
Lorenzo Alibardi and Paul F.A. Maderson

**ABSTRACT** Histochemical and TEM analysis of the epidermis of *Sphenodon punctatus* confirms previous histological studies showing that skin-shedding in this relic species involves the periodic production and loss of epidermal generations, as has been well documented in the related Squamata. The generations are basically similar to those that have been described in the latter, and their formation involves a cyclic alternation between \(H252\) - and \(H251\) -keratogenesis. The six differences from the previously described squamate condition revealed by this study include: 1) the absence of a well-defined shedding complex; 2) the persistence of plasma membranes throughout the mature \(H252\) -layer, including the oberhautchen; 3) the concomitant presence of lipogenic lamellar bodies and PAS-positive mucous granules in most presumptive \(H251\) -keratinizing cells; 4) the presence of the secreted contents of these organelles in the intercellular domains of the three derived tissues, the homologues of the squamate mesos, \(H251\) - and lacunar cells; 5) the paucity of lamellated lipid deposits in such domains; 6) the presence of keratohyalin-like granules (KHLG) in the presumptive lacunar, clear, and oberhautchen cells. In toto, the absence of many of the precisely definable, different pathways of cytotogenesis discernible during squamate epidermal generation might be interpreted as primitive for lepidosaurs. However, when the evolutionary significance of each of the six differences listed is evaluated separately, it becomes clear that the epidermis of *S. punctatus* possesses primitive amniote, shared and derived lepidosaurian, and some unique characters. This evaluation further elucidates the concept of a lepidosaurian epidermal generation as a derived manifestation of the sauropsid synapomorphy of vertical alternation of keratin synthesis and shows that further study of keratinocyte differentiation in the tuatara may contribute to our understanding of the origin and evolution of \(H251\) -keratinization in sauropsid amniotes. J. Morphol. 256: 111–133, 2003. © 2003 Wiley-Liss, Inc.

**KEY WORDS:** tuatara; *Sphenodon*; Lepidosauria; squamate; epidermis; keratin; evolution

Skin shedding in lizards and snakes involves the periodic formation and loss of epidermal generations. Over the past 40 years the underlying cyclic cellular activities have been well documented by light (LM) and electron microscopy (EM) (reviewed by Maderson, 1985; Landmann, 1986; Maderson et al., 1998). Fundamentally, squamate epidermis is a stratified squamous epithelium wherein populations of daughter cells derived from a morphologically homogeneous stratum germinativum (Maderson et al., 1972) manifest a conspicuous vertical alternation between two distinct keratogenic pathways, emphasizing either \(H251\) - or \(H252\) -proteins. Although mechanisms directing this alternation remain uncertain (Maderson, 1985, pp. 555–558), many studies have confirmed the presence of morphologically and functionally distinct epithelia associated with the transitions from one pathway to the other.

During the transition from \(H252\) - \(\rightarrow\) \(H251\) -keratogenesis, the flattened cells of the mesos layer are laid down. The mature tissue houses the barrier to cutaneous water loss (CWL) (Lillywhite and Maderson, 1982), a function that depends on mesos granules, a type of lipogenic lamellar body (LB). Transmission electron microscopic (TEM) study shows that, in mammals, LBs exocytose their contents into the extracellular domain, where they form lipid-rich, bilayered structures (Menon et al., 19921996; Menon and Ghadially, 1997; Menon and Menon, 2000).

During the transition from \(H251\) - \(\rightarrow\) \(H252\) -keratogenesis, a shedding complex is laid down. During the preshed renewal phase, membranes of cells of the clear...
layer (the innermost component of the outer generation, the next to be shed) develop interdigitations with those of the subjacent oberhautchen (the outermost component of the inner generation that will be exposed next) whose eventual separation permits shedding. These events are barely discernible by LM. Details of membrane interdigitation and the resultant microornamentation demand, respectively, TEM and scanning electron microscopic (SEM) analysis (Maderson et al., 1998).

Vertical alternation of β- and α-keratogenesis in the epidermis is a sauropoid synapomorphy and lepidosaurian skin shedding is its best-known manifestation (Maderson and Alibardi, 2000). The existence of two unique squamate tissues (mesos layer and shedding complex) associated with the alternating transitions raises questions regarding their evolutionary origin, answers to which must be sought in the closest living relative of squamates, the relic sphenodontid species, the New Zealand tuatara (Sphenodon punctatus).

An LM study of tuatara epidermis (Maderson, 1968) confirmed earlier reports of the presence of epidermal generations. It was found that, in contrast to its syncytial form and chromophobia in squamates, the mature β-layer retains its stratified squamous morphology so that its tinctorial properties grade insensibly into those of the α-layer. Presence of a mesos layer was suggested by artifactual splitting of mature corneous tissues during tissue processing. Identification of a shedding complex was uncertain due to lack of renewal phase material (see below). By LM, an oberhautchen was discernible only as a refractive line on the surface of the β-layer: the paucity of microornamentation was later established in an SEM study (Petersen, 1984).

The present TEM study extends identification of epidermal components in Sphenodon punctatus. New data 1) permit more precise comparison with those of squamates and extend understanding of the lepidosaurian epidermal generation, 2) elucidate the evolutionary origin of the squamate barrier to CWL and shedding complex, and 3) offer insights into the origins of amniote keratinization.

MATERIALS AND METHODS

Pieces of caudal skin (1–3 mm long for TEM, 5 mm for histology) were sampled from six specimens of Sphenodon punctatus. Three were 3-month-old juveniles hatched in the laboratory from eggs collected on Stephens Island, Cook Strait, New Zealand, by authority of the NZ Department of Conservation (Thompson, 1990). The other three were adults captured during the summer on Stephens Island (Alibardi, 1992). Status of individuals within shedding cycles was unknown.

Samples for TEM were fixed in 2.5% glutaraldehyde in Ringer’s (pH 7.4–7.6), stored in Ringer’s (12–16 h), postfixed in 1% osmium tetroxide (2 h), dehydrated in graded ethanol, embedded in Epon, and sectioned on an ultramicrotome. Semithick sections of normal skin from different regions of the tail were stained with 0.5% toluidine blue. Some sections were double-stained with eosin for 3–10 sec over a hotplate, washed, and then stained with 0.5% toluidine blue. Others were deplastincized with sodium-ethoxide prior to staining with PAS and 1.0% alcian blue to detect mucosubstances (Troyer, 1980). Thin sections collected on 200–100 mesh copper or nickel grids were stained with uranyl acetate and lead citrate and examined with a Philips CM 100 electron microscope.

Larger pieces of tail (5–10 mm) were fixed in 10% formaldehyde, ethanol dehydrated, cleared in xylene, and wax embedded. Sections 6–10 μm were stained with hematoxylin and eosin.

RESULTS

Gross Morphology

Tail scales vary in size and shape depending on the region examined (Fig. 1). Those on the characteristic mid-dorsal ridge are heavily keeled. While most dorsolateral scales overlap little or not at all, the larger medioventral scales are slanted and partially overlapped, permitting identification of outer and inner surfaces and hinge regions (Maderson, 1964).

Light Microscopic Observations

Data presentation — definitions of three epidermal cytologies. With material from only six animals available for study, some aspects of cyclic epidermal activity were not represented. Additionally, a description of semithick sections (Figs. 2–6) is confounded by 1) great variability in tinctorial properties of keratinized tissues within and between specimens, and 2) unique cytologies. These factors complicate correlation with previous LM studies (Maderson, 1968) and comparison with squamate cycle stages (Maderson, 1985; Maderson et al., 1998, fig. 1).

To facilitate communication, we define six different epidermal cytologies in our material as Conditions One, Two, and Three. Each definition is accompanied by a brief comment on the general relation of the condition to the squamate cycle. The definitions are followed seriatim by LM descriptions of 1) aspects of keratinized tissues of the outer generation common to all specimens, 2) the inner generation in one specimen, and 3) the stratum germinativum and suprabasal cells in all six. These descriptions permit tentative assignment of each of the six specimens to a stage of the squamate cycle. In accounts of TEM and histochemical data for each condition that complete the results, the assignments permit specific identifications of cell types that are justified in the Discussion section.

