin samples from a number of other snakes occurring in Hong Kong and Great Britain were also examined. These included *Ptyas korros* Schlegel (Rat Snake), *Bungarus fasciatus* Schneider (Banded or Common Krait), *B. multicinctus* Blythe (Many Banded Krait), *Naja naja* Linnaeus (Cobra), the sea-snakes *Hydrophis cyanocinctus* Daudin, and *Pelamis platurus* Linnaeus, *Natrix natrix* Linnaeus (Grass Snake) and *Vipera berus* Linnaeus (Adder). The classification of these animals follows Romer (1961) and Smith (1954). The fresh samples were fixed in Bouin’s fluid and dehydrated in ethyl alcohol.

Epidermal tissues are difficult to prepare for histological examination and, at first, difficulties were encountered due to loss or displacement of the keratinized layers. It was found that normal clearing agents such as benzene, dioxan and xylene caused hardening and subsequent fragmentation on cutting. To overcome this, specimens were cleared for 24 h in chloroform, embedded in 56°C paraffin wax, and the blocks, knife and knife-holder deep-frozen until just before sectioning at 10 μ. The sections were mounted on albumen-smeared slides, stretched on a hot-plate, and then firmly blotted with damp filter paper. After oven-drying for at least 48 h, the slides were gently “flamed” before staining. This method enabled the sections to be stained successfully by most of the normal histological techniques with minimal loss of sections and with only minor displacement of the keratin layers.

The following stains were used: Ehrlich’s haematoxylin and eosin; Heidenhein’s haematoxylin and orange G; Aniline blue and orange G; Heidenhein’s Azan technique; Meyer’s haematoxylin, phloxine, alcian blue, and orange G (Dane & Herman, 1963; this is referred to in the text as the phloxine stain).

**Observations**

The general structure of the skin of snakes and lizards has been described elsewhere (Maderson, 1964). It consists of a series of elevated scales continuous with each other at a hinge region. Each body scale has an outer surface, and an inner surface which overlaps the adjacent scale. Only the epidermal components of the scale participate in the shedding process. The epidermis consists of a stratum germinativum, and a number of layers of living cells and keratinized tissues, which are derived from it. The cells which comprise the keratinized tissues, are not, however, produced and shed continuously, like those of the mammalian epidermis. The stratum germinativum undergoes periodic phases of cell division; the products of any two such successive phases may at times co-exist, one outside the other, forming what are termed the outer and inner epidermal generations.
During the course of one complete cycle, six stages of development, each characterized by a specific histological appearance, can be described. The division of a continuous process into separate stages is inevitably somewhat arbitrary, but is employed here for ease of description. Five of the six stages which are described below can be correlated with changes in the external appearance of the animals. The terminology used is discussed on p. 107.

Stage 1 (Fig. 1 and Plate 1(a))

The first stage is defined as that which occurs immediately after the animal has sloughed, when the skin colours are bright and the spectacle of the eye is clear.

The epidermis consists of two parts, a superficial outer epidermal generation (OG) and a basal stratum germinativum (sg).

The outer epidermal generation consists of two layers of keratin and two layers of living cells. There is an outermost layer of homogenous keratin which may be lightly or heavily impregnated with melanin granules depending on the density of the epidermal melanocytes at that point (Rahn, 1941). This keratinized layer is here termed the \( \beta \)-layer (\( \beta_0 \)); the serrations which can be seen on its free edge characterize the Oberhautchen (Obo).

Beneath the \( \beta \)-layer is a layer of loose keratinized material which stains bright pink in sections stained with eosin, and orange in phloxine stained sections; this is here termed the \( \alpha \)-layer (\( \alpha_0 \)).

The cells of the first living layer immediately beneath the \( \alpha \)-layer have granular contents, and are rectangular, lying with their long axes parallel to the surface of the skin (plto). They are firmly attached to the \( \alpha \)-layer, and under oil immersion, keratinized material may be seen between adjacent cells. Beneath this cell layer, and above the stratum germinativum, there is a single layer of ovoid living cells (pclo); these have relatively large dark-staining nuclei and the cell contents usually have a granular appearance in haematoxylin and eosin-stained sections (Goslar, 1958).

The second part of the epidermis, the stratum germinativum (sg), is the lowermost cell layer and lies directly against the collagenous connective tissue of the dermis. The component cells are almost perfectly cuboidal, and both nucleus and cytoplasm are granular and dark-staining (Goslar, 1958).

