

Fine autoradiographical study on scale morphogenesis in the regenerating tail of lizards

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Summary. Regenerating scales in lizards originate as pockets in the epidermis instead of epidermal elevations as during embryo development. The morphogenesis of scales in the regenerating tail of the lizards *Anolis* and *Lampropholis* was studied after peritoneal injection of ^3H -thymidine. The tracer was localized in the forming epidermis after progressive post-injection times, by means of autoradiography on plastic sections. After 4-5 hours post-injection of ^3H -thymidine, the radioactivity was localized in the basal layer. After 2 to 4 days post-injection labelled cells were seen in the basal and intermediate spinosus layers but not in the uppermost keratinizing layers. Labelled cells were seen in the differentiating cornifying layers (pre- β and pre- α) 6-8 days post-injection. At 12-14 days post-injection almost no radioactivity was seen in the basal layer or in the living part of the epidermis. A few labelled cells were present in the dense keratinizing layers of the sloughing wound and interscale lacunar layers. This study shows that scale formation and morphogenesis in the regenerating tail is brought about by a localized cell proliferation along the regenerating epidermis. In the forming scales the percentage of labelled cells in the distal side (future dorsal part of the new scale) is much higher than in the proximal side (the future ventral side of the scale), so that overlapped scales are generated.

Key words: Lizard, Scale formation, Regenerating tail, Electron microscopy, Autoradiography

Introduction

During tail regeneration in lizards the new epidermis progressively differentiates new scales which resemble, with different degrees from species to species, the original ones (Quattrini, 1952, 1954). In most lizard species, the regenerated scales are different from the original ones both in macroscopical and microscopical

appearance (Quattrini, 1952; Werner, 1967). Despite this difference, sensory organs can also be reformed in the regenerated tail skin (Hughes and New, 1959; Maderson, 1971).

Scale morphogenesis during regeneration of the tail in lizards presents some interesting problems. In fact, regenerating scales begin as epidermal invaginations instead of external elevations as in normal development (Maderson, 1965, 1985; Dhouailly and Maderson, 1984). This modality of scale morphogenesis shows some analogies with the initial stages of hair development in mammals, due to the epidermal migration into the dermis (Bryant and Bellairs, 1967).

The knowledge of scale morphogenesis during regeneration appears particularly interesting since squamate epidermis contains an outer layer of cornified cells containing β -keratin, resembling bird keratin, and an inner cornified layer made up by α -keratin, which resembles mammal keratin (Alexander and Parakkal, 1969; Alexander, 1970; Maderson et al., 1972; Maderson, 1985).

The vertical sequence of β and α keratinized layers in the normal squamate skin has been divided into six arbitrary stages (Maderson, 1965, 1985; Landmann, 1979).

Though the general development of the regenerating scales of lizards is known (Hughes and New, 1959; Shah and Chakko, 1967; Cox, 1969; Liù and Maneelly, 1969), many important details on the fine cytology, keratin types, morphogenesis, timing and cell migration during the scaling process are missing.

The present research describes all these processes using the fine resolution of semithin plastic sections and autoradiography. This study shows that only the very early stages of scale formation resemble hair morphogenesis in mammals and that the two processes are completely different. In fact, the following differential pattern of cell division, cell shaping, differentiation and the spatial aggregation of cells in the proximal side (the future ventral side of the scale) and the distal side (the future well-keratinized dorsal side of the scale) of the

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invaginations, produces asymmetric and overlapping scales.

Materials and methods

Adult individuals of the American iguanid lizard *Anolis carolinensis* and of the Australian scincid lizard *Lamphopholis delicata* and *L. Gouchenoti* were studied. The animals were kept in the laboratory, the former species at 25-30 °C and the latter at 22-24 °C, and fed with insect larvae.

Some animals were peritoneally injected with a single dose of ³H-thymidine (10-15 µC/gBW; ³H-thymidine Amersham, 29 Ci/mmole or 60-90 Ci/mmole) and tissues sampled after 4-5 hours post-injection as reported later.

In other specimens, the original tail was removed by induced autotomy, and from the tail stump a new tail was left to regenerate until about 21-25 days later, when the length of the regenerating tail was ranging from about 3 to 6 mm. At this stage the animals received a single intraperitoneal injection of ³H-thymidine as previously reported.

Regenerating tails were fixed after 4-5 hours, 2-4, 6-8, 12-14 and 20 days post-injection. The cool fixative (0-4 °C) consisted of a 2.5% solution of glutaraldehyde and 0.5% acrolein in reptile Ringer solution at pH 7.2-7.4. After about 8 hours in cold fixation the tissues were immersed in 1% OsO₄ for about 1-2 hours, dehydrated in alcohol and embedded in epon or spurr resins. After sectioning with LKB or Reichert ultramicrotomes, semithin sections containing regenerating scales were collected on clean slides. Some thin sections, to be studied with the electron microscope, were collected with a wire loop and deposited over slides previously coated with collodium. The sections were coated and prepared for electron microscopic autoradiography (Weakly, 1981).

Semithin sections were coated in a darkroom with Ilford K5 nuclear emulsion, while thin sections (for electron microscopy) were coated with Ilford L4 nuclear emulsion. Exposure time ranged between 5-7 weeks