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Modifications of the dermis during scale regeneration in the lizard tail

L. Alibardi
Department of Histology and Embryology, University of Sydney, Sydney, Australia

Summary. During scale morphogenesis in the regenerating tail of lizards (*Anolis* and *Lampropholis*) the structure of the dermis undergoes changes in relation to the ingrowth of epidermal papillae to form the new scales. Cell proliferation in the dermis, as revealed by the uptake of ^{3}H-thymidine, is high in the prescaling region of the regenerating tail but lower than the proliferation in the epidermis. Under the epidermis of the scaling region dermal cell proliferation rapidly drops down under the distal (apical) and proximal (caudal) sides of the infolding epidermal papillae. Dermal fibroblasts take up ^{3}H-proline in high amounts, especially in the forming deep dermal layer, where many collagen fibrils are laid down forming dense connective. Electron microscopic study revealed that «anchoring filaments» link the basement membrane of the epidermis with the deep dermis, in particular in the sinking hinge region. As a result of the higher proliferation of the epidermis with respect to the dermis (heterochrony) and the presence of dermo-epithelial «anchoring filaments», the superficial laminar epidermis sinks into the dermis to produce new scales. The epidermal downpushing is evidenced by a characteristic distortion of the dermal fibrils under the distal and the proximal sides, and in the hinge region of the forming scales.

Key words: Scales, Dermis, Tail regeneration, Lizard, Autoradiography

Introduction

During tail regeneration in lizards new scales are reformed in a proximo-distal fashion (Bryant and Bellairs, 1967; Shah and Chakko, 1968; Cox, 1969; Liu and Maneely, 1969).

The mode of scale regeneration is different from the embryological morphogenesis of the scale (Dhouailly and Maderson, 1984) and is brought about by a localized cell proliferation along the epidermis of the regenerating tail (Alibardi, 1994).

The dermis under the forming scales is initially made of a loose connective tissue that progressively differentiates into a superficial discontinuous loose layer and into a deep dense connective one (Quattrini, 1953, 1954; Shah and Chakko, 1968; Liu and Maneely, 1969).

Because of the importance of the dermis to the differentiation of epidermal structures like scales, hairs and feathers (Sengel, 1986), it is important to know the detailed behaviour and the rearrangement of the dermis during tail regeneration and scale-genesis. The concentration of melanocytes in the dermis under the dorsal side of the regenerating scale and the almost complete absence of melanocytes in the ventral side of the scale (Alibardi, 1994), suggest that an intense rearrangement of the dermis during scale morphogenesis takes place.

To this purpose the dermis of regenerating lizard tails has been studied by a detailed microscopic and autoradiographical study using ^{3}H-thymidine and ^{3}H-proline.

Materials and methods

This study was conducted on adult specimens of two lizard species, one american (*Anolis carolinensis*) and the other Australian (*Lampropholis delicata*). The animals were maintained in terraria at 22-30 °C with a 12-hour photoperiod, and fed with insect larvae.

After the amputation of about 1/3 proximal of the tail, the animals were left undisturbed in order to regenerate their tails. After about three weeks post-amputation the animals (16 *Anolis* and 14 *Lampropholis*) were injected with 50-70 µl of ^{3}H-thymidine in a physiological saline solution (specific activity 60-90 Ci/mM or 29 Ci/mM, Amersham), in order to receive a total of 10-15 µCi/gBW. Tissues were sampled 4-5 hours post-injection, and then at 2, 4, and 6 days.

Another 8 animals (*Lampropholis*) were injected with 50-100 µl of ^{3}H-proline in saline solution (specific activity 24 Ci/mM, Amersham) in order to receive a total injection of 10 µCi/gBW. Sampling was done 1 hour postinjection.

Offprint requests to: Dr. Lorenzo Alibardi, Dipartimento di Biologia, Universita di Padova, Via Trieste 75, 35121 Padova, Italy

The tissues (regenerating tail) were immediately fixed in a cold solution of glutaraldehyde-acrolein in phosphate buffer 0.2M at pH 7.4-7.6 (glutaraldehyde 2.5%, acrolein 0.5%). After 8 hours, the tissues were post-fixed in 2% osmium tetroxide for 2 hours, washed in buffer for 30 min, dehydrated and embedded in either epon or spurr resin.

Sections were taken, both for light microscopy and transmission electron microscopy as previously described (Alibardi, 1994). Briefly, semithin or thin sections were collected from an ultramicrotome using a wire loop, and floated over collodium precoated slides. After drying, the slides were coated (in a darkroom equipped with a safelight filter Ilford 904) with Ilford Nuclear Emulsions for autoradiograph (K4 or L5). The slides were exposed for 1 to 5 months and then developed with Kodak D19 and fixed with Agfa fixer.

The collodium membrane was stripped from the glass slide, floated on distilled water and the thin sections were picked up with copper grids, and lightly stained in uranyl acetate and lead citrate. For light microscopy autoradiography, the developed and fixed sections on the slides were lightly stained in 0.5% toluidine blue.

Other thin sections, derived from the same embedded blocks used for autoradiography, were collected with the ultramicrotome (LKB nova or ultratome III or Reichert ultracut) on copper grids, and stained with uranyl acetate and lead citrate according to the standard procedure.

Grids with thin sections were observed with a Jeol CX 100 and with Hitachi 600 electron microscope.

The light microscope autoradiographical quantification was made counting the number of ^{3}H-thymidine-labelled cells (4-5 hours, 2, 4 and 6 days post-injection) out of 300 unlabelled cells in the different areas reported in Fig. 1. Throughout the text the percentage of labelled cells (%L) only refers to ^{3}H-thymidine-labelled cells.

Results

The apical blastema under the epidermis (area A in Fig. 1) is made by irregularly-arranged mesenchyme-like cells, a few of them labelled with ^{3}H-thymidine (Fig. 2).

The ^{3}H-thymidine %L at 4-5 hours post-injection was quite low (0.5-2.5% in *Anolis*, see Fig. 1). This value lowered toward zero in the following 2-4 and 6 days post-injection.

In longitudinal section, the forming dermis under the prescaling epidermis at about 200-400 μm from the apical tip (area B in Fig. 1) showed fibroblasts that were oriented perpendicularly to the basement membrane of the epidermis (Figs. 3, 4).

In cross section, the long axes of these cells were

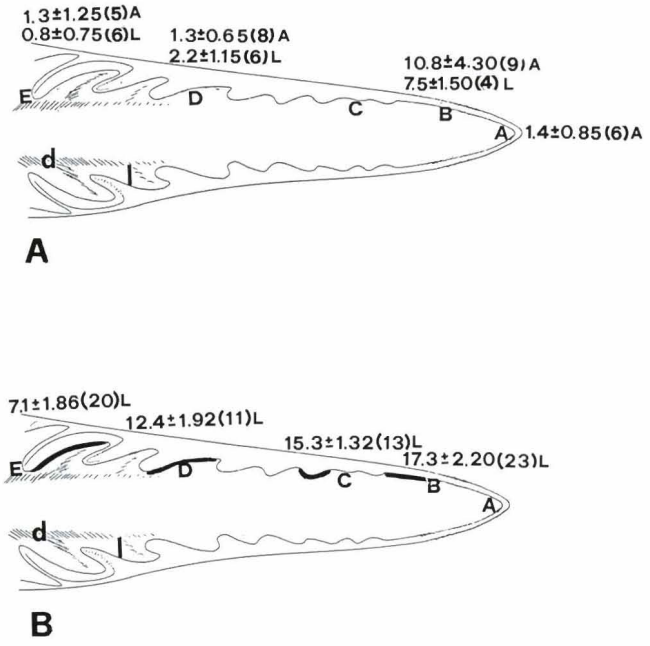


Fig. 1. Diagrammatic drawing of regenerating lizard tail. A: blastema; B: dermis under the prescaling epidermis; C: dermis under the wave-like scaling epidermis; D: dermis under the deepening scales (>20 epithelial cells deep); E: dermis under the differentiating scales (ß-layer differentiation). In **Fig. 1A**, the number in the respective areas represents the %L at 4-5 hours post-injection of ^{3}H-thymidine. In **Fig. 1B** the numbers in the respective areas (+SD) refer to the number of dermal cells/100 μm of basement membrane (thick lines). In parenthesis the number of sampled sections are reported. d: deep dense dermis; l: superficial loose dermis; A: *Anolis*; L: *Lampropholis*.

Fig. 2. *Anolis*. ^{3}H-thymidine-labelled mesenchymal cell 4 hours post-injection in the blastema close to the apical epidermis. Bar= 2 μm.