This stage in the sloughing cycle may be conveniently described as the “resting condition”. Towards the end of the stage, however, slight variations occur in the two layers of living cells of the outer epidermal generation. The cells of the lower layer (pclo) become rectangular, and take the place of the other cell layer (plto) whose component cells become
flattened and incorporated into the $\alpha$-layer. At the end of this stage there may appear to be only two layers of living cells in the epidermis; the stratum germinativum, and the other ($\rho\text{clo}$) associated with the outer epidermal generation.

**Stage 2 (Fig. 2 and Plate I(b))**

The outward appearance of the animal is similar to that in stage 1. Material showing the second stage can only be collected by estimating the approximate date on which an animal is due to enter the cloudy phase (stage 3) and by taking daily biopsy samples commencing four or five days before the anticipated date.

During the second stage, the stratum germinativum undergoes an intense cycle of proliferation and forms a new presumptive inner epidermal generation (PIG) which lies between the stratum germinativum (sg) and the outer epidermal generation (OG). The $\beta$- and $\alpha$-layers of the outer epidermal generation ($\beta_0$ and $\alpha_0$) are unchanged in their appearance since the previous stage. The outermost layer of living cells (Fig. 1, $\rho\text{lo}$) is now almost completely incorporated into the $\alpha$-layer and the innermost layer of living cells (Fig. 2, $\rho\text{Obi}$) is closely juxtaposed to its nuclear remains.

The first layer of daughter cells produced from the stratum germinativum comes to lie immediately beneath the outer epidermal generation; these ovoid cells lie with their long axes parallel to the surface of the skin, and both their cytoplasm and their nuclei are slightly granular ($\rho\text{Obi}$). The second layer of daughter cells still retain their rounded appearance and some may be seen lying halfway between the level of the stratum germinativum and the ovoid cells just described. Numerous cells undergoing mitosis are visible in the stratum germinativum at this time.

During the two or three days of this stage, some 10 to 12 layers of flattened cells are laid down in the presumptive inner epidermal generation (PIG). The superficial layers begin to differentiate while the stratum germinativum is still proliferating.

Towards the end of the stage the cells of the stratum germinativum, which were uniformly cuboidal throughout stage 1, lose their alignment and sometimes appear to be slanted at an angle towards the skin surface.

**Stage 3 (Fig. 3 and Plate I(c))**

This stage is characterized externally by a slight overall dulling of the animal's coloration, and in particular by a slight cloudiness of the spectacle of the eye. The layers of new cells produced in the preceding stage show further signs of differentiation.
The keratinized portions of the outer epidermal generation are unchanged in their histological appearance ($\beta_0$ and $\alpha_0$). The outermost layer of living cells (plto) is now represented only by a line of pycnotic nuclei adhering to the $\alpha$-layer. The rectangular living cells of the innermost layer still retain their previous shape, but their staining characteristics have changed. The cytoplasm no longer stains and the conspicuous nuclei appear to lie in a vacuole; because of this feature the layer is termed the "clear layer" (clo). The $\alpha$-layer of the outer epidermal generation and the two associated cell layers lying beneath it are here collectively termed the "stratum intermedium" (sio). This stratum is the region through which the "split" occurs at sloughing between the old outer epidermal generation and the newly-formed inner epidermal generation.

![Diagram of epidermis](image)

Fig. 3. Diagrammatic drawing of the epidermis of the outer scale surface in the stage 3 condition. (For abbreviations, see p. 112.)

During this stage, the cells of the inner epidermal generation (PIG), which were derived from the stratum germinativum during stage 2, begin to keratinize; regional divisions become apparent in the layers of flattened living cells. The most superficial cell layer (Obi) lying immediately beneath the outer epidermal generation has a very characteristic appearance and is easily recognizable in sections. It consists of a line of rectangular cells with dark-staining ovoid nuclei lying in a granular cytoplasm. In sections stained with haematoxylin and eosin this layer may appear to be refractive, but in phloxine, and azan-stained sections, it takes up a trace of alcian or aniline blue suggesting the presence of mucopolysaccharides. A slight thickening of the outermost cell walls may sometimes be observed, although this becomes more apparent in stage 4. This layer (Obi) will become the Oberhautchen associated with the inner epidermal generation.

The layers of flattened living cells of the inner epidermal generation lying between the future Oberhautchen and the stratum germinativum, are, at this time, roughly divisible into three regions. The outermost cell layers ($p\beta_1$) stain very darkly pink in haematoxylin and eosin sections and orange in phloxine sections. All the component cells are very flattened and contain deep-staining granular contents. The cell walls are thickened and there are
numerous "tonofibrils" (Mercer, 1961) running between adjacent cells. All the cells have flattened nuclei, the degree of flattening increasing the more superficial the cells lie. At the time of their origin from the stratum germinativum these cells acquired copious intracellular deposits of melanin granules which are now visible.