Condition One, seen in samples from two juveniles and one adult, shows an incomplete outer generation above flattened basal cells (Fig. 2). It reflects a resting stage of squamates.

Condition Two, seen in samples from two adults, shows a complete, but immature, outer generation above cuboidal/columnar basal cells (Fig. 3). This is identified as a Stage 2 of squamates.
Condition Three, seen in one juvenile, shows a complete, but immature, outer generation, an incomplete inner generation, and a germinal layer (Figs. 4, 5). This reflects a renewal phase but unique features of the inner generation prohibit direct equation with any single squamate stage.

Histology of the corneous tissues of the outer generation (Figs. 2–6). The mature β-layer's surface is slightly waved: some toluidine blue-stained sections show a dark line with occasional serrations. The entire tissue is intensely toluidinophilic in material from juveniles (Fig. 2), but stains lighter, or not at all, in adults (Fig. 3). In wax sections, treated with eosin, it stains only slightly, or not at all, whatever the animal's age. A distinctive lamellate, tile-like pattern is visible in lightly stained sections (Fig. 6). On the outer scale surface, the β-layer is thicker in adults (>5 cell layers) (Figs. 3–5) than in juve-
niles (3–5 cell layers). The β-layer is always thinner in the inner scale surface and hinge region, sometimes only one cell thick.

An artifactual split traversed by thin strands, variously stained, always occurs at the base of the β-layer (Figs. 2–4). Such were interpreted as LM indications of a mesos layer (Maderson, 1968) but TEM data demand reevaluation of direct homology.

The α-layer is thinner in the three specimens showing Condition One (Fig. 2) than in the other specimens (Figs. 3–5). The flattened, toluidinophilic (but eosinophilic) cells have a typical stratified squamous epithelial form at high magnification.

Some extremely flattened, apparently keratinized, cells visible at the base of the α-layer in Condition Three (Fig. 5) are the innermost components of the outer generation: their specific identities are discussed after presentation of their ultrastructural features.

**Histology of the inner generation (Figs. 4, 5).**

The inner generation and basal cells in Condition Three vary greatly. This might reflect a centrifugal gradient of cytodifferentiation across the outer scale surface during the renewal phase (cf. squamates, Maderson et al., 1998, p. 16), but additional unique features in *Sphenodon punctatus* would defy interpretation without TEM data. While it would be inappropriate to offer specific identifications of the various cell populations in Figure 5 before such data are presented, any attempt to present them without reference to the entire tissue would be confusing. Here, we identify regions A–D in Figure 5 and offer brief comments on the LM appearance of each.

**Fig. 7. Sphenodon punctatus.** Low-power TEM micrograph of innermost tissues in Condition One epidermis showing part of a flat germinal cell (B) with basement membrane (arrowheads). A single suprabasal cell with organelles typical of an immature keratinocyte lies between the stratum germinativum and immature α-layer (α). The latter comprises only four cells that become flatter towards the mesos region (MR). Bar = 1 μm.

**Fig. 8. Sphenodon punctatus.** Medium- to high-power TEM micrograph of α-layer (α) and mesos region (MR) in Condition One epidermis. The gradually increasing degree of flattening of α-cells (Fig. 7) is seen again. In some extracellular spaces desmosomal remnants (arrows) and occasional lipid lamellae can be discerned. Bar = 200 nm.

**Fig. 9. Sphenodon punctatus.** High-power TEM micrograph of indistinct transition between α-layer (bottom) and mesos region (top) in Condition One epidermis. All cells have filamentous contents and similarly thickened marginal regions (arrows). Occasional, scattered images of extracellular lipid lamellae can be seen in these tissues. Bar = 200 nm.
Region D comprises 5–8 layers of living cells. The deepest are polygonal: the others are increasingly elongate and flattened away from the basal layer. A shedding stage assignment for Condition Three is deferred until the germinal and suprabasal cells have been described.

**Histology of the stratum germinativum and suprabasal cells.** The deepest living cells in Condition One (Fig. 2) differ from those in the other two conditions (Figs. 3–5). Germinal cells are so flat that ovoid nuclei lie with long axes paralleling the skin surface and suprabasal nuclei are sparse. This form characterizes the squamate “perfect resting stage” (PRS), but the ultrastructure of superjacent keratinized tissues defines a post-shed resting stage (see Discussion).

The cuboidal/columnar basal cells and polygonal suprabasal cells in Condition Two (Fig. 3) have rounded nuclei, often with prominent nucleoli. Epidermal melanocytes are arborized, with widely dispersed pigment granules. Some pycnotic, flattened nuclei occur at the base of the α-layer. All these features suggest direct homology with very early renewal phase (Stage 2) of the squamate cycle.

Chromophobia of columnar basal cells in Condition Three (Figs. 4, 5) resemble that seen in squamate epidermis during mesos maturation (late Stage 5 to early 6), but features of regions B and C suggest an earlier stage in the renewal phase. Ultrastructural data do not resolve this anachronistic aspect of tuatara epidermis, but emphasize that cytodifferentiation here is less precise than in squamates.

**Ultrastructural and Histochemical Observations**

**Condition One (Figs. 7–9).** Resting on a basement membrane, flattened germinal cells have many desmosomes where they contact suprabasal cells (Fig. 7) and other organelles typical of undifferentiated amniote keratinocytes. The suprabasal cells resemble presumptive α-cells. The superjacent 10–16 layers of keratinized cells become progressively thinner in ascending vertical sequence (Figs. 7–9). The first 5–6 are moderately electron-dense. Their irregular profiles show marginal layers 10–17 nm wide, desmosomal remnants, and intercellular lipid deposits (Figs. 7, 8). A pattern of electron-lucent, 6–8 nm-wide filaments against a darker background matrix (lowermost cells) changes to one with 15–20 nm filaments — all features resembling mammalian α-keratinization. Sequential flattening of the remaining 5–10 layers produces cells <0.1 μ deep (Figs. 7–9) that resemble those in mature squamate mesos layer, but there is no clear distinction between “α” and “mesos” cells in the tuatara.

Ultrastructural features of the mature β-layer common to all three conditions are described below. **Condition Two (Figs. 10–17).** Figure 10 confirms LM data (Fig. 3) showing differences in general form of living cells from those in Condition One (Fig. 2). While bundles of 6–8 nm filaments in suprabasal cells (Figs. 11, 13, 15) imply an α-keratogenic capacity comparable to that of presumptive α-cells in Condition One (Fig. 7), several features indicate additional synthetic capacities.

The electron-lucent, amorphous contents of vesicles blebbing from the maturing (trans) face of the widely distributed Golgi complexes coalesce to form two types of electron-dense bodies: small ovoids, and larger, irregular or roundish units (Figs. 11–14). The small ovoid vesicles (0.2–0.3 × 0.09–0.15 μm) show an internal lamellar pattern on a finely granular background (Figs. 11–13). Lamellar periodicity, measured as the distance between adjacent dense lines, was usually 6.0–6.5 nm but ranged up to 8.0 nm. These organelles are identified as lamellar bodies (LBs) (Menon and Ghadially, 1997). Coarse granulation in the larger (0.2–1.0 μm) bodies resembles mucous granules (MGs) of amphibian skin (Parrakal and Matoltsy, 1964; Lavker, 1974). Contents of both LBs and MGs appear to be discharged into the extracellular domain at the base of the α-layer (Fig. 15) in regions where occasional cells with numerous MGs occur (Fig. 16).

Cells on the α-layer’s inner aspect (Figs. 10, 13), whose recent addition to it is suggested by condensed chromatin in the electron-dense nuclei, are not as flat as more superficial cells: they resemble transitional cells of mammalian stratum corneum.

The ultrastructure of the other keratinized tissues resembles that seen in Condition One. PAS staining (Fig. 17) is weak or negative in the mature β-layer, but positivity increases steadily towards the base of the α-layer. Occasional living cells with large PAS-positive granules are apparently those that contain numerous MGs (Figs. 16, 17).