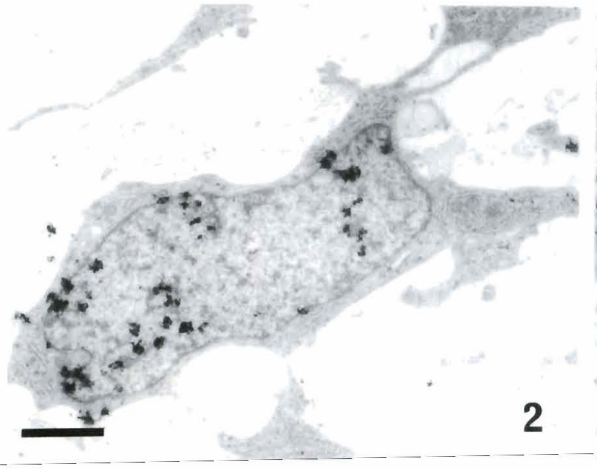
Fig. 3. *Anolis*. Forming dermis under the prescaling epidermis (E) where basal cells are labelled 4 hours post-injection of ^{3}H-thymidine. Fibroblasts (arrows point to some labelled ones) appear perpendicularly oriented toward the basal epidermis. Bar= 15 μm.

Fig. 4. *Anolis*. Electron microscopic view of perpendicularly oriented fibroblasts (one is labelled 4 hours post-injection of ^{3}H-thymidine) and their cellular processes (small arrows). Bar= 2 μm.

Fig. 5. *Anolis*. Cross section of prescaling epidermis featuring unlabelled and labelled fibroblasts (f) after 4-5 hours post-injection of ^{3}H-thymidine, with a circular disposition (small arrows) under the epidermis (E). Bar= 15 μm.

Fig. 6. *Lampropholis*. Cross section of pre-scaling epidermis showing circularly oriented (small arrows) labelled fibroblasts (f) 1 hour post-injection of ^{3}H-proline. E: epidermis. Bar= 10 μm.

Fig. 7. *Lampropholis*. Dividing fibroblasts (arrow) in the superficial dermal layer. Collagen bundles (arrowhead) run among the cells. Bar= 1.5 μm.



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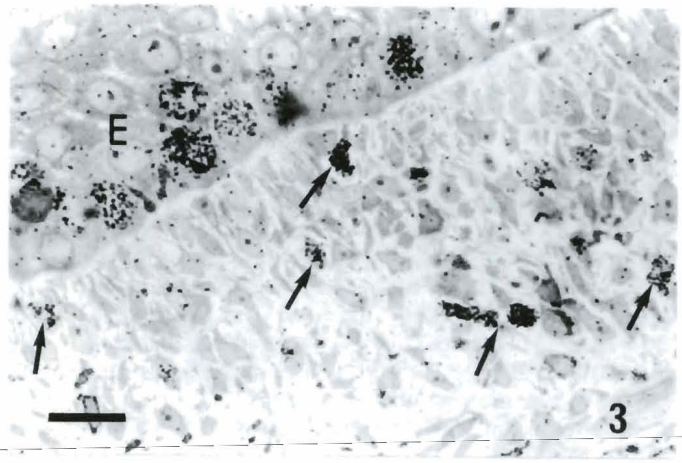

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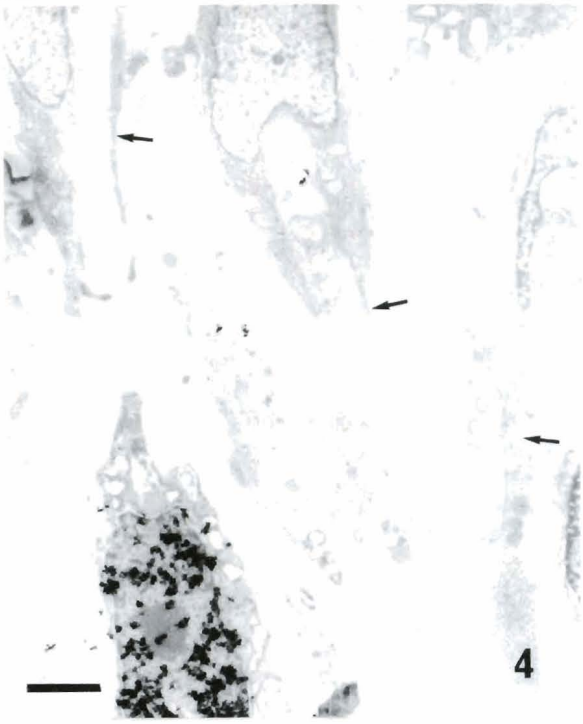

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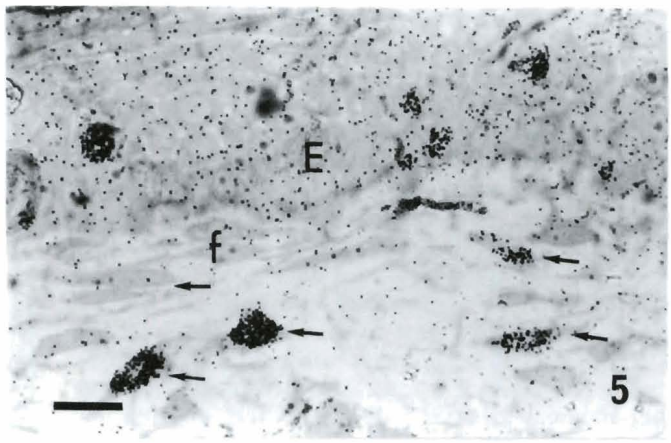

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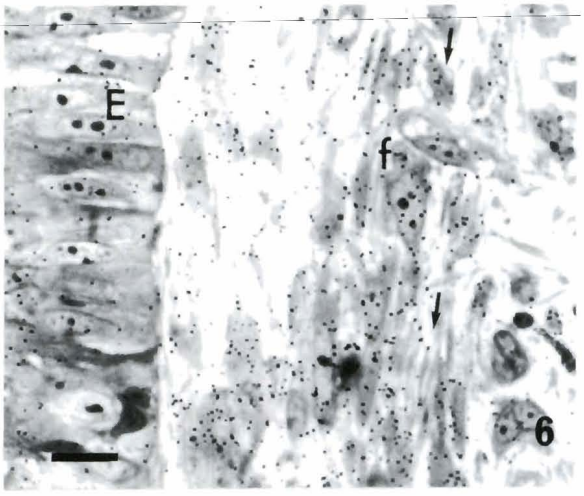

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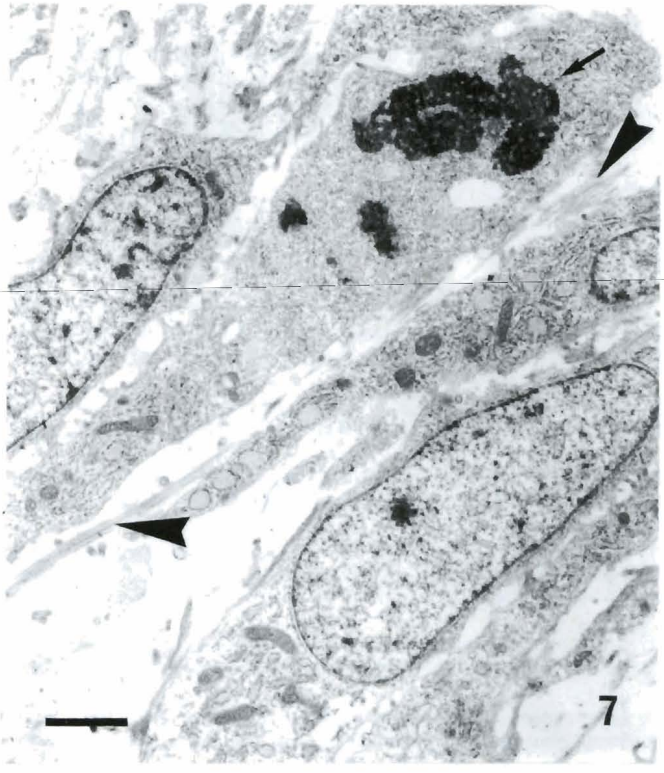

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circularly oriented and they took up ^{3}H-proline in large amounts one hour post-injection (Figs. 5, 6). The ^{3}H-thymidine %L in these areas (B in Fig. 1) was quite high (6.5-15.0% in *Anolis;* 6.0-9.0 % in *Lampropholis*), and dividing fibroblasts were sometimes encountered (Fig. 7). In this area at 2-4 days post-injection the ^{3}H-thymidine %L was 7.3-13.3 in *Anolis.* At 6 days post-injection in *Lampropholis* it was still high (4.6-12.0%) but the intensity, as number of trace grains per nucleus, was decreased with respect to 4-5 hours post-injection.

In the dermis under the wave-shaped epidermis at the beginning of the scaling (near the area C in Fig. 1), the ^{3}H-thymidine %L was also high 4-5 hours post-injection (Fig. 8). Area C showed symmetrical epidermal papillae that gradually became asymmetric in area D.