The middle region (pei) beneath that described above stains very lightly pink in haematoxylin and eosin sections and slightly blue in phloxine sections. Here the nuclei are less flattened, the cell contents less granular, and melanin is absent.

The innermost region (ili) of the inner epidermal generation consists of two or three layers of living cells lying immediately outside the stratum germinativum. The cells vary in shape from ovoid to round, they have dark-staining, relatively large, nuclei and very lightly-staining cell contents.

The cells of the stratum germinativum have altered considerably in shape. They have lost their previous cuboidal shape and are now columnar with their long axis at right angles to the surface of the skin. The nuclei are no longer darkly staining, and the cell contents do not stain. Some mitotic figures are visible at this stage.

**Stage 4 (Fig. 4 and Plate II(a))**

At this time the colours of the intact animal are at their duldest. The spectacle over the eye is almost opaque and the animal is usually inactive and quiescent. The loss of colour is due to the rapid establishment of a layer of new tissue between the inner and outer epidermal generations in the region of the stratum intermedium (sio).

![Diagram](image)

**Fig. 4.** Diagrammatic drawing of the epidermis of the outer scale surface in the stage 4 condition. (For abbreviations, see p. 112.)

The β-layer of the outer epidermal generation is unchanged from its previous condition. The uppermost portion of the α-layer is also unchanged, staining pink (haematoxylin and eosin sections) or orange (phloxine sections) as before. The lower portion of the α-layer
and the associated cell layers have undergone radical changes. Both the layer of nuclear remnants (Fig. 3, plto) and the cellular clear layer (Fig. 3, clo) have disappeared and in their place is seen a region of blue-staining tissue (azan and phloxine sections) consisting of a series of protoplasmic strands with large lacunae between them (Fig. 4, lto) and with scattered nuclei borne on the strands. The extent of this region of blue-staining tissue depends on the part of the scale being examined. If the section is taken either transversely or longitudinally through the distal two-thirds of the outer scale surface, the lacunar nature of the lower part of the stratum intermedium can only be determined with difficulty under oil immersion. On the outer surface of the proximal third of the scale, and all over the inner scale surface and hinge region, however, the histological structure is quite different. Here the stratum intermedium is greatly increased in depth, the strands are readily apparent, and there are not only scattered nuclei to be seen, but also an entirely different type of cell. The irregular amoeboid shape of these latter, and the staining characteristics of their intra-cellular granules in haematoxylin and eosin sections identify them as eosinophil granulocytes. These cells appear in the dermal blood vessels at the beginning of stage 2, and, after leaving the blood vessels subsequently migrate up through the stratum germinativum, passing between the flattened cells of the presumptive inner epidermal generation and come to lie in the stratum intermedium of the outer epidermal generation (Fig. 4, sio).

In *Elaphe* and *Natrix* the migration of the eosinophils through the lower epidermal layers has only been observed on the inner scale surfaces, and in the hinge regions, but in *Pelamis*, and especially in *Hydrophis*, they migrate up through the epidermis over the whole surface of the scale (see Plate III(a)). There is also a considerable difference in the size of these eosinophils in the various genera. In *Elaphe* they are fairly small, measuring approximately 8 μ–11 μ across. In *Hydrophis* (Plate III(a)), however, they are much larger and measure approximately 13 μ–17 μ across, so that two or even three layers of flattened cells may be partially obscured by one of these migrating cells. Positive identification of these cells in the mature stratum intermedium of the outer scale surface of *Elaphe* and *Natrix* is, however, uncertain.

The Oberhautchen of the inner epidermal generation (Fig. 4, Obi) has also undergone considerable differentiation since stage 3. The cytoplasm no longer stains blue in azan and phloxine sections, but is chromophobic. The nuclei of the cells are pycnotic and often seem to lie in a central vacuole. The outer edge of the cells against the stratum intermedium is highly refractive, and shows signs of keratinization as evidenced by the orange-staining in phloxine sections, and the pinkish appearance in haematoxylin and eosin sections. Examination of this outer edge under oil immersion shows oblique intra-cellular structures resembling teeth (Fig. 4). As keratinization proceeds, the individual cells are split into pieces, apparently by the continued oblique growth of these structures. The pieces continue to adhere to the outer surface of the keratinizing β-layer (see below) and it seems that the splitting gives rise to the sculpturings by which early workers characterized the Oberhautchen.