**Condition Three (Figs. 18–26).** The many unique features are best approached by noting the orientation within the entire epidermis (Figs. 4, 5) of a low to medium power TEM (Fig. 18) that straddles the tissues of region A through the uppermost tissues of region C (Fig. 5). There is extreme variation in the form of contacts between the electron-lucent cells, ranging from flat to a complex interdigitation (Fig. 18). Plasma membranes are thickened with deposits of electron-dense material 8–12 nm wide. Desmosomal remnants are visible throughout, but many cells remain separated by spaces 10–15 nm wide (Fig. 19). The cytoplasm of a single cell oriented obliquely across Figure 18 has a “basket-weave” pattern similar to that seen in squamate presumptive β-cells (Maderon et al., 1972, 1998; Maderon, 1985). The round or ellipsoid inclusions forming this pattern are similar in electron-lucency to the more homogeneous cytoplasm of the adjacent, more flattened cells. They may be associated with bundles of filaments converging on desmosomes. They merge
with one another in cells as nuclear heterochromatin increases, ribosomes diminish, and the cytoplasm becomes swollen and vacuolated. At higher resolution (Fig. 19) it is sometimes possible to measure a granular pattern of 3–5 nm electron-lucent filaments. A similar pattern is also discernible in the outermost cells of the mature layer (Figs. 6, 20). In mature cells (Figs. 19, 20) occasional melanosomes and irregular electron-dense areas are seen, but 10–20 nm-thick filaments are absent. All these features suggest that Figure 18 shows cells in various stages of keratogenesis and that the inclusions are packets of keratin filaments (Alibardi, 1998a).

During final maturation the cell extremities (Figs. 18, 19) fit together producing the characteristic form of the mature layer (Fig. 6).

The variability in membrane form (see above) can be seen on a single cell at the surface of the β-layer of the OG (Fig. 20), where the almost continuous electron-dense thickening of the inner leaflet, 8–12 nm wide, highlights occasional short (0.15–0.20 μm) projections. By definition, this is the oberhautchen, and the projections are the sparse microornamentation reported by Peterson (1984).

Interpreting regions A–C (Fig. 5) as sequential steps in β-layer differentiation permits identification of the extremely flattened cells in region D. Because sparse intracellular LBs and their extracellular products occur throughout most α-keratogenic tissues, cells in region D must be precursors of the keratinized tissues in Figures 7–9.

Identification of differentiating β-cells of the inner generation permits further comment on oberhautchen fine structure (Fig. 20). Because most of the remaining keratinized components of the outer generation in Condition Three are unchanged from Condition Two, attention can focus on flattened cells at the base of its α-layer (Fig. 5). These show diverse TEM images (Figs. 21–24). The morphology of cell–cell contact varies from region to region (Figs. 21, 22) and across single cells (Fig. 24). The cytoplasm is generally more electron-dense than that of subjacent β-cells (more obvious in Figs. 21 and 24 than in Fig. 22), and a
lighter 3–5 nm filament pattern is just discernible against a finely granulated matrix. Usually, scattered, round or irregular, electron-dense granules (Fig. 23) distinguish them from “typical"/H9252-cells in Sphenodon punctatus. Desmosomal remnants occur frequently and in regions of greatest membrane complexity (Fig. 22) conspicuous intercellular gaps (perhaps magnified by sectioning) occur.

A similar mixture of features suggesting both α- and β-keratogenic capabilities in the cells described above characterizes the most superficial cells of the β-layer of the outer generation (Fig. 25).

DISCUSSION

Lepidosaurian Epidermal Generation: Introductory Comments

Squamate epidermal generations are complex units with outer β-, and inner α-keratogenic, regions. When mature, the former (including the superficial oberhautchen) is syncytial but its multicellular origin is evident during differentiation. Mature α-keratogenic tissues retain cellular form and derive from four definable cell types (Maderson, 1985, table I). This picture of the basic squamate epidermal generation as comprising six distinct cell types has been well substantiated. Its application to Sphenodon punctatus in LM studies (Maderson, 1968) seemed justified and, until recently, it seemed that epidermal generations were basically similar in all lepidosaurs (Maderson et al., 1998).

This study shows that the epidermal form in Sphenodon punctatus differs from previous TEM accounts of squamate EGs in two locations, both involved with transitions from one pattern of keratogenesis to another. The β- → α-, and α- → β-transitions concern, respectively, the locations of the squamate mesos layer and shedding complex, the two specialized tissues whose evolutionary origin we sought to elucidate. Interpretation of differences revealed by TEM is hindered by the unknown status of individuals within their shedding cycles and the limited material. We encountered other unexpected difficulties whose resolution necessitates review of the general pattern of cyclic epidermal activity in lepidosaurs before we address the evolutionary issues.

Cyclic Events in Tuatara and Squamate Epidermis: Equating Conditions With Stages

Recent research on lepidosaurian skin has followed premises established over a century ago (reviewed by Lange, 1931). Earlier workers defined what was lost from the body as an outer generation and wrote of its replacement by a newly formed inner generation. They recognized the most superfi-
cial generation component as the oberhautchen and the innermost as the clear/granular layer. The latter, and indeed several other components of a generation, were variously named. A nomenclature based on LM study (Maderson, 1965) avoided terms likely to cause confusion with mammalian epidermis, e.g., stratum intermedium, s. spinosum, or s. granulosum, but was necessarily modified as EM data appeared (Maderson et al., 1972, 1998). However, because interpretation of cellular identity depends on how the system is conceptualized, new data raise new problems. The first report on squamate epidermis, based on longitudinal biopsies (Maderson, 1965), conceptualized its cyclic histogenesis as resembling the mammalian hair cycle with “resting phases” alternating with “renewal phases.” On the assumption that an entire generation was produced during a renewal phase, suprabasal cells present at shedding were interpreted as presumptive lacunar and clear cells that matured concurrently with inner generation differentiation during the next renewal phase (loc. cit.).

The original conceptualization was revised when later studies permitted more precise analysis of cell lineage and elucidated cycle dynamics. Although the capacity for cyclic activity is intrinsic to the epidermis (Flaxman et al., 1968), frequency of its expression is controlled by hormones that alter the duration of the perfect resting stage (PRS) (Maderson et al., 1970a; Maderson, 1985). Because longitudinal biopsying of lizards such as Anolis carolinensis that shed every 14–16 days reveals no PRS morphologies, epidermal form in infrequent shedders, e.g., most snakes, can be predicted by noting the animal’s status within a cycle. Such data have been incorporated into a model of the still incompletely understood mechanisms involved in switching between two keratogenic patterns (Maderson, 1985, pp. 557–558, fig. 10). The model proposes that a late resting stage morphology reflects a downregulated “α-synthesizing signal” and an upregulated “β-synthesizing signal,” while a Stage 4 morphology (Maderson et al., 1998, fig. 1) reflects the reverse.

The squamate PRS is definable morphologically. An incomplete outer generation, comprising mature β-, mesos, and α-layers, and one layer of suprabasal cells lies above a stratum germinativum, whose cells are conspicuously flattened. It is uncertain whether the system is absolutely quiescent when a PRS occurs (Maderson, 1985, pp. 552–557). While extensive longitudinal biopsies from Elaphe g. guttata revealed occasional “proliferating histologies” (Zucker, 1977), 87% of 328 field-collected specimens of two lizard species showed PRS (Maderson et al., 1970b). Differences in epidermal structure between Sphenodon punctatus and squamates could reflect differences in patterns of cyclic activity underlying generation production. In the absence of data concerning renewal phase duration in S. punctatus, we can approach questions pertaining to the evolutionary origin of the squamate shedding complex and mesos layer by comparing epidermal morphologies reflecting their formation in the two taxa. This necessitates equating the three conditions described here for S. punctatus with squamate stages (Maderson et al., 1998, fig. 1).

Only Condition Two equates directly with a designated squamate stage, Stage 2 of early renewal, as indicated by a mature α-layer and subjacent, arborized, epidermal melanocytes. The cytology (Fig. 3) is identical to that of two snakes, Constrictor...
constrictor (Szabo et al., 1973, fig. 6) and *Elaphe o. quadrivitta* (Maderson, 1985, fig. 6G). Unique features of living cells beneath the H9251-layer (Figs. 10 – 17) distinguish them from suprabasal cells in Condition One (Fig. 7).