The ^{3}H-thydimidine %L rapidly dropped at 4-5 hours post-injection in area D (under the down growing epidermal papilla), in *Anolis* (0.6-1.9%) and more slowly in *Lampropholis* (1.0-3.5%). This was seen both in the superficial loose dermis and in the forming deep dense dermis. In area D epidermal papillae were asymmetric, i.e. the distal side of the papilla (facing the tail tip, see Fig. 1) had over 20 cells and was longer than the proximal side (facing the tail stump). The first sign of keratinization was seen in area D.

In the scaling area (D in Fig. 1) the ^{3}H-thymidine %L in the dermis slightly decreased 2 days post-injection in *Anolis* (0.5-1.5%) but remained constant, or slightly increased after 6 days post-injection in *Lampropholis* (2.5-3.6%). Also in this case, while at 2 days post-injection a reduction of the nuclear intensity of labelling was not appreciated, after 6 days from the injection the number of trace grains per nucleus was reduced with respect to 4-5 hours post-injection.

The fibroblasts in areas C and D took up high levels of ^{3}H-proline 1 hour post-injection, and the electron microscopic analysis showed that trace grains were concentrated mainly in the perinuclear Golgi area (Figs. 9, 10).

Under the epidermal basement membrane, characteristic round clear spaces were seen (Figs. 8, 9). These spaces were resolved with the electron microscope as enlarged cavities that represented tunnels circularly oriented along the cross perimeter of the tail. These tunnels contained scarce amorphous material (Figs. 11, 12).

The tunnels often resulted from the cavitation between the terminal arms of the fibroblasts that contacted the epidermis (Figs. 11, 12).

Cell processes and collagen fibrils in these areas were oriented perpendicularly to the epidermis and joined to the basement membrane (Fig. 13). Melanocytes were spread irregularly under the forming epidermis (areas A, B and C in Fig. 1), particularly in the dorsal part of the regenerating tail, and some appeared labelled with ^{3}H-thymidine (Figs. 11, 12, 14).

In the dermis under the sinking epidermal papillae, numerous blood vessels were observed, but their precise disposition with respect to the epidermis was not determined.

Initially, the fibroblast closer to the epidermis showed a perpendicular orientation toward the epidermis (Fig. 15). Later, with the deepening of the epidermal papillae, the previous orientation was lost both in the dermis under the proximal side (facing the old tail) and the distal side (facing the tail tip) of the epidermal papillae (Fig. 16). In these areas (D in Fig. 1) melanocytes were by far more concentrated under the distal side of the epidermal papillae (where most of ^{3}H-thymidine-labelled cells were seen) than in the proximal side (Figs. 17, 18). These melanocytes sent their pigmented processes into the basal layer of the epidermis (Fig. 19).

Also in the area E of Fig. 1 the ^{3}H-thymidine %L 4-5 hours post-injection was low (0- 2.5 % in *Anolis*; 0-1.5% in *Lampropholis*). Area E was characterized by the formation of a compact layer of keratin (ß) in the middle of the regenerating scale. At 2 days post-injection in area E of *Anolis* the ^{3}H-thymidine %L was still low (1.6-2.8%).

In area E at 6 days post-injection in *Lampropholis*, where the dermis was differentiated in superficial and deep layers (area E of fig. 1, Fig. 16), the ^{3}H-thymidine %L was 4.9-11.0% in the superficial loose dermis and 3.5-9.2% in the deep dense dermis.

Fig. 8. *Anolis.* Dermis of the scale anlagen showing may labelled fibroblasts (1 hour post-injection of ^{3}H-thymidine) under the curved epidermis (E). Small arrows point to pale spaces under the basement membrane. Bar= 15 µm.

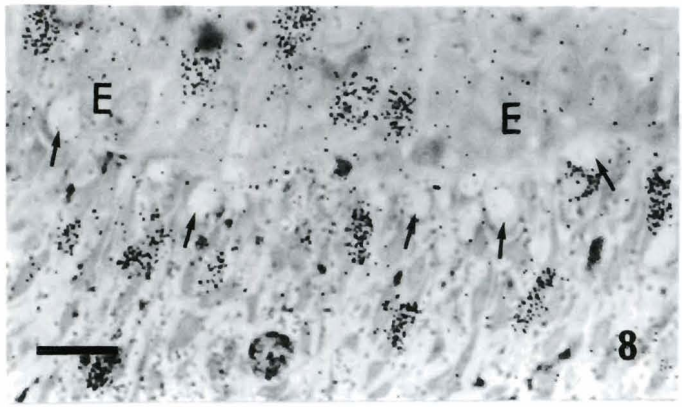
Fig. 9. *Lampropholis.* Dermal regions under prescaling epidermis showing highly labelled and perpendicularly oriented fibroblasts (f) 1 hour post-injection of ^{3}H-proline. E, epidermis. Small arrows point to some empty spaces under the epidermis. Bar= 15 µm.

Fig. 10. *Lampropholis.* ^{3}H-proline labelled fibroblasts from an area similar to figure 9. After 1 hour post-injection the silver grains are most concentrated in the perinuclear Golgi area (G). Small arrows point to collagen fibrils. Bar= 2 µm.

Fig. 11. *Lampropholis.* Electron microscopic view of the boundary between epidermis (E) and perpendicularly oriented dermal fibroblasts (f) in the prescaling epidermis. Many collagen fibrils (perpendicular or cross sectioned, see arrows) join to the basement membrane. Circular «empty spaces» (S) are seen between the terminal arms of the fibroblasts. Arrowheads point to cross-sectioned melanocyte arms. Bar= 1.5 µm.

Fig. 12. *Lampropholis.* Dermal fibroblasts with a basket-like appearance (arrows) under the epidermal scale anlage (E, in area C of Fig. 1). Together, terminal fibroblast arms and melanocyte elongations are seen (arrowheads) with the amorphous matrix within the clear spaces (S). Bar= 2 µm.