The division of the rest of the inner epidermal generation (IG) into three regions is now even more apparent than it was in stage 3. The outermost region (pβi) immediately beneath the Oberhautchen, now stains far more darkly with all the stains used; the details of the individual cells are partially obscured and the nuclei are so flattened that they are only just visible. The greatest differentiation between this region and the region beneath
(a) Epidermis of outer scale surface of *Elaphe taeniura* showing the stage 1 condition. During histological preparation the α-layer of the outer epidermal generation has split so that the β-layer (with part of the α-layer attached to it), lies away from the living tissues of the epidermis which are covered by the remainder of the α-layer. Ehrlich's haematoxylin and eosin.

(b) Epidermis of outer scale surface of *Elaphe taeniura* showing the stage 2 condition. The same type of artefact as was described in Fig. 1 is again seen. Note the mitotic figure in the stratum germinativum. Ehrlich's haematoxylin and eosin.

(c) Epidermis of outer scale surface of *Elaphe taeniura* showing the stage 3 condition. The β-layer of the outer epidermal generation is not shown in the photograph. The clear layer is visible as a light band above the lightly striated Oberhautchen of the inner epidermal generation. Ehrlich's haematoxylin and eosin.

[To face page 104.]
PLATE II(a). Epidermis of the outer scale surface of *Elaphe taeniura* showing the stage 4 condition. The $\beta$-layer of the outer epidermal generation is not shown in the photograph. The clear layer is still represented as a clear band, but is not cellular at this time. Note the difference in the staining reactions of the presumptive $\beta$-layer and $\alpha$-layer of the inner epidermal generation. Phloxine stain.

(b) Epidermis of the outer scale surface of *Elaphe taeniura* showing the stage 5 condition. The gap between the outer and inner epidermal generations is caused by the breakdown of the tissues of the stratum intermedium. Ehrlich's haematoxylin and eosin.
PLATE III. See caption overleaf.
PLATE III(a). Epidermis of outer scale surface of *Hydrophis cyanocinctus* showing stage 3–4 condition and the migration of eosinophils. The keratinized tissues of the outer epidermal generation are not shown in the photograph. The lacunar nature of the stratum intermedium can be seen. Some eosinophils lie on the boundary between the two keratinizing parts of the inner epidermal generation, but some are also to be seen passing between the cells of the irregular stratum germinativum. Ehrlich’s haematoxylin and eosin.

(b) Epidermis of the outer scale surface of *Elaphe taeniura* showing the stage 6 condition just before sloughing. The inner epidermal generation shows an artefact similar to that described in Plate I(a). Ehrlich’s haematoxylin and eosin.
HISTOLOGICAL CHANGES IN THE EPIDERMIS OF SNAKES

The outermost region stains heavily orange with the phloxine B and orange G components of this stain indicating the presence of pre-keratin (Dane & Herman, 1963), whereas the region beneath (p₂i) presents a laminated appearance staining a uniform light blue. The nuclei in this lower region are slightly less flattened, and may even retain a slightly ovoid shape nearer the stratum germinativum. Phloxine-stained sections do not show the lowermost region (lili) very distinctly but in sections stained with haematoxylin and eosin two or three layers of ovoid cells are clearly seen. These have slightly granular cell contents and the nuclei are fairly clear. Under oil immersion the cells in the most superficial layer are seen to be more rectangular in appearance and are in close apposition to the lowermost cell layer of the middle region (p₂i).

The cells of the stratum germinativum remain columnar and the nuclei and cell contents take up very little stain.

Stage 5 (Fig. 5 and Plate II(b))

About five to eight days before sloughing occurs, the colours of the snake suddenly become bright again and the spectacle of the eye is completely clear. This stage corresponds with the breakdown of the lacunar tissue of the stratum intermedium (sio).

The keratinized portions of the outer epidermal generation remain unchanged.

Stage 5 (Fig. 5 and Plate II(b))

About five to eight days before sloughing occurs, the colours of the snake suddenly become bright again and the spectacle of the eye is completely clear. This stage corresponds with the breakdown of the lacunar tissue of the stratum intermedium (sio).

The keratinized portions of the outer epidermal generation remain unchanged.

The stratum intermedium (sio) associated with the outer epidermal generation may still show blue-staining lacunar tissue in places but for the most part the strands have broken down and all that remains are keratinized strands with adpressed nuclei. The eosinophils
are likewise reduced in number and are frequently absent. There is, therefore, a gap between the outer epidermal generation and the Oberhautchen of the inner epidermal generation (Fig. 5 and Plate II(b)).