Neither histological (Figs. 4, 5), nor ultrastructural (Figs. 18 – 24), criteria permit equation of Condition Three of *Sphenodon punctatus* with a specific squamate stage. The tripartite tinctorial properties (regions A–C, Fig. 5) of the immature β-layer reflect cytogenetic patterns never before observed in juxtaposed vertical sequence. Condition Three (which shows cytologies seen from early Stage 4 to late Stage 5 in squamates; Maderson et al., 1998, fig. 1) is a “mid-renewal phase” that would precede the pre-sloughing condition described previously (Maderson, 1968, figs. 4, 5).

Condition One (Fig. 2), a morphology never previously recorded in *Sphenodon punctatus*, is difficult to interpret. The keratinized outer generation tissues are unlike those in Conditions Two (Fig. 3) and Three (Fig. 4). The apparent absence of an α-layer in LM preparations, in contrast to the three layers of keratinized α-cells revealed by TEM (Fig. 7), reflect a tissue immaturity that defines a squamate post-shed RS, not a PRS. While ultrastructural identification of the single suprabasal cell as a presumptive α-cell confirms that the α-layer is still immature, the extreme basal cell flattening characterizes a PRS. These mutually exclusive data imply that α-layer histogenesis in the tuatara must differ from that described for squamates, a possibility that requires reevaluation of previous studies.

On the basis of autopsy samples from two animals, Gabe and St. Girons (1964, p. 12) give a schema of epidermal form in *Sphenodon punctatus*. In it, the keratinized tissues of the outer generation are divided into three squamous strata in vertical

![Fig. 21. *Sphenodon punctatus*. Medium-power TEM micrograph Condition Three epidermis showing the junction between innermost aspect of outer generation (top 2/3) and outermost aspect of inner generation (bottom 1/3). Granulation size is similar in electron-dense and electron-lucent areas of cells of outer generation: their membranes are linear. Although cytoplasm of cells of mature β-layer (βI) of inner generation exactly resembles that in Figures 18–20, here their electron-dense, thickened membranes emphasize linearity of cell surfaces (cf. Fig. 22). Bar = 500 nm.](image)

![Fig. 22. *Sphenodon punctatus*. Medium-power TEM micrograph Condition Three showing a quite different image of interface between outer (top) and inner (bottom) generations than that shown in Figure 21. Cells closest to upper margin of figure (α) are identical to those at base of α-layer of outer generation in Conditions One and Two (Figs. 7, 8, 15). Cells at bottom of figure (β) are identical to those in Figures 18–21 and are clearly part of newly formed β-layer of inner generation, as evidenced by arrowed electron-dense areas. Cells at center of micrograph (SP) show a mixture of features mentioned, with complex membrane interdigitations and incorporated dense granules (double arrowheads). Large arrowheads indicate possible splitting regions. The striking, regional differences between membrane form as shown here and in Figure 21 can be understood more readily by reference to Figure 26. Bar = 1 μm.](image)
sequence: such are discernible in accompanying photomicrographs. However, the forms of the 5–6 layers of suprabasal and germinal cells in the schema do not reflect the photomicrographs (loc. cit., Pl. I, figs. 1, 2). The latter resemble our Condition Two. Gabe and St. Girons (1964, p. 14) argue that their animals had shed “recently.” We disagree for three reasons. First: Their photographs show arborized melanocytes (cf. Fig. 3). Second: Suprabasal cells (their “stratum intermedium [granulosum]”) are said to be “…assez pauvres en grains keratohyaline … comparables a celles que signale Goslar chez \textit{Na}trix…” (“…somewhat sparsely provided with kerato-hyalin granules … comparable to the situation Goslar has demonstrated in \textit{Na}trix…”). Goslar (1958, 1964) noted minute, intracellular, dark-stained “keratohyalin granules (KHG)” in a tissue later identified by TEM as the mesos layer in this snake (Landmann, 1979). Barely discernible by LM, these organelles are in fact LBs and/or MGs (Figs. 10–16). Third: Gabe and St. Girons’ (1964) allusions to basal mitoses do not necessarily imply a post-shed RS when proliferative activity is diminishing — they could equally describe its resumption at the onset of a renewal phase (Maderson, 1985, fig. 10). The possibility that Gabe and St. Girons’ specimens showed our Condition Two is supported by other data.

Neither Gabe and St. Girons (1964) nor Maderson (1968) evaluated allusions to classical reports of tuatara epidermis in Lange’s (1931) review because of uncertainties concerning the number of animals studied, their unknown cycle status, quality of fixation, lack of photographic record, and nomenclatural confusion. All these problems are confounded by a third translation from the original German!

It can now be seen that Lange (1931), whose treatment of each topic evaluates data and/or interpretations within and between living reptilian taxa, makes important points. Emphasizing that layering of mature keratinized tissues characterizes lepidosaurs, he notes that staining of the squamate components now termed the β-, mesos, and α-layers is more distinct than that of their homologs in \textit{Sphenodon punctatus}. His remarks in several contexts imply that data known to him for this species were limited. When describing the squamate oberhautchen, he does not mention a tuatara shedding complex. His description of undifferentiated, reptilian keratinocytes implies a familiarity with the form defined here as the squamate PRS, but he does not allude to such in the tuatara. Rather, he emphasizes that its complex dermo–epidermal junction, germinal, and suprabasal cells more resemble those of crocodilians and chelonians than those of squa-
In describing nonsquamate epidermis he uses the term stratum spinosum and, in comparative allusions to that of mammals, he speaks of “tonofibrils.” In classical LM the latter denoted desmosomes, intercellular junctions not fully characterized until TEM became a research tool 35 years later. Because TEM reveals extensive desmosomes among immature keratinocytes in mammalian stratum spinosum (Matoltsy, 1986) we conclude that Lange (1931) knew only of post-shed, or late RS material, in S. punctatus.

We reexamined LM samples from dorsal, ventral, and gular regions of 14 specimens reported previously (Maderson, 1968). Of 12 whose fixation permitted analysis of unkeratinized tissues, four were pre-shed, and eight showed “resting stages.” Of the latter, four post-shed RS morphologies were uniform in all regions sampled and the other four showed slight regional variation. Although LM alone precludes quantitation of α-layer development (cf. Figs. 2, 7), living cells lacked PRS morphologies. Suprabasal cells, identifiable by TEM as presumptive α-cells (Figs. 2, 7), were discernible in all specimens and were illustrated in both “post-shedding” (resting stage) and “pre-sloughing” (Stage 6) categories in Maderson (1968).

A summary of “squamate equivalent” cycle stages known for Sphenodon punctatus (Table 1) shows that nine of 20+ reported individuals showed renewal phase histologies. Of six reported here, 50% were in renewal and 50% showed the paradoxical Condition One. The tuatara is an infrequent shedder: In nature it sheds once (Hazley, 1982; M.B. Thompson, pers. obs.), and in captivity twice per year (Mertens, 1971). Allowing for the uncertain number of individuals known to Lange (1931), by comparison with squamates it is surprising that approximately 45% of individuals known in toto should show renewal phase histologies. Three mutually compatible possible explanations could be resolved by studies involving longitudinal skin biopsies of living animals of known cycle status. 1) Sphenodon punctatus has a renewal phase longer than the 14 days documented for several squamates (Maderson, 1985). The fact that 33% of Maderson’s (1968) specimens were “pre-shed” does not necessarily support this possibility. Because samples from different body regions were similar, we may conclude that the tuatara sheds piecemeal following a pan-body, synchronized renewal phase, as do many lizards (Maderson et al., 1998). 2) The early renewal histology seen in 20% of 20+ specimens might reflect stress. Field-captured squamates kept for a few days before biopsy show a disproportionate percentage of early renewal stages (Maderson, pers. obs.). No data are available concerning the pre-sacrifice history of animals reported by Gabe and St. Girons (1964) and Maderson (1968). 3) The site of origin of the skin samples reported here might have influenced normal cell dynamics. For the six animals, 50% were in renewal and the other 50% showed the anomalous Condition One. We describe normal skin just proximal to neogenic scales on regenerating tails. Nothing is known of the possible effects of such proximity on normal skin (Alibardi and Maderson, 2003).