Fig. 13. *Lampropholis.* Infolding epidermal papilla (E) with ^{3}H-thymidine-labelled basal cells (small arrows). Filamentous dermal processes (large arrows) contact the epidermal basement membrane. B: blood vessel; K: outer keratin. Bar= 10 µm.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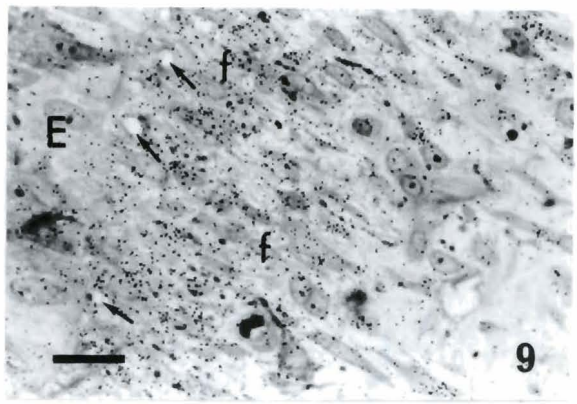
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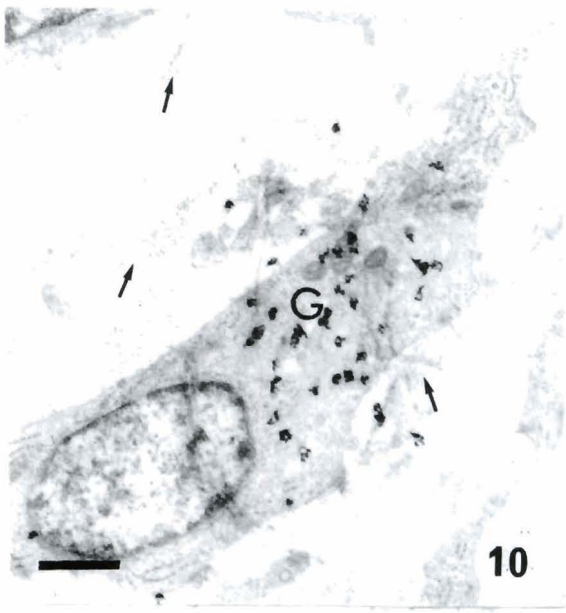
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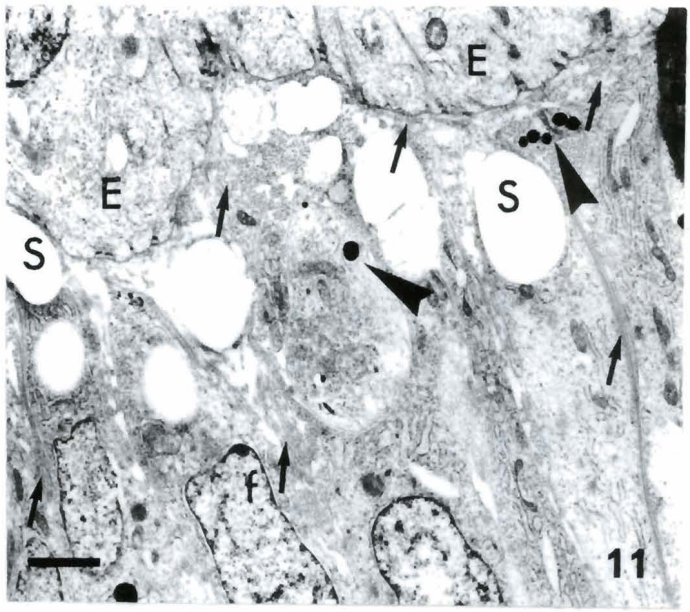
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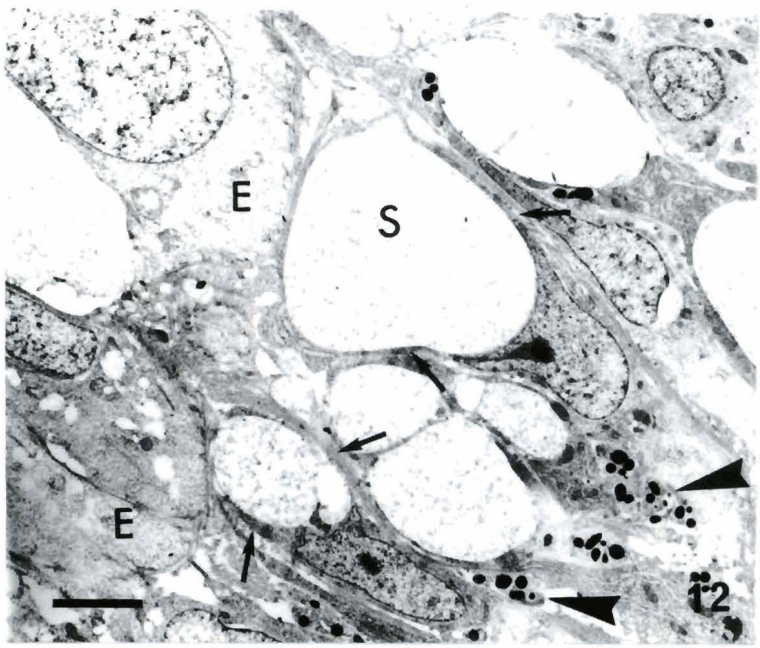
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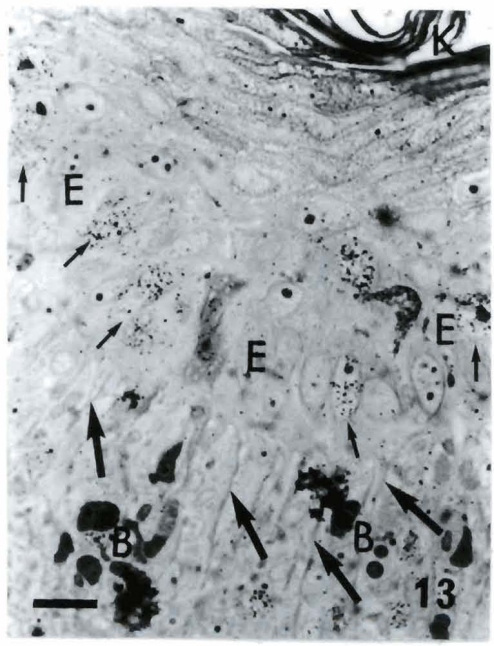
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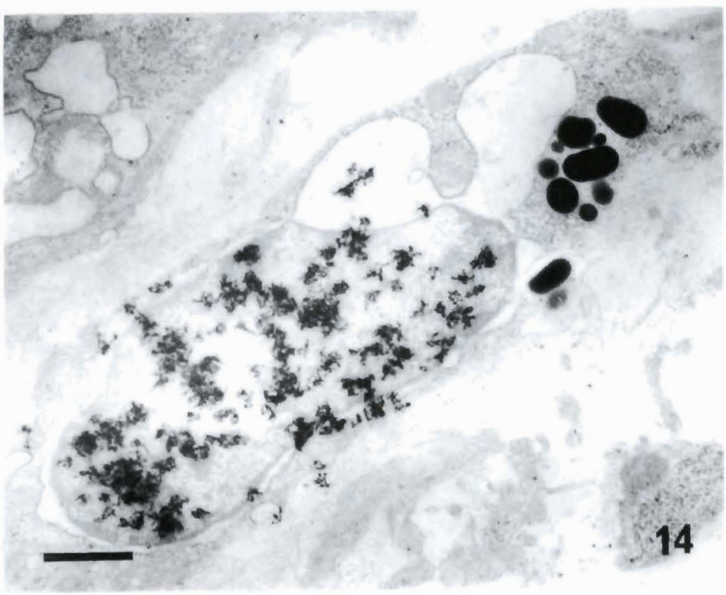

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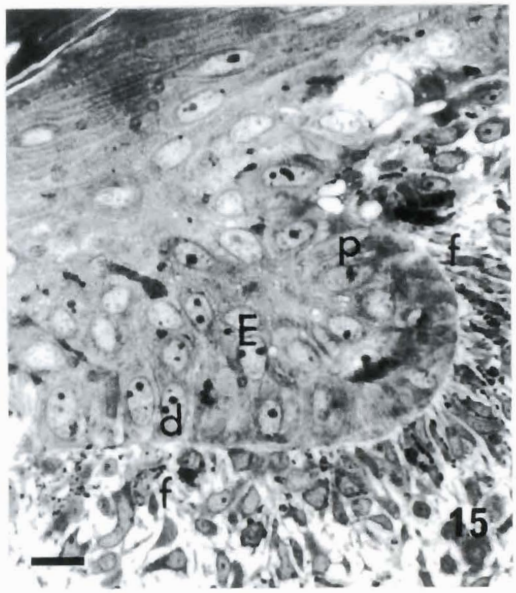

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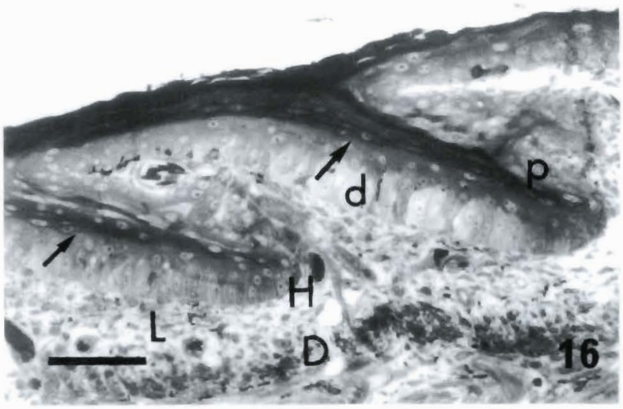

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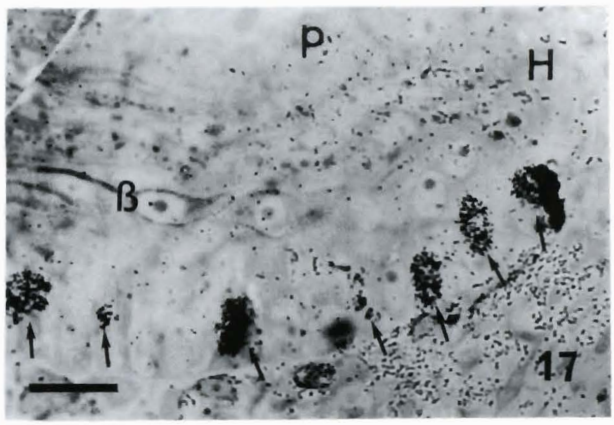

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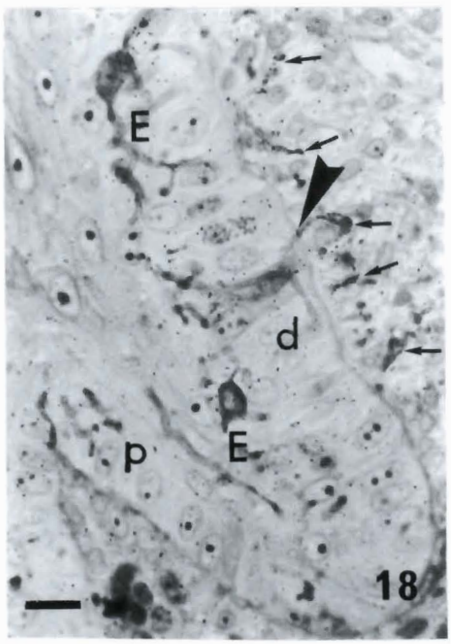