In the inner epidermal generation (IG) the process of keratinization has carried on down through the superficial layers so that the outer region now presents the same histological appearance as the β-layer of the outer epidermal generation, except for traces of pycnotic nuclei which disappear in stage 6. This homogeneous keratinized material with its deposited melanin is here termed the definitive β-layer of the inner epidermal generation (Fig. 5, βi). There are sometimes slight indications of orange staining in the lower part of the β-layer, but the region becomes uniformly yellow in phloxine sections as the keratinization process is completed.

The cells of the middle region of the inner epidermal generation (Fig. 4, ρaxi) lose the blue-staining properties seen in stage 4, and now stain orange in phloxine sections indicating the presence of prekeratin as was seen in the outermost region in the previous stage. Just as the thickness of the inner epidermal generation decreased with the onset of the keratinization process, this middle region also becomes thinner as its component cells become more flattened with increasing keratinization. In sections stained with haematoxylin and eosin the reduction of this dark pink-staining region is especially apparent. This region can now be identified with the α-layer of the outer epidermal generation which was described earlier.

At this time there are still two or three layers of living cells between the α-layer (αi) and the stratum germinativum (sg). The most superficial of these cell layers later becomes completely keratinized and incorporated into the α-layer. The component cells of the other two layers are still rounded. As previously mentioned, the two lower layers will undergo differentiation right up to the end of the next “stage 1”, when they will form part of the stratum intermedium of the next outer epidermal generation to which they will belong. These cells (illi), and the overlying α-layer can therefore be termed the presumptive stratum intermedium (psii) of the inner epidermal generation.

At this time the cells of the stratum germinativum are regaining their definitive cuboidal shape and take up stains more readily.

Stage 6 (Fig. 6 and Plate III(b))

Sloughing occurs from four to seven days after the disappearance of the cloudiness of the animal’s colour, the variation depending on the health of the snake and environmental factors. In a healthy animal the old keratin will be shed in toto (Klauber, 1956) and the process is completed within 24 hours. Imminent sloughing is detected by a slight renewal of dulling of the animal’s colour and if the snake is held firmly, the outer keratin feels loose. This loosening is brought about by the complete breakdown of the physical connection between the outer and inner epidermal generations, the split occurring within the stratum intermedium.

The structure of the epidermis immediately before sloughing is shown in Fig. 6. The keratinized portions of the outer epidermal generation (OG) are unchanged from their previous appearance and both the β- and α-layers are still visible. On the lower surface of the α-layer a line of pycnotic nuclei is apparent. It is uncertain whether these nuclei are derived from the cells of the clear layer of the stratum intermedium, or from the eosinophils.
The histological appearance of the inner epidermal generation is the same as that described for the outer epidermal generation in stage 1. When the original outer epidermal generation (OG) is shed then the cycle will be complete and the old "inner epidermal generation" (IG) will become the new outer epidermal generation (Fig. 1, OG).

Fig. 6. Diagrammatic drawing of the epidermis of the outer scale surface in the stage 6 condition. (For abbreviations, see p. 112.)

Discussion and conclusion

Nomenclature

Although many of the terms used here have been employed by other workers, they have been used ambiguously and without precise definition. All the terms used here have been redefined and it is desirable to homologize as far as possible the present terminology with that of earlier workers (Fig. 8). This homology can best be illustrated with reference to Figs 4 and 5 which show the epidermis in its histologically most complex condition. For comparative purposes Maurer's (1895) figure of the squamate epidermis is reproduced here as Fig. 7. It should be noticed that the presence of the innermost of the three epidermal generations figured by Maurer (Fig. 7) has not been confirmed, as far as is known, by any subsequent worker (see p. 110).

The most superficial part of the "outer epidermal generation" (Figs 4 and 5, OG) bears characteristic sculpturings. This region is here termed the Oberhautchen (Obo) following the terminology of Maurer (1895) and Schmidt (1914). It is the first part of each successive generation to keratinize and it appears to play an important part in the sloughing process (see below). The keratinized material of which the Oberhautchen is part is here termed the β-layer (βo), corresponding to the "layer of β-type keratin" described by Rudall (1947), and the keratinized material termed the α-layer (αo) corresponds to the "layer of α-type keratin" described by the same author.