α-Keratogenic Cells in Tuatara Epidermis: Lipogenesis, Mucogenesis, and Keratohyalin-Like Granules (KHLGs)

Condition Three shows approximately 20 layers of keratinized cells between the base of the β-layer of the outer generation and the inner generation. This increase over the number present in Condition One and Two shows that the outer generation is complete and mature in Condition Three. The steady increase in thickness from without inwards revealed by TEM (Figs. 7–10, 13, 18, 21, 22, 24) is one of several “gradients” discernible in the α-keratogenic cells. In Condition One (Figs. 7–9), intra- and extracellular deposits of lipids and mucus are heaviest in more superficial cells. Although organelles responsible for their deposition (lamellar bodies [LBs] and mucous granules [MGs]), have only been seen in Condition Two (Figs. 10–17), late maturing, keratinized, outer generation tissues in Condition Three (Figs. 5, 21–24) show less deposited lipid and mucus than do “typical” α-cells (Figs. 7–13). Because lipogenic LBs occur throughout α-keratogenic tissues in the tuatara, a “mesos layer” is not definable by “mesos granules” (Roth and Jones, 19671970; Landmann, 19791986). Hereafter, we use the term “mesos region” to distinguish the most superficial, flattened cells (Figs. 7–9) from subjacent α-cells.

Most of the late-maturing α-keratogenic tissues comprise typical α-cells (cf. Figs. 7–13, 21), but occasional cells (possibly in the innermost layer (Figs. 22, 24)) show tortuous membranes typical of β-cells (Fig. 18), and other ubiquitous features resemble the latter. The uniformly granular cytoplasm seen in Condition Three (Figs. 21–24) derives from aggregation of keratohyalin-like granules (KHLGs, Alibardi,
1998b) around tonofilament bundles and β-keratin packets. Such mixed α- and β-keratogenic capacities also occur in the most superficial of the subjacent “β-keratogenic tissues.”

**β-Keratogenic Cells in Tuatara Epidermis: β-Layer and the Shedding Complex**

 Persistent cell membranes distinguish the mature β-layer from that of squamates, and absence of syncytiality may explain the unique cytology in Condition Three (Figs. 5, 18). In contrast to squamates, the boundaries of all β-keratogenic cells, not just that of the outer-facing oberhautchen membrane (Maderson et al., 1998), are thickened (Figs. 18, 19, 21–23). The deposits of electron-dense materials, of unknown chemical nature, decrease as cells mature (Fig. 18), implying involvement in the reduction and loss of membrane tortuosity during flattening.

 The basis for the toluidinophilia and basophilia of differentiating β-cells, properties mostly lost at maturity, is uncertain, although copious ribosomes may be partly responsible. Certainly, the mature tissue is never as chromophobic as is that in squamates. Similarly, the basis for the replacement of toluidinophilia by eosinophilia during keratogenic cells in $Sphenodon punctatus$ resembles squamates.

 Less discrete than in squamates, the distinction between tissues of the innermost outer generation and outermost inner generation varies regionally (cf. Figs. 21, 22). Because cells show mixed α- and β-keratogenic features, the α→β-“switch” is clearly less precise than in squamates. The absence of the rectilinear, i.e., square, columnar, or rectangular in section, cellular morphologies that characterize the cells of the clear layer and oberhautchen of squamates precludes identification of a shedding complex (Maderson, 1985, figs. 6G–K, 7A–E; Maderson et al., 1998). The sparse, irregular microstructure seen with TEM at the β-layer surface in $Sphenodon punctatus$ supports Peterson’s (1984) SEM observations and raises important functional questions.

**Cellular Mechanisms of Generation Loss in Lepidosaurous**

 Reviewing squamate skin shedding, Maderson et al. (1998) noted that loss of mature epidermal tissues in large flakes, or sheets, characterizes lissamphibians and reptiles, in contrast to insensible desquamation in endotherms. Weakening of desmosomal connections between living cells during maturation characterizes both modes of loss. The existence of mature, dead tissues at the body surface depends on a stratified squamous form augmented by adhesive properties of residual desmosomal proteins and possibly extracellular lipids. Epidermal replacement is needed to: 1) increase the surface area of the body to accommodate somatic growth (all vertebrates); 2) repair abrasive damage caused by environmental contact (all terrestrial tetrapods); and 3) maintain the barrier to CWL (most amniotes). A pelage (mammals), or plumage (birds), provides the physical protection necessary for both the organism, and the barrier tissues, but β-keratinized tissues covering reptilian scales serve both these roles (Maderson et al., 1998). In lepidosaurs, scale overlap also provides integumentary flexibility so that, in this clade, the sauropsid synapomorphy of vertical alternation of β- and α-keratogenesis manifests in periodic, pan-body skin shedding (Maderson and Alibardi, 2000).

 Protection of immature, squamate β-keratogenic, and mesos tissues relies on the functional outer generation’s being held in place by the three-dimensional complexity of the integument (Maderson et al., 1998, p. 17). Separation of the two components of the shedding complex involves a precise sequence of behavioral, tissue, cellular, and molecular events. The review noted the absence of a shedding complex in $Sphenodon punctatus$. However, beyond noting that synchronized shedding could occur in the absence of morphological specialization (Maderson et al., 1998, pp. 20–21), the shedding mechanism could not be addressed. New TEM data, correlated with SEM observations (Peterson, 1984; Maderson and Pauwels, pers. obs.) and new data concerning KHLGs (Alibardi, 1999a, 2000a,b, 2001b; Alibardi et al., 2000) permit further comment.

 Occasionally seen in the innermost α-keratogenic tissues (Figs. 22, 24), deposits of electron-dense materials associated with exceptional membrane tortuosity (Fig. 18) are unique features of immature β-keratogenic cells in $Sphenodon punctatus$. Both features are lost during final maturation (Figs. 18, 21). The electron-dense deposits resemble the “marginal layer” (“cornified envelope”) of mammalian keratinocytes (Polakowska and Goldsmith, 1991). This may imply that mechanisms underlying flattening of β-keratogenic cells in $S. punctatus$ more resemble those of more primitive α-keratogenic cells (Maderson and Alibardi, 2000) and could explain the similarity between the surfaces of the tuatara’s mature β-layer and mammalian stratum corneum (Maderson et al., 1998, p. 20). Comparison of Peterson’s (1984) figures with SEMs of squamates (Maderson and Pauwels, pers. obs.) reveals that the tuatara oberhautchen, bearing only occasional pits, lacks impressions of overlying clear layer cells (Irish et al., 1988). Lacking the rectilinear form of juxtaposed, squamate, oberhautchen cells, the exceptionally convoluted margins of homologous cells in the tuatara overlap. They lack consistent orientation: “Where a shift in orientation occurs, the cell margins lie at right angles to each other and interdigitate irregularly.” (Peterson, 1984, p. 43, emphasis added). These data facilitate interpretation of our TEMs.
Figure 26 shows juxtaposed, immature generations in Sphenodon punctatus. Tortuosity of β-cell membranes decreases during maturation so that TEM shows flat surfaces (Figs. 18, 21) with occasional convoluted profiles (Fig. 22). In squamates, TEM reveals vertically oriented membranes in clear layer and oberhautchen (Maderson et al., 1972, figs. 9–11; Landmann, 1979, figs. 23, 30; Alibardi and Thompson, 1999a, figs. 12, 13, 23, 24), but comparable sections in S. punctatus show their oblique orientation. In the absence of regularly spaced interdigitations (serrations, spines, setae, etc. forming squamate microornamentation [Maderson et al., 1998]), loss of desmosomal adhesion during final cell maturation separates adjacent units along a splitting zone (Fig. 26). Synchronized in some unknown fashion, separation probably results from a specific sequence of events as in squamates (Maderson et al., 1998). While no descriptions of tuatara removing flakes of outer epidermal generation from the body are available, such could be torn away, so that previously opposed oberhautchen cells assume a tiled appearance (Peterson, 1984, fig. 1E).
Fig. 27. Similarities and differences in mature generation structure among three taxa of living lepidosaurs: lack of relevant data precludes inclusion of the amphibians. Features unique to *Sphenodon punctatus* (dark backgrounds); features shared between taxa (medium backgrounds and faint grid-lines between cells); features unique to “lizards” and/or snakes (light backgrounds). Explanatory notes appearing in cells: 1 α-keratin persists in electron-dense areas of β-layer (Alibardi, 2000a; Alibardi et al., 2000). 2 Horizontal level of cells or tissues being discussed — columns towards right are progressively deeper. 3Phase/stage of cycle when first cells of indicated tissues can be discerned. 4Phase/stage of cycle when they are morphologically and functionally mature. 5 Post-shed — this component is revealed as old shed is removed from body surface. 6Post-shed — final maturation of this tissue, probably involving dehydration of cells, occurs approximately 10–20 h after shedding (Maderson et al., 1998). 7Tuatara loses a unit homologous with squamate generation that has “outer” and “inner” surfaces but, because shedding complex is not discernible, the most superficial and the deepest surfaces can only be defined respectively as “oberhautchen” or “clear/granular layer” by their topographic position. 8Many possible cytologies exist in various “lizard” genera ranging from that in desert iguanids (Maderson and Licht, 1967) to *Anolis carolinensis* (Maderson et al., pers. obs.). 9Sea snakes (Hydrophidae) have a stratified squamous β-layer, a derived condition (Maderson, 1985). 10Mesos layer, with characteristic mesos granules, was originally defined in snake epidermis (Roth and Jones, 1967). Previous assumptions that a homologous tissue exists in all lepidosaurs negated by fact that LBs and derived extracellular lipid lamellae may be distributed throughout α-keratogenic tissues in tuatara and many lizards (Alibardi, Dujsebayeva, van Wyk, pers. obs.). In future, the terms “mesos layer” and “mesos granules” should be used only in descriptions of snake epidermis. For other clades, we recommend that very flattened cells lying beneath β-layer and above “typical” α-cells should be termed the “mesos region” with included lipogenic organelles recognized as LBs. 11This mucogenicity does not involve distinct granules. Data compiled from various sources in Literature Cited.
Form and Evolution of Lepidosaurian Epidermal Generations