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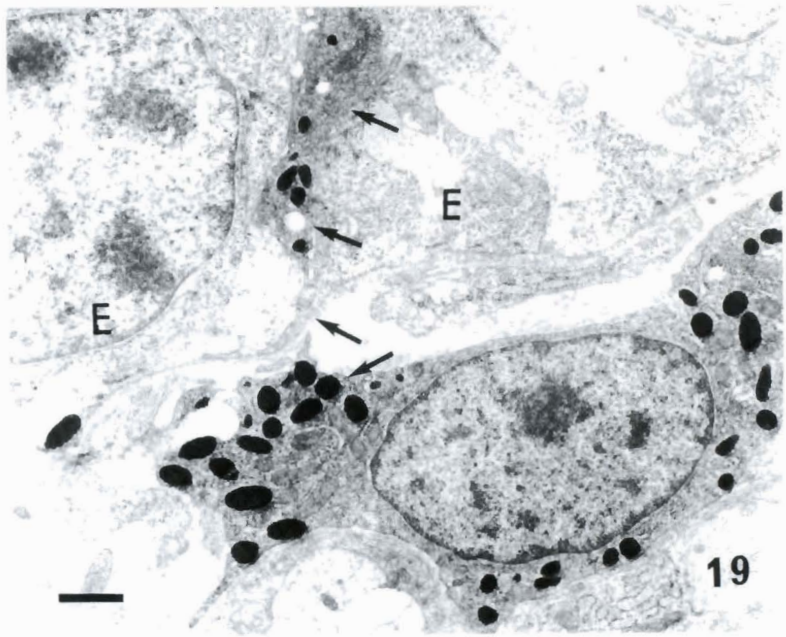

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Fig. 14. *Anolis.* Highly ^{3}H-thymidine-labelled melanocyte in the prescaling dermal layer. Bar= 1 μm.

Fig. 15. *Lampropholis.* Initial epidermal papilla (E) with subjacent radiate layer of dermal fibroblasts (f). d: distal side; p: proximal side. Bar= 10 μm.

Fig. 16. *Lampropholis.* Forming scales showing ß-keratin formation in the middle line (arrows). The dermis appears distinct in a loose superficial (L) and dense deep (D) layer. d: distal side of the scales; H: hinge region; p: proximal side. Bar= 20 μm.

Fig. 17. *Anolis.* Epidermal ^{3}H-thymidine-labelled cells (small arrows) are seen at the distal side of a deepening scale where dermal melanocytes are concentrated. No labelled cell is seen in the proximal side (p). H: hinge region; ß: forming epidermal ß-layer. Bar= 10 μm.

Fig. 18. *Lampropholis.* Epidermal (E) melanocytes within the basal layers of the distal side of forming scales (d). Dermal melanocytes are mainly located under the distal side (small arrow). The arrowhead points to the arm of a dermal melanocyte that penetrates into the epidermal layer. p. proximal side. Bar= 10 μm.

Fg. 19. *Lampropholis.* Electron microscopic view of a dermal melanocyte under the epidermis of the distal side of a papilla. The arrows points to a melanocyte arm which penetrates among the basal epidermal cells (E). Bar= 1 μm.

In area D (and E) a loose superficial and interpapillar dermis, and a deeper dense dermis progressively appeared, with the deeper dermis being richer in ^{3}H-proline labelling 1 hour post-injection (Figs. 1, 16, 20-22). The deep dermal layer showed the extracellular fibrils oriented in a circular direction along the tail perimeter, as observed in cross section (Figs. 5, 6, 22).

Under the deep compact dermis the flat layer of the perimuscular (subdermal) connective tissue was seen.

The extracellular fibres were more concentrated and showed a circular orientation in the deep dermis, and in this layer the uptake of ^{3}H-proline was higher than in the superficial dermis. In the latter, an irregular disposition of the fibrils was observed.

The cell density under the epidermis (counted as number of cells along a distance of 100 μm of basement membrane) was different in areas B, C, D and E (Fig. 1B). In fact, the cell density progressively decreased moving from area B (prescaling, mean 17.3) to area C (mean 15.3), then area D (mean 12.4) and to area E (mean 6.8).

In areas D and E, where the superficial and the deep dermal layers were already differentiated, the latter was joined to the forming epidermal papillae by some long filaments (Figs. 13, 20, 21). The electron microscopic observation showed that the dermis under the distal side of the forming scales was mainly constituted by irregularly-oriented fibroblasts to form a loose connective tissue, while a few fibroblasts were oriented along the basement membrane (Figs. 22-24). This irregular connective tissue resembled the mesenchymal tissue of the apical blastema which contained amorphous substance and few collagen fibril (Figs. 2, 25). A few «anchoring filaments» contacting the basement membrane were seen (Fig. 24).

Bundles of collagen fibrils and thin cell processes (anchoring complexes, see Dhouailly and Maderson, 1984) were seen to constitute most of the long filaments previously seen joining the dermis to the basement lamina of the epidermis (Figs. 13, 23, 24).

Though it was not quantified, these «anchoring complexes» were located under the prescaling and early scaling epidermis (area B) at random. In the deepening scales (areas C, D and E in Fig. 1) the «anchoring complexes» were more frequently seen around the downgrowing hinge region of the forming scales than under the distal and proximal sides of the forming scales.

The collagen (banded), reticular (not banded: maybe elastic) fibrils entered into the amorphous components of the basement membrane, and contacted the dense basement lamella (Figs. 26, 27). The amorphous material of the basement lamina faced the hemidesmosomes of the pale epidermal cells and of the occasional darker epidermal cells. Epidermal tonofilaments converged into the spot hemidesmosomes and appeared in a sort of «tensile» continuity with the dermal collagenous fibrils.

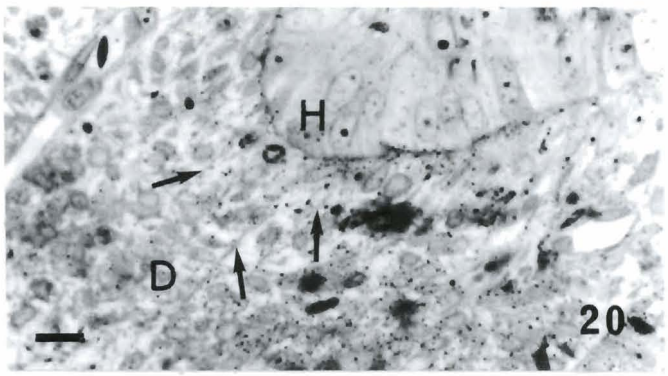
Fig. 20. *Lampropholis.* Hinge region of forming scale (H) near the deep dermal layer (D) where grains from ^{3}H-proline are seen. Thin filaments (arrows) join the hinge region to the deep dermis. Bar= 10 μm.

Fig. 21. *Lampropholis.* Formed deep dermis layer (D) under the hinge region (H) of a maturing scale. Thin filaments (arrows) join the deep dermis to the hinge region (H). Bar= 10 μm.

Fig. 22. *Lampropholis.* Cross section through the distal side of maturing scale 1 hour post-injection of ^{3}H-proline. The superficial loose dermis (L) shows collagen fibrils perpendicularly or irregularly oriented. The deep dermis (D) shows circularly oriented labelled cells and fibrils. E: epidermis of the distal side of the scale. Bar= 10 μm.

Fig. 23. *Lampropholis.* Dermal fibroblast with parallel orientation under the epidermis (E) of the distal side in a forming scale. Numerous collagen fibrils (arrows) join the fibroblast to the basement membrane. Bar= 1 μm.