In this work the stratum intermedium is regarded as a composite structure with three separate parts—the innermost portion of the α-layer, a layer of pycnotic nuclei (Fig. 3, plto), and a layer of cells termed the "clear layer" (Fig. 3, clo). The last two layers give rise to the "lacunar tissue" (Fig. 4, lto). Zimmermann & Pope's (1948) description of the origin of their stratum intermedium (which, according to their description, consists of
several layers of cells) from the outer part of the newly-forming rattle, seems to support Schmidt's (1914) text observation that his "shedding cells" (see p. 110) were derived from the outer cell layers of his inner epidermal generation. This view cannot be supported, because apart from the included eosinophils, the tissues which comprise the stratum intermedium as defined here, originate from the base of each epidermal generation. On the other hand, Zimmermann & Pope's description of the stratum intermedium in the rattle of *Crotalus* just before a new segment is added, does correspond to what is here described as "lacunar tissue" (Fig. 4, Ito).

The "inner epidermal generation" (Figs 4 and 5, IG) corresponds to Maurer's (1895) "second epidermal generation" (Fig. 7, Gen. 2); this region is also indicated by a variety of names by the other authors (see Fig. 8). The Oberhautchen of the inner epidermal generation (Figs 4 and 5, Obi) corresponds to Maurer's "Oberhautchen of the second epidermal generation" (Fig. 7, Ob. 2). Schmidt (1914) figured a second Oberhautchen in this region, but did not comment on the significance of the sculpturings which characterize this layer. Although he noted the presence of intra-cellular striations, Maurer (1859) appears to concur with Kerbert's (1876) suggestion that the sculpturings result from the "overlapping" of adjacent cells that was said to occur during keratinization. This view cannot be maintained as the intra-cellular origin of the serrations can be observed during stage 4 and "overlapping" of keratinizing Oberhautchen cells has never been observed. Goslar (1958) described certain structures at the border of the old and new epidermal generations in *Natrix* which are quite different from the structures described here in *Elaphe*. Goslar stated that the structures he described vary considerably in form and distribution. The observations made in this study suggest a uniformity of structure, and the serrations have always been found on all parts of the outer scale surface. A number of the reactions of this region to standard histological techniques described by Goslar (1958) are not confirmed by the present author.

Maurer (1895) and Schmidt (1914) did not separate the inner epidermal generation into two regions which would correspond to the β- and α-layers described here. Goslar (1958), however, indicated that the "compact tissue of the young horny layer" (β-layer here) showed a higher degree of S—S activity than the underlying "loose tissue of the
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Tissues described but no specific name given

OLD EPIDERMAL GENERATION
NEW EPIDERMAL GENERATION/YOUNG HORNY LAYER
OBERHAUTCHEN/INNER SHEDDING CELLS
COMPACT HORY LAYER
LOCALITY
Keratinized tissues not divided into regions

**Fig. 8.** Table showing the homology of the terms used in this work with those of previous workers (see Discussion).

† Maurer described three epidermal generations; this observation has not been confirmed by any subsequent author.
‡ The stratum intermedium, in the sense the term is used here, is a composite structure involved in the sloughing mechanism. It is not strictly homologous with any single layer described by previous workers (see Discussion).
§ In the older German texts, the stratum germinatium is sometimes referred to as the basal layer of the stratum Malpighii, when this latter term is used to describe all the innermost, unkeratinized cells of the epidermis. However, it is sometimes referred to as the stratum Malpighii.
|| These terms, which are more commonly used in descriptions of the mammalian epidermis were used somewhat ambiguously by Zimmermann & Pope (1948) in their description of the keratinization of the rattle of rattlesnakes. As the terms have rather specific meanings in mammalian histology their use in the present context is not desirable (see Discussion).
young horny layer” (α-layer here), during the keratinization phase. This concurs with Matoltsy’s (1962) and Barnett & Sognnaes’ (1962) statements that hard, fibrous keratins tend to contain a high percentage of fully polymerized cystine groups.

In their description of the keratinization of the new segment in the rattle of *Crotalus*, Zimmermann & Pope (1948) used the terms stratum profundum, stratum spinosum, and stratum granulosum. The histological detail they gave is insufficient to permit a precise homology with the regions described here. As the terms they used have specific meanings in mammalian histology, their use in the present context is not desirable. Neither Rudall (1947) nor Bechtel (1957) considered the processes of cell differentiation in the inner epidermal generation.

The most important difference between Maurer’s (1895) observations and those presented here concerns the “first epidermal generation” (Fig. 7, Gen. 1). Maurer claimed that three fully differentiated epidermal generations were seen at the same time. Under certain circumstances, a snake will undergo a cloudy phase and not slough, so that two fully keratinized “outer” epidermal generations and a normal inner epidermal generation may be seen in the same sections. This condition is probably pathological and a series of biopsy samples from healthy animals confirms Schmidt’s (1914) statement that no more than two epidermal generations exist in any part of the epidermis at the same time.