Tuatara generation structure differs from that of squamates (Fig. 27) in that all the components have a stratified squamous epithelial form reflected in the absence of β-layer syncytiality and a shedding complex, while β-keratin packets, LBs, MGs, and KHLGs are not restricted to specific cell populations. In toto, these differences reflect the lack of precision in transitions from β- → α- and α- → β-keratogenesis long thought to characterize lepidosaurs (Baden and Maderson, 1970). They do not merely suggest a possible primitive condition for the clade; they also elucidate the evolution of amniote keratinization and its functional correlates.

Discussions of soft-tissue evolution involving comment on the adaptive significance of character states require an explicit phylogeny and an understanding of form and function. In these contexts, any attempt to explain the origin and evolution of the squamate shedding complex, and barrier to CWL, faces problems similar to those pertaining to feathers (Maderson and Homberger, 2000).

The systematic problem here is that while the status of the monospecific Sphenodontidae and families of “lizards,” Amphisbaenians, and Ophidians are widely accepted, their interrelationships remain uncertain (see references and discussion in Evans, 1995, 1998; Lee, 1997). The database concerning functional morphology of lepidosaurian skin is incomplete. Scale diversity within and between taxa awaits evaluation (Maderson and Alibardi, 2000) and for amphisbaenians data concern only glands (Jared et al., 1999). This study documents epidermal structure in Sphenodon punctatus but, in contrast to an extensive, albeit disjunct, literature for several squamates that permits comparison with other sauropsid amniotes (Lillywhite and Maderson, 1982,1988; Menon and Menon, 2000), we know nothing of its physiology.

Recent EM studies have extended knowledge of cytodifferentiation of squamate epidermal generations and biochemical and molecular data concerning reptilian keratins are becoming available (Ali-
**Figure 28.**

<table>
<thead>
<tr>
<th></th>
<th><strong>A</strong></th>
<th><strong>B</strong></th>
<th><strong>C</strong></th>
<th><strong>D</strong></th>
<th><strong>E</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tuatara Epidermis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Keratins</strong></td>
<td>α-</td>
<td>α + β</td>
<td>α + β</td>
<td>α + β</td>
<td>α + β</td>
</tr>
<tr>
<td><strong>Distribution</strong></td>
<td>Homogeneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Actual Histology</strong></td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>Syncytial β-layer</td>
<td>Syncytial β-layer</td>
</tr>
<tr>
<td><strong>Epidermal generations</strong></td>
<td>N.A.</td>
<td>N.A.</td>
<td>PRESENT 6 cell types</td>
<td>PRESENT 6 cell types</td>
<td></td>
</tr>
<tr>
<td><strong>Transition zone</strong></td>
<td>N.A.</td>
<td>Occasional weak zones</td>
<td>POORLY DEFINED S-C absent</td>
<td>WELL DEFINED S-C present</td>
<td>WELL DEFINED S-C present</td>
</tr>
<tr>
<td><strong>Oberhautchen MO</strong></td>
<td>N.A.</td>
<td>N.A.</td>
<td>Sparse/Absent</td>
<td>Very complex</td>
<td>Very complex</td>
</tr>
<tr>
<td><strong>Marginal layer matrix material</strong></td>
<td>In α-keratogenic cells</td>
<td>In α- and β- keratogenic cells</td>
<td>In α- and β- keratogenic cells</td>
<td>In oberhautchen</td>
<td>In oberhautchen</td>
</tr>
<tr>
<td><strong>Histidine-Rich Protein (HRP)</strong></td>
<td>? ancestral molecule</td>
<td>Derived from KHLGs</td>
<td>Derived from KHLGs</td>
<td>Derived from KHLGs</td>
<td>KHLGs absent: HRP in CLO</td>
</tr>
<tr>
<td><strong>α-keratogenic lacunar tissue (LTO) + clear layer (CLO)</strong></td>
<td>N.A.</td>
<td>N.A.</td>
<td>KHLGs and β-packets: full keratinization</td>
<td>Specifically Variable</td>
<td>LTO viable and Mucogenic: keratinized CLO</td>
</tr>
<tr>
<td><strong>Transition zone</strong></td>
<td>N.A.</td>
<td>Occasional strong zones</td>
<td>Well defined at upper mesos region</td>
<td>Well defined at upper mesos region</td>
<td>Well defined at Upper mesos layer</td>
</tr>
<tr>
<td><strong>Mucogenic capacity</strong></td>
<td>XXXXX</td>
<td>XXXX</td>
<td>XXX</td>
<td>XX</td>
<td>X</td>
</tr>
<tr>
<td><strong>Lamellar Bodies in α-keratogenic Tissues</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present in mesos region and α-layer</td>
<td>Present in mesos region and α-layer</td>
<td>Restricted to mesos layer</td>
</tr>
<tr>
<td><strong>Barrier to CWL</strong></td>
<td>?????</td>
<td>?????</td>
<td>Probably all α-keratogenic tissue</td>
<td>Probably all α-keratogenic tissue</td>
<td>Located in mesos layer</td>
</tr>
</tbody>
</table>
bardi, 2000a; Alibardi et al., 2000; Sawyer et al., 2000). Most data derive from study of three lizards (Gekko gecko [a gekkonine gekkonid], Anolis carolinensis [a polychrotid iguanian], and Podarcis muralis [a lacertid]), and two snakes (Constrictor constrictor [a boied], and Natrix natrix [a colubrid]). Although these five species represent major squamate radia-
tions, two caveats are important.