Fig. 24. *Lampropholis.* Loose dermis under the distal side of a deepening epidermal papilla (E). Small «anchoring collagen bundles» (arrows, see the inset, x 15,000) and cell processes are close or contacting the basement membrane (B). Bar= 2 μm.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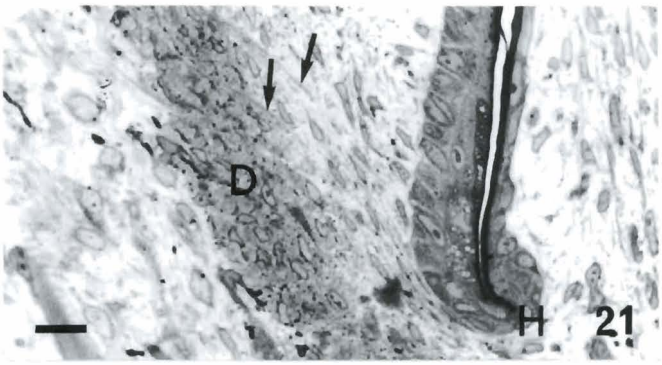
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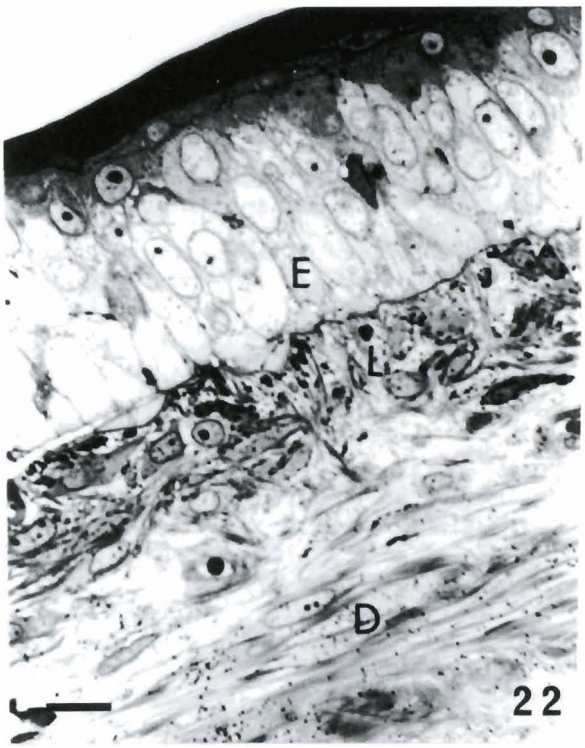
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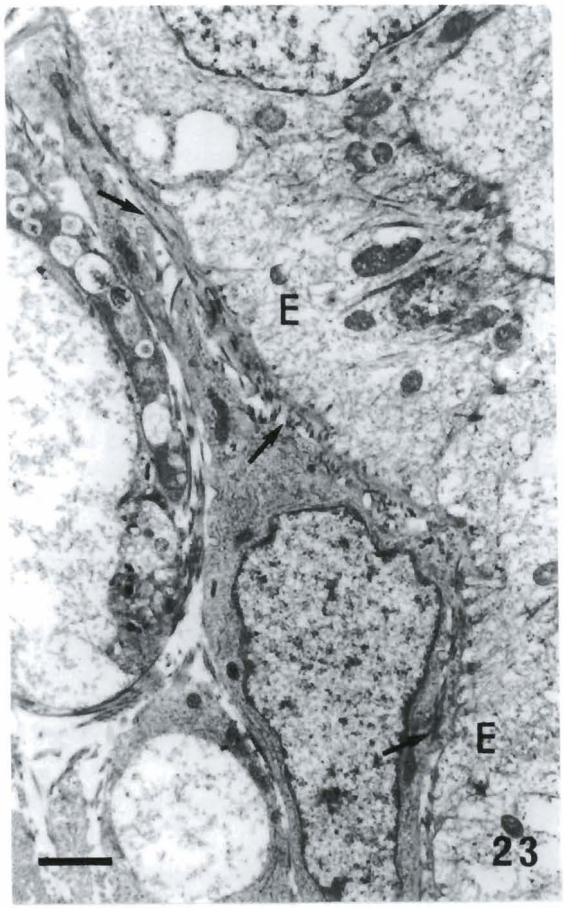
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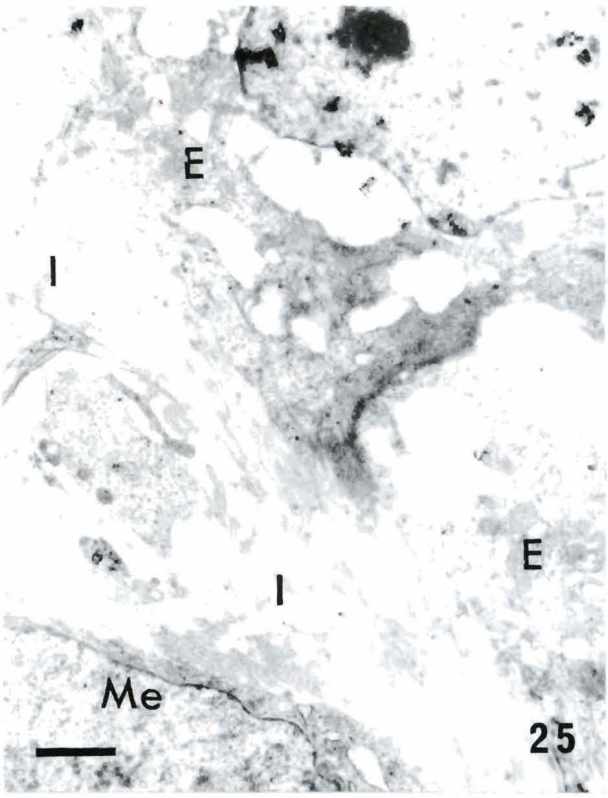
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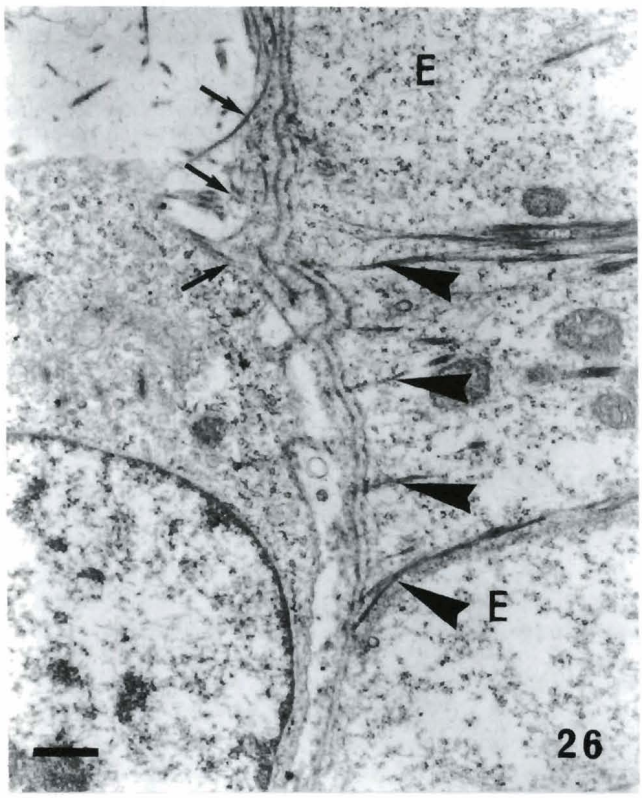
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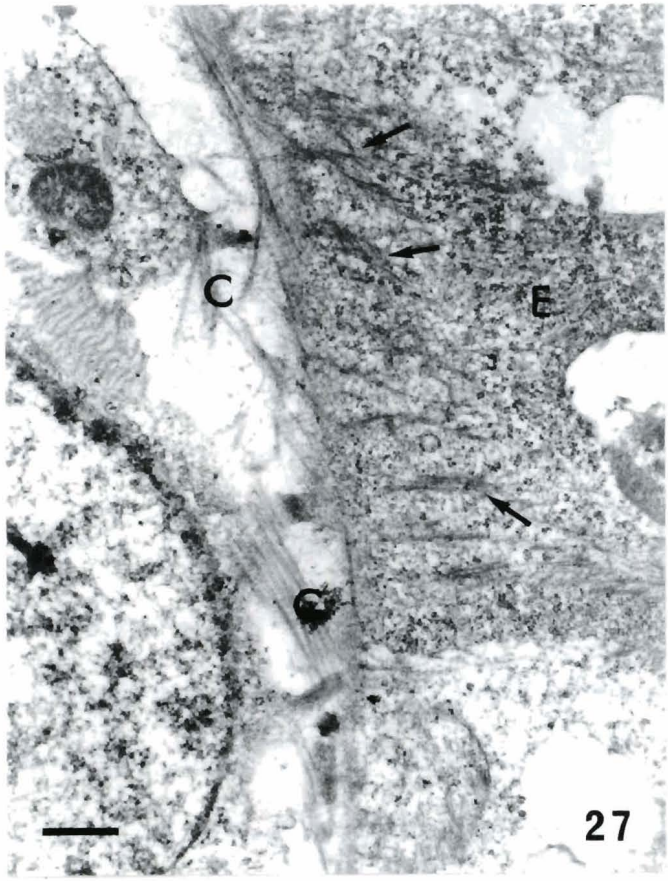
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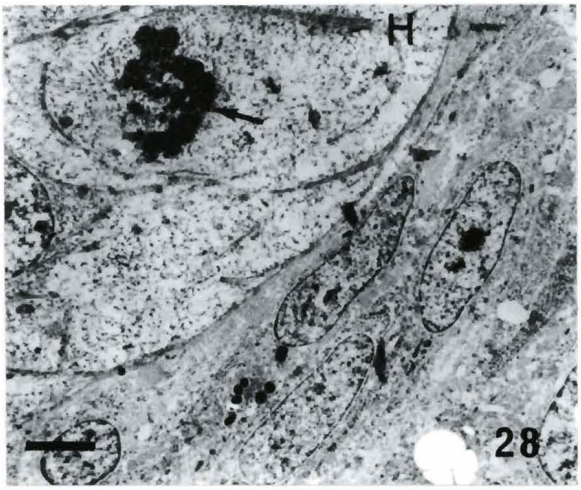
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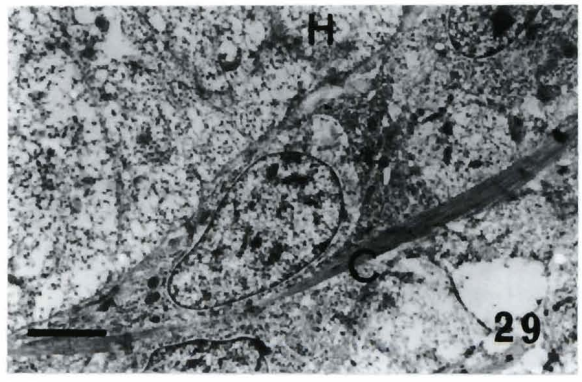
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Fig. 25. *Anolis.* Mesenchymal cell (Me) under the apical epidermis (E) where an ^{3}H-thymidine labelled cell is visible (zone A in Fig. 1). Neither «anchoring filaments» nor a definite basement membrane are seen, but irregular fibrils among the amorphous intercellular matrix are visible (I). Bar= 1 μm.