The homologies discussed here are presented in a tabulated form in Fig. 8.

**The shedding mechanism**

The mechanism by which the outer epidermal generation becomes separated as the slough from the underlying inner epidermal generation may now be considered.

Maurer (1895) stated that shedding was brought about by the “drying-up” of the incompletely keratinized cells of his stratum intermedium (clear layer). Schmidt (1914) described two layers of “shedding cells”; an “outer layer” (possibly the clear layer), and an “inner layer” which appears to be the Oberhautchen (Obo) of the inner epidermal generation. There is a discrepancy between Lange’s (1931) and Goslar’s (1958) interpretation of Schmidt’s (1914) observation as to where the split actually occurs between the two epidermal generations. Lange (1931) stated that it occurred in the clear layer, while Goslar (1958) stated that it occurred beneath this layer. The former interpretation seems more likely as Schmidt (1914) claimed that the nuclei of the clear layer remained on the shed keratinized tissue; pycnotic nuclei have been described in this position (p. 106). Schmidt (1914) said further that the remains of the cells look like “fibrils” on the Oberhautchen of the inner epidermal generation. Unfortunately it is not possible to be certain of the exact relationship between Schmidt’s “fibrils” and the sculpturings described here. The difficulty of interpreting Goslar’s (1958) own observations has already been mentioned.

A further problem concerning the shedding mechanism is raised by the possible role of the eosinophil granulocytes which appear in the epidermis. Although blood cells of this type are well-known to histologists, they are not mentioned specifically in the German literature. The “shining” cells which Kerbert (1876) described in the dermo-epidermal region in carmine-stained sections, and which he compared to those seen in pathological human tissue were probably eosinophils. Maurer (1895), Schmidt (1914) and Goslar (1958) all referred to granular cells in the epidermis, but the granules were not described as eosinophilic, and all three authors compared them to the Leydig cells of Amphibia. However, Schmidt (1914) also described another type of cell with granules borne in a
protoplasmic network, and stated that they were to be found in groups in between the two epidermal generations in the hinge region. From the figure these cells look very like eosinophils, but Schmidt claimed that the granules were keratohyalin. Such cells have not been seen in the present study. None of the German authors suggested that any of the cells they described were associated with sloughing.

The migration of eosinophils from the dermis through the lower regions of the epidermis to the stratum intermedium has been previously described by Zimmermann & Pope (1948) in Crotalus, and Bechtel (1957) in Elaphe g. guttata. Although Pienaar (1962) inferred that the unusually high eosinophil count in the circulating blood of one of his snakes was due to an undetected worm infection (eosinophilia being a common symptom of such an infection), and the presence of eosinophils in the human epidermis is well-known as a symptom of allergic skin disorders (de Gruchy, 1958), there appears to be little doubt that their periodic appearance in the snake epidermis is a normal feature of the sloughing process. Zimmermann & Pope's (1948) figures suggest that the eosinophils are quite large in Crotalus, but in Elaphe and Natrix they are small and easily overlooked.

Suggested sloughing mechanism

The observations made in this study make it possible to suggest a mechanism responsible for sloughing.

By stage 3, the cells of the clear layer are the only living elements belonging to the outer epidermal generation. Their intimate attachment to the lower surface of the α-layer has been emphasized. Also by stage 3, the Oberhautchen of the inner epidermal generation is seen as a clearly differentiated layer. The outer surfaces of its component cells are closely applied to the cells of the clear layer. The indications of mucopolysaccharide material in this region revealed by standard histological techniques confirm the detailed histochemical study by Goslar (1958). After comparing his results with those of other workers, Goslar concluded that these materials reside in the intercellular cement which binds the living cells of the clear layer, and of the Oberhautchen, to their neighbours and to each other. The morphological evidence therefore suggests that the boundary between the cell layers described above, is the only region where the inner and outer epidermal generations are joined to one another. If the connection between the cells of the clear layer of the outer epidermal generation and the cells of the Oberhautchen of the inner epidermal generation, was destroyed, then the outer epidermal generation could be shed from the body.