First, because the spinulate oberhautchen in gekkonids and polychroits, independently derived in the two clades (Irish et al., 1988), is different than microornamentation in other lizards (Alibardi, 1999b, 2000b; Alibardi and Thompson, 1999a) and snakes (Alibardi, 2002a; Maderson and Pauwels, pers. obs.), we must be cautious in making generalizations con-
cerning factors involved in membrane interdigitation between oberhautchen and clear layer cells (Maderson et al., 1998). Second, the shedding complex is so unlike any other epidermal specialization that it has a uniqueness comparable to that of a feather (Maderson and Alibardi, 2000), or a hair (Maderson, 1972a). Sim-
larities of the clear layer shared by the five best-
studied species do not track other features of their 
α-keratogenic tissues. In all non-eublepharine (Mad-
erson, 1972b) gekkonids, but in only some polychroits (Maderson, pers. obs.), the superjacent lacunar tissue resembles that of snakes. In Anolis carolinensis (Mad-
erson and Licht, 1967), but not in gekkonids (Mader-
sen, 1966), the similarity includes eosinophil invasion 
of epidermal tissues during the renewal phase. Such similarities (and others regarding microornamenta-
tion [Harvey and Maderson, 1999; Maderson and Pau-
wels, pers. obs.]) are homoplasies, not reflecting phyl-
etic relationships. Lack of understanding of their 
functional significance constrains identification of evo-
lutionary trends.

Although epidermal generations are a lepidosau-
rian synapomorphy (Maderson and Alibardi, 2000), 
there are differences between lizards and snakes (Fig. 27). Given systematic problems, possible biases in the database, and unresolved questions concern-
ning possible adaptive diversity in lizards inter alia, 
and snakes, and/or amniote keratinization sui gene-
ris, their evaluation demands a broad context.

Consideration of 13 epidermal characters (Chars. 01–13, Fig. 28) shows that Sphenodon punctatus 
shares some character states with basal amniotes and/or basal sauropsids (Chars. 01–03, 07), and some with squamates. Ill-defined generations, lack of a shedding complex and microornamentation, and mixed α- and β-keratogenic capacities (Chars. 04– 
06, 09) are primitive for lepidosaurs. However, its 
mesos tissues resemble those of lizards (Chars. 10, 
12). Evolutionary patterns can be tentatively ident-
ified when this mosaic of character states is consid-
ered in relation to 1) other aspects of vertical alter-
nation of keratogenesis in sauropsids (Maderson and Alibardi, 2000), and 2) current knowledge of vertebrate keratinocyte differentiation (Fig. 29).

Landmann’s (1986) skepticism concerning syncl-
tial β-keratogenic tissues in nonsquamates has been 
validated for two chelonians, a crocodilian and a bird 
(Alibardi and Thompson, 1999b-c2000; Alibardi, 
2002b), while other studies (Alibardi and Thompson, 
1999a; Alibardi, 2000b,2001a) confirm synclinality to be a squamate synapomorphy. Found only in tet-
rapods (Matoltsy, 1987), a marginal layer (“cornified 
envelope”) is a primitive feature of amniote 
α-keratogenic cells (Char. 07, Fig. 28). It is absent 
from the cells of all other sauropsidan β-keratogenic 
tissues except for the squamate oberhautchen (Fig. 
28). The presence of this envelope in all such cells in 
Sphenodon punctatus implies that the β-layer in 
this species is the most primitive β-keratogenic tissue known. Mixed organelles in cells in the α- 
→ β-transition zone (Chars. 05, 09, Fig. 28) tell us that 
lack of a shedding complex, and imprecisely con-
trolled vertical alternation of keratogenesis, are 
primitive for sauropsids. Coexistence of sparse 
KHLGs with β-packets in lizard oberhautchen cells 
(Alibardi, 1999b, p. 263) implies that final refine-
ment of the squamate α-→ β-transition occurred 
after a well-defined shedding complex appeared in 
the evolution of this clade. These conclusions are 
relevant to the evolution of amniote keratinization.

**Epidermal Keratinization in Amniote Vertebrates**

The rapid advances in our understanding of epi-
dermis, especially keratinization and lipids, are evi-
denced by treatments of similar topics in Lyne and 
Short (1965), Sengel (1976), Bereiter-Hahn et al. 
(1986), Sawyer (1987), Goldsmith (1991), and 
Chuong (1998). Chronologic review of any one topic 
shows that advances come first from studies of mam-
malian (usually human) cells and comparative data 
follow. Such a sequence can produce misunder-
standing when complex functional issues are involved. 
With our new understanding of amniote phylogeny 
(Maderson and Alibardi, 2000, fig. 2), an implied 
evolutionary “history” of amniote keratinization 
based on chelonian α-keratogenesis (Matoltsy, 1987) is 
no longer tenable.

Stratified squamous epidermis of basal amnioni 
comprised α-keratogenic cells that synthesized la-
mellar bodies (LBs) while retaining ancestral, ana-
mniote mucogenicity (Maderson and Alibardi, 2000, 
fig. 2) to some extent (Chars. 11, 12, Fig. 28): we can 
have no direct knowledge of its barrier efficacy. In 
gross form, the tissue may have resembled that of 
living bufonid anurans, although, of course, LBs (loc.
cit.), and histidine-rich protein (HRP) and filaggrin 
are absent from these lissamphibians (Fig. 29, Ali-
bardi, pers. obs.).

Filaggrin derives from the histidine-rich profilag-
grin of mammalian keratohyalin granules (KHGs) 
that also contain a cystine-rich protein (Resing and 
Dale, 1991). It forms the matrix within which 70-nm
Fig. 29. Epidermal cell differentiation in living vertebrates compared to that of lepidosaurian reptiles (highlighted for comparison with Figs. 27 and 28). Abbreviations and notes appearing in cells: A, absence of pattern of cell differentiation, organelle or molecule; L, organelles or molecules large and/or extensively distributed; M, organelles of medium size; na, not applicable; P, presence of pattern of cell differentiation, organelle or molecule; S, organelles or molecules large and/or extensively distributed; ?, data unavailable.

1 Mucogenicity, a primitive feature of vertebrate epidermal cells, is seen in parakeratotic buccal epithelia and other "mucous membranes" associated with alimentary and urogenital openings of all amniotes. Otherwise absent from epidermis of mammals and archosaurians, MGs have been described in keratogenic tissues in turtles and lepidosaurs.  

2 Marginal layer (cornified cell envelope) is here identified primarily on basis of TEM study. Biochemical data identifying constituent molecules ( involucrin, loricin and small proline-rich proteins) and role of transglutaminases in their assembly are available only for mammalian tissues (Polakowska and Goldsmith, 1991; Akiyama et al., 2000). 

3 Free lipid droplets have been reported in amphibian epidermal cells, but their origin is unknown. In mammalian hair trichohyalin granules, HRP is much reduced by comparison with KH, but both contain arginine. 

4 Although biochemical data are lacking for Sphenodon punctatus, the similarity of its KHLGs to those of other sauropsids suggests a relation between the HRP component and the transition from α- to β-keratinization. 

5 Cornified" tissues described in gnathostomatous amniotes are difficult to define: their strongly mucogenic cells contain 70-nm filaments, but lack lamellar bodies (LBs). Those of lissamphibia possess a marginal layer, but lack HRP or filaggrin (Alibardi, pers. obs.). Provisionally, we restrict the term "α-keratinization" to orthokeratotic tissues in amniotes. 

6 Presence of a marginal layer in all β-keratogenic cells in S. punctatus is unique among all such tissues. 

7 Free lipid droplets, of unknown origin but not derived from LBs, have been found in β-keratogenic tissues in several sauropsid species. 

8 Positivity to antibodies against HRP-derived filaggrin and/or TEM evidence of KHLGs has been reported in oberhautchen cells (Alibardi, 2001, 2002a, pers. obs.; this study). Data compiled from various sources in Literature Cited.
filaments coalesce, while the cystine-rich protein contributes to the marginal layer (loc. cit.). Respectively, the two proteins provide the strength and hydrophilia of mature mammalian keratinocytes, between which lie lipid lamellae derived from lamellar bodies (Menon and Menon, 2000). Desmosomal remnants, aided by the adhesive properties of those lipids, facilitate the ordered stacking of mature squames (Odland, 1991) that ensures that mammalian stratum corneum forms a flexible surface to the body, while simultaneously providing physiological and mechanical protection. The last-named role is augmented by the pelage (Maderson et al., 1998).