Fig. 26. *Lampropholis.* Collagen fibrils contacting the basement membrane (arrows) of the epidermal distal side of a forming scale (E). The epidermal tonofilaments converge on spot hemidesmosomal terminals (arrowheads) facing the dense basal lamella. Bar= 0.5 μm.

Fig. 27. *Lampropholis.* Dermal collagen fibrils (C) contacting the basement membrane under a dark epithelial cell (E) rich in tonofilament bundles (arrows). Bar= 0.5 μm.

Fig. 28. *Lampropholis.* Flat curved fibroblasts under the hinge region (H) of a forming scale. The arrow points to a dividing epidermal cell. Bar= 2.5 μm.

Fig. 29. *Lampropholis.* Curved bundle of collagen (C) under the hinge region (H) of a forming scale. Other collagen bundles are cross sectioned. Bar= 2 μm.

Collagen and reticular fibrils merging with the basement membrane (lamina densa) were also seen under the hinge region and the proximal side of the deepening epidermal papilla.

In the hinge region, the cells were oriented along the crescent-shaped line of the basement membrane, while the extracellular collagen fibrils were criss-crossed in a lattice pattern around the epidermal papillae (Figs. 16, 22, 28, 29).

Also, under the proximal side of the forming scale, cells and extracellular fibrils or bundles of fibrils (anchoring complexes), were mostly oriented in a parallel fashion (often criss-crossed) with respect to the basement membrane (Figs. 30, 31).

Perpendicular anchoring filaments were occasional or absent under the proximal side.

Finally, the ultrastructural analysis of the forming deep dense connective tissue showed that fibrocytes were intensely labelled with ^{3}H-proline 1 hour post-injection (Figs. 20, 22, 32). A strand of dense dermis also penetrated into the core of the scale aiming toward the tip of the scale (Figs. 1, 16). Labelled fibrocytes containing a diminished number of trace grains per nucleus at 6 days post-injection were also recorded with the electron microscope in the deep dermis (Fig. 33). At maturity, these cells were tightly surrounded with bundles of collagen fibrils that filled up most of the intercellular spaces, while occasional elastic fibres were seen (Fig. 34).

Discussion

Effects of cell proliferation

This study produces further evidence that during tail regeneration in lizards the morphogenetic mechanism that leads to scale neogenesis is due to the proliferation of the epidermis and that the dermis appears to have a role in directing the epidermal sheet downward, probably by means of the «anchoring complexes».

In a previous study, Liu and Maneely (1969) hypothesised that the outer keratinized layer of the epidermis was preventive and the soft dermis permissive of epidermal infolding. However, an ultrastructural study (Alibardi, personal observations) has shown that the initial shoft epidermis in the scaling region is composed of «soft and pliable» α-keratin. This latter type of keratin does not constitute a preventive mechanical barrier to the upward elevation of the epidermis. Besides, during embryogenesis (Dhouailly and Maderson, 1984; Maderson, 1985) even the keratinized periderm that coats the embryonic skin, does not prevent the epidermal elevations that foward the formation of the first scales. Therefore, an α- or ß-keratin layer is very unlikely to prevent the elevation of the epidermis into papillae.

At present, the forces that cause the infolding of the epidermis into the dermis to produce new scales appear most linked to the different rate of cell proliferation between the epidermis and the dermis of the regenerating tail (heterochrony). In fact, previous studies

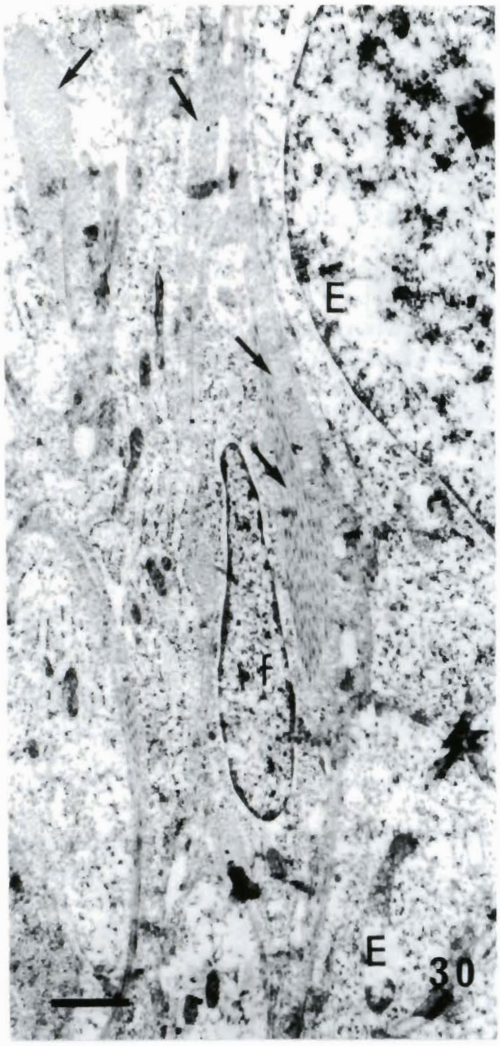
Fig. 30. *Lampropholis.* Flat fibroblast (f) and collagen fibrils (arrows) with a parallel-oblique orientation under the epidermis (E) of the proximal side of a forming scale. Bar= 1 μm.

Fig. 31. *Lampropholis.* Other flat fibroblast (f) attached to the basement membrane of an epidermal cell (E) on the proximal side. Collagen bundles (large arrows) are mostly cross sectioned. Bar= 1 μm.

Fig. 32. *Lampropholis.* Fibroblast of the forming deep dermis 1 hour post-injection of ^{3}H-proline. Trace grains (arrowhead) are most concentrated in the region occupied by the Golgi complex. C: collagen fibrils. Bar= 0.5 μm.

Fig. 33. *Lampropholis.* ^{3}H-thymidine-labelled fibroblast in the deep dermis at 6 days post-injection. Cross-sectioned extracellular collagen fibrils (arrowheads) are visible. Bar= 0.5 μm.