By stage 4, the migrating eosinophils have reached the stratum intermedium. Wintrobe (1946) and Godlowski (1953) confirm Zimmermann & Pope's (1948) suggestion, that eosinophils in amniotes contain proteolytic enzymes. It seems possible that if these enzymes were released in the hinge region, where most of the eosinophils are seen, they could then "digest" their way round the whole scale, destroying the intercellular cement and cell membranes of the clear layer on their way. The significance of the intracellular splitting of the Oberhautchen cells which gives rise to the characteristic sculpturings can not be understood at the present time. The presence of these sculpturings on the β-layer of the first epidermal generation seen in the embryo (Maderson (1965)), before any stratum intermedium or eosinophils are seen suggests they could not be the result of enzyme activity. Such a process of slow digestion of the intercellular bonds would explain the formation of the "lacunar tissue" seen in stage 4, and the presence of pycnotic
nuclei adhering to the outer epidermal generation when a natural slough is examined. Goslar (1958) emphasized that once histodifferentiation of the inner epidermal generation began, the keratinized portions of the outer epidermal generation were easily lost during histological preparation. The continued adherence of the clear layer to the underlying Oberhautchen after the rest of the outer epidermal generation has become detached during preparation of material showing a stage 2 or 3 condition, supports the idea that prior to the invasion of the stratum intermedium by the eosinophils, the living cells in this region are held together by strong intercellular bonds. Final substantiation of the hypothesis must await conclusive demonstration of a period of enzyme activity within the stratum intermedium.

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Figs. 1 to 6

Fig. 7

Key to abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>$\alpha$</td>
<td>$\alpha$-layer of inner epidermal generation</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$\beta$-layer of inner epidermal generation</td>
</tr>
<tr>
<td>$\beta_i$</td>
<td>$\beta$-layer of outer epidermal generation</td>
</tr>
<tr>
<td>clo</td>
<td>Clear layer of stratum intermedium of outer epidermal generation</td>
</tr>
<tr>
<td>IG</td>
<td>Inner epidermal generation</td>
</tr>
<tr>
<td>lli</td>
<td>Three layers of innermost, living cells associated with the inner epidermal generation</td>
</tr>
<tr>
<td>lto</td>
<td>Lacunar tissue of stratum intermedium associated with the outer epidermal generation</td>
</tr>
<tr>
<td>Obi</td>
<td>Oberhautchen of inner epidermal generation</td>
</tr>
<tr>
<td>Obo</td>
<td>Oberhautchen of outer epidermal generation</td>
</tr>
<tr>
<td>OG</td>
<td>Outer epidermal generation</td>
</tr>
<tr>
<td>PIG</td>
<td>Presumptive inner epidermal generation</td>
</tr>
<tr>
<td>p$\alpha$</td>
<td>Presumptive $\alpha$-layer of inner epidermal generation</td>
</tr>
<tr>
<td>p$\beta$</td>
<td>Presumptive $\beta$-layer of inner epidermal generation</td>
</tr>
<tr>
<td>pclo</td>
<td>Presumptive clear layer of stratum intermedium of the outer epidermal generation</td>
</tr>
<tr>
<td>ptelo</td>
<td>Presumptive lacunar tissue of the stratum intermedium of the outer epidermal generation</td>
</tr>
<tr>
<td>pObi</td>
<td>Presumptive Oberhautchen of the inner epidermal generation</td>
</tr>
<tr>
<td>psii</td>
<td>Presumptive stratum intermedium of the inner epidermal generation</td>
</tr>
<tr>
<td>sg</td>
<td>Stratum germinativum</td>
</tr>
<tr>
<td>sio</td>
<td>Stratum intermedium of the outer epidermal generation</td>
</tr>
</tbody>
</table>

Gen. 3 | Third epidermal generation |
Gen. 2 | Second epidermal generation |
Gen. 1 | First epidermal generation |
st. corn. 1 | Stratum corneum of first epidermal generation |
st. corn. 2 | Stratum corneum of second epidermal generation |
st. corn. 3 | Stratum corneum of third epidermal generation |
st. int. 1 | Stratum intermedium of first epidermal generation |
st. int. 2 | Stratum intermedium of second epidermal generation |
st. int. 3 | Stratum intermedium of third epidermal generation |
Ob. 1 | Oberhautchen of first epidermal generation |
Ob. 2 | Oberhautchen of second epidermal generation |
Ob. 3 | Oberhautchen of third epidermal generation |
st. germ. | Stratum germinativum |
REFERENCES


Appendix

Since this paper went to press, a second part has appeared to Goslar’s study (Goslar, H. G. (1964). *Acta Histochem.* 17: 1-60). Although Goslar describes enzyme activity at the ‘frontier layer’ (probably the stratum intermedium of this work), there is still some doubt as to the exact relationship between his regions and the structures described here, particularly in the context of the sculpturings described in this work.