Demonstration of HRPs in epidermis of representative species of all sauropsids implies that such characterized basal amniotes (Char. 08, Fig. 28), but their functions are problematic. Soon after amorphous granules lacking a surrounding membrane were reported in lizard α-keratogenic tissues, Maderson et al. (1972, p. 196) noted that their acidophilia contrasted with the basophilia of mammalian KHGs. This distinction was confirmed (Maderson, 1985; Alibardi, 1995) and the acronym KHLGs (keratohyalin-like granules) adopted (Alibardi, 1998b). The presence of KHLGs in lizard clear layer suggested they were involved in the formation of microornamentation (MO) by facilitating interdigitation with oberhautchen cells (Alibardi, 1999a,b, 2000b, 2001b). This does not explain: 1) KHLG presence in normal lacunar cells in some species (Maderson et al., 1970b), or their enhanced expression therein in vitro (Flaxman et al., 1968) or posttrauma (Maderson, 1985); 2) a range of sizes of KHLGs that does not correlate with MO size or shape (Maderson and Pauwels, pers. obs.); 3) the absence of KHLGs from snakes that have MO (Alibardi, 2002a); 4) the presence of KHLGs in Sphenodon punctatus that lacks MO. Available data on sauropsid KHLGs derive from TEM, histochemical, and autoradiographic studies. Although future biochemical analysis may show them to be as heterogeneous as are mammalian KHGs (Resing and Dale, 1991), for the present we can draw some tentative conclusions.

Keratinization and the Evolution of Epidermal Barrier Function in Amniotes

In the stratified squamous epidermis of basal amniotes (Column A, Fig. 28), there must have been a molecule ancestral to some HRP component of both KHGs and KHLGs (granular, see data for snakes [Alibardi, 2002a]). Because α-keratogenesis in most living reptiles involves mucous granules, and often only modest expression of intercellular lipid lamellae, the ancestral tissue may have resembled amniote parakeratotic buccal epithelia (Fig. 29). Whatever its barrier efficacy, its delicate structure would have constrained invasion of an abrasive terrestrial environment. The theropsidan, and sauropsidan, amniote lineages (Maderson and Alibardi, 2000, fig. 2), employed different strategies to protect barrier tissues (Maderson et al., 1998; Maderson, 2002).

At some point in theropsidan evolution, a tough, flexible stratum corneum resulted from involvement of KHGs in α-keratogenesis (Maderson, 1972a, 2002). Barrier efficacy was enhanced by continuous renewal of stacked cells with copious extracellular lipid lamellae: the barrier tissues were further protected when a pelage evolved (Maderson, 2002). There are differences in epidermal HRP metabolism between the “naked skin” of Homo sapiens and hirsute mammalian species (Resing and Dale, 1991). Because such might be explicable in the context of differing modes of protecting barrier tissues, they warrant further investigation, although, clearly, the hominid condition is secondarily derived.

The evolutionary origin of the sauropsid strategy to cope with a terrestrial environment (Maderson and Alibardi, 2000) is elucidated by the expression of vertical alternation of keratogenesis in Sphenodon punctatus, whose β-keratogenic tissues are the most primitive yet described. Because KHLGs were seemingly associated with α-keratinization in basal sauropsids (Char. 08, Fig. 28), at first sight their coexpression with β-keratin packets in S. punctatus (Char. 09, Fig. 28) is puzzling. However, both organelles occur in occasional cells (Figs. 21, 22) in the innermost α-keratogenic generation tissues (Fig. 27), a region where fewer mucous granules (MGs) and lamellar bodies (LBs) occur than in “typical” α-cells. While no data suggest a causal relationship between the apparently simultaneous evolutionary origin of KHLGs and the new β-keratinaceous protein (Maderson and Alibardi, 2000), the expression of both precludes the presence of MGs and LBs (Fig. 29).

Does the imprecise vertical alternation of keratogenesis in tuatara epidermis reflect the basal sauropsid condition? This question cannot be answered without data concerning temporal aspects of epidermal cycling in this relic species: relevant information may emerge from an ongoing study of cytodifferentiation in chelonic carapace involving longitudinal biopsies (Alibardi, pers. obs.). With the evolutionary origin of the squamate shedding complex, many as-yet unexplained changes occurred in KHLG morphology, presumably implying altered patterns of HRP metabolism. Varied KHLG expression in innermost α-keratogenic tissues in lizards, and absence therefrom in snakes (Figs. 27, 28), offer a variety of models for study, the results of which could elucidate the role of “sauropsid” HRP in squamate epidermal generations and perhaps enhance our understanding of its role in the evolutionary origin of β-keratinization.

The sharp morphological transition visible within tissues associated with the β → α-transition in lepidosaurs (Char. 10, Fig. 28) is due in part to the absence of MGs and LBs from cells where 30 nm keratin fila-
ments predominate. While syncytiality of the mature squamate β-layer emphasizes this discontinuity, TEM has revealed a similarly well-defined inner boundary of β-keratogenic tissues in chelonians and crocodilians (Alibardi and Thompson, 1999b,c, 2001), avian scute scales (Sawyer et al., 2000), even feathers (Maderson and Alibardi, 2000). In all lepidosaurs, especially snakes, there is extreme flattening of subjacent α-keratogenic cells and thickened membranes are characteristic. Marginal layer retention in mature, tuatara, β-keratogenic cells is the only cytological feature shared with cells of the mesos region (Sphenodon punctatus and lizards) or mesos layer (snakes). Because the latter are the α-keratogenic tissues where both MGs and LBs occur (Fig. 27), similarities and differences in cells and organelles may be related to the evolution of the barrier to CWL (Chars. 11–13, Fig. 28).

In the 25 years since the function of cell/molecular, lipid/protein complexes in amniote epidermis as barriers to CWL became known, descriptive and experimental studies have revealed differences in barrier tissue morphology and rates of CWL in mammals, birds, and squamate reptiles (Menon and Menon, 2000; Tu et al., 2002). However, we may not state that a particular organization of any tissue is functionally inferior, or superior, to that of another — both are the products of natural selection that equip a particular species for life in its native habitat. In the context of lepidosaurian evolution, it makes little difference that we can never know the form, or efficacy, of barrier tissues in ex-lution, it makes little difference that we can never native habitat. In the context of lepidosaurian evo-
tution — exists in all lacertilians awaits EM confirma-
tion (Maderson and Pauwels, pers. obs.). Maderson et al. (1998) showed that useful cytological information can derive from TEM study of shed skins and such material can yield biochemical data (Thorpe and Giddings, 1983). Such future studies could re-inforce the clues to the origins of sauropsid β-keratogenesis provided here, but information concerning temporal aspects of epidermal cell cycling in all such tissues is urgently needed.

ACKNOWLEDGMENTS

Dr. Alibardi’s fellowship to stay in New Zealand came from the NZ University Grant Committee in exchange with the Italian MAE. He thanks Dr. V.B. Meyer-Rochow (University of Waikato, Hamilton, NZ) for hosting him and for his important role in getting a permit to work on the tuatara. We thank Drs. M. Thompson (University of Sydney, NSW, Australia), A. Cree (Victoria University, Wellington, NZ), and Mr. J. Govey (NZ Department of Conservation), who made possible the collection of specimens during a visit to Stephen’s Island. Mrs. Luciana Dipietrangelo’s photographic skills are greatly appreciated. The authors thank Dr. Susan Evans (University College, London) and Dr. George Rogers (University of Adelaide, Australia) for comments on an earlier draft of the manuscript.

LITERATURE CITED


Harvey HB, Maderson PFA. 1999. Homoplasies as inadequately described character states: a case study involving squamate skin. Am Zool 39:203A.


Malakhov DV, Dujeabeyeva TN. 2001. Cornified scalation of Utegnia shpinari (Seymouromorpha, Discosauriscidae) and radiation of the family during the Paleozoic. Biota 2:157–162.


Menon, GK, Ghadially R, Williams ML, Elias PM. 1992. Lamellar bodies as delivery systems of hydrolytic enzymes: implications...