Fig. 34. *Lampropholis.* Mature deep dermis featuring fibroblast (f) surrounded by many collagen bundles. The large arrows point to cross-sectioned elastic fibrils. Bar= 1 μm.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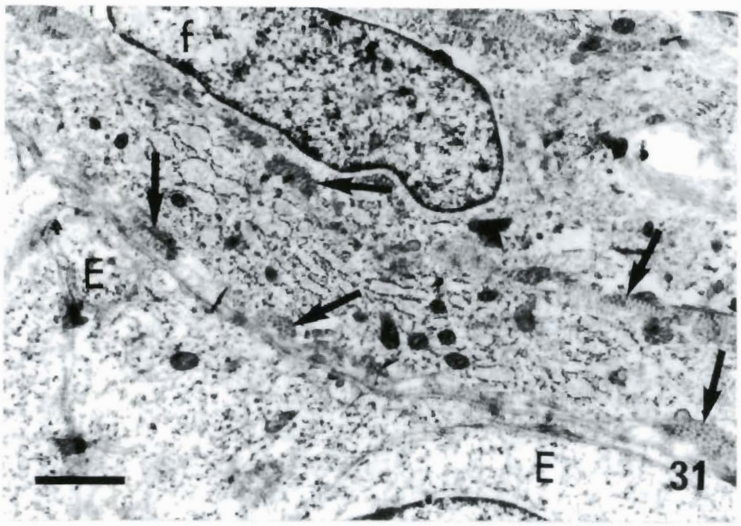
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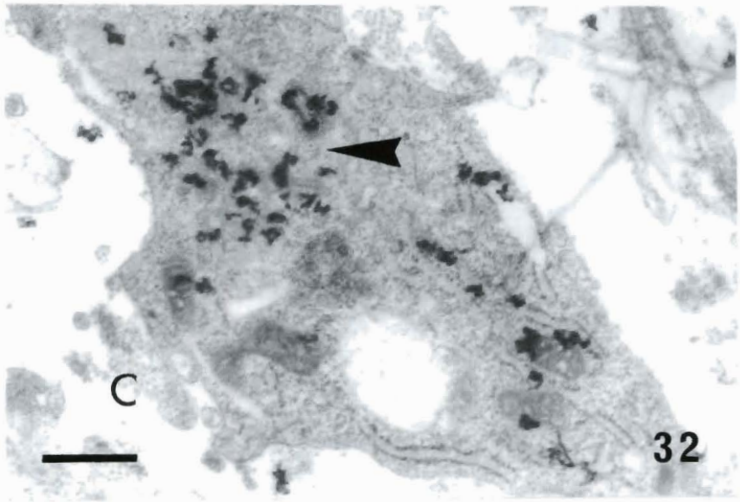
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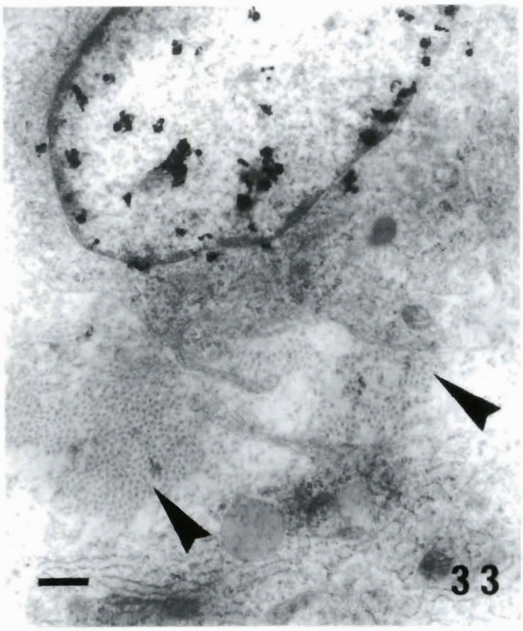
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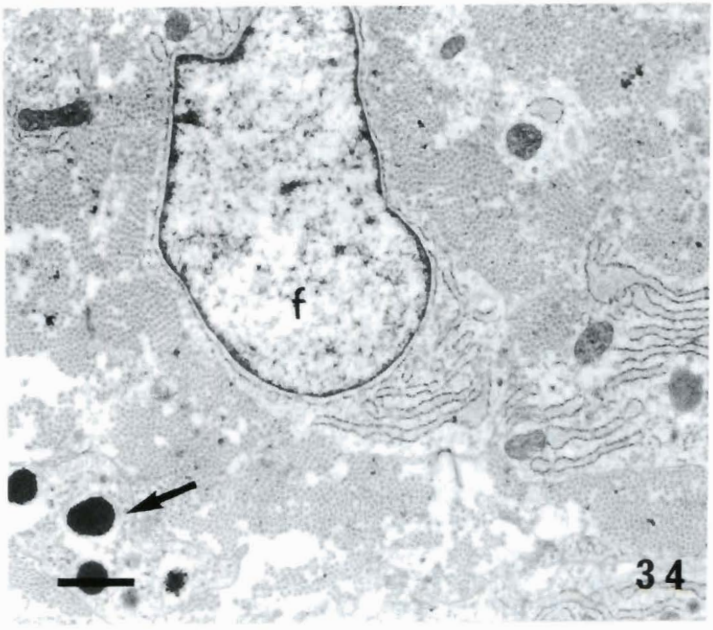
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(Alibardi, 1994) have shown that the epidermis in the prescaling region (area B in Fig. 1) has an H3-thymidine %L (average 11.8-14.8% that rises to 42.8% in the basal stratum) higher than the %L of the dermis of the underlying dermis (average 7.5- 10.8%, as reported in this study). The difference in the rate of proliferation is even higher between the epidermis and dermis in areas D and E, where the reason for this drop in cell multiplication is not known. A low rate of cell proliferation in the dermis of *Anolis* was previously seen by Cox (1969).

During avian and mammal scale, feather and hair development a more or less definite proliferation and dermal condensation takes place under the epidermis or the placodes (Wessels, 1967; Sawyer, 1972; Sawyer et al., 1986; Sengel, 1986).

In Reptiles, dermal condensations are not seen, neither during embryogenesis nor regeneration (Maderson, 1965, 1985, Bellairs, 1972) and the dermis does not affect the pattern of keratinization of the epidermis (Flaxman, 1972).

It is not known why there is a localized proliferation during scale regeneration that determines the epidermal infoldings and the prevalent proliferation in the epithelial cells of the distal side of the downgrowing scale (Alibardi, 1994). A relation to blood vessel arrangement in these regions (Quattrini, 1954; Hughes and New, 1959) has not been traced, but it is known that vascularization is associated to papillae formation (Minelli, 1983).

As in lizards, an asymmetrical rate of cell proliferation has also been recorded in birds in the outer and inner surfaces of the scutate scale primordia (Saywer, 1972; Tanaka and Kato, 1983).

Although fibroblasts in the prescaling area actively multiply, they do not proliferate very much under the deepening epidermal papillae (area D in Fig. 1). This is also documented from the relatively constancy or increased %L found in the areas at 2-6 days post-injection. On the contrary, in a fast proliferating tissue the labelling (%L) would be reduced among the daughter cells at 6 days post-injection.

The low proliferation of fibroblasts (both in the superficial and deep dermis) produces a decrease in cell density under the sinking epidermis (Fig. 1B) and may leave room for the folding epidermis.

Dermal remodelling

The dermis may direct the expanding epidermis of the regenerating tail downward by means of the «anchoring complexes», especially seen in the hinge regions. The dynamic role of these filaments during the formation of scales is not known, but they have been mainly found localized in the interscale-hinge regions of lizards (Dhouailly and Maderson, 1984), in the interplacode regions of the scutate scales of chick embryo (Sawyer, 1972; Haake and Sawyer, 1982) and in the interfeather germ regions of the chick (Kallman et al., 1967).

Either collagen or fibronectin are present in these complexes (Haake and Sayer, 1982), which may exert a traction on the overlaying epidermis. It is not known whether elastic fibrils are components of the anchoring filaments.

The high ^{3}H-proline uptake in the deep dermis suggests that it is from this stratum that most of the collagen fibrils are formed, in particular the long anchoring filaments that reach the basement membrane of the epidermis.

The downgrowing movement of the epidermal papilla produces a «distortion» of the «palidase» arrangement of fibroblasts observed in the anterior area of the regenerating tail (area B in Fig. 1). In fact, the intense cell proliferation in the stratum basale of the distal side of the forming scale very likely produces a distension of the basement membrane surface, and therefore the former linkage points of the dermal fibroblasts to the basement membrane would move apart, producing a change of orientation of the fibroblast axes.

As a result of the differential growth between the epidermis and dermis (heterochrony) the fibroblasts under the distal and proximal sides of the scale would bend under the downgrowing epidermis. Both fibroblasts and collagen fibrils change their orientation; from perpendicular («palidase fibroblasts») they turn into a horizontal or irregular arrangement (Liu and Maneely, 1969).

Another morphological sign of this process is the disappearance, in the scale regions, of the clear tunnels of the «basket-like fibroblasts» present in the prescaling region. The widening of the basement membrane stretched the fibroblast arms from a perpendicular position (Figs. 11, 12) into a horizontal one (Fig. 23).

The remodelling of the dermis under the sinking distal side might be brought about by enzymatic lysis of the intercellular matrix (collagenase, hyaluronidase, etc). In fact, the dermis under the distal side resembles the loose irregular connective tissue in the scale core during lizard development (Dhouially and Maderson, 1984; Maderson, 1985). In spite of the fact that a previous study recorded higher concentrations of the lysosomal acid phosphatase in the regenerating dermis, no differences were reported in the dermis of the two sides (Shah and Chakko, 1966). The present electron microscopic analysis did not reveal a particular richness of lysosomes in the proximal side dermal cells, even if the anchoring filaments were found at a lesser amount than in the hinge region and extracellular fibrils appeared somewhat degraded.

If a loosened connective tissue is present under the distal side, the melanocytes might preferentially move about and concentrate in this region (the future dorsal side of the scale) more than at the proximal side of the scales, which appears more compact (Alibardi, 1994).

The fibroblasts and their extracellular fibres around the hinge region under the «dislocation pressure» of the downgrowing epithelium appear distorted and curved to

follow the roundish shape of the hinge region.

Also, under the proximal side of the scale the orientation of the cells appears mainly parallel to the basement membrane while the extracellular fibres are both parallel to the basement membrane and criss-crossed in a lattice pattern. Under the proximal side of the scale, fibroblasts appear stretched along the collagen fibrils that converge toward the hinge region into the deep dermis and have completely lost the perpendicular orientation seen in the prescaling region.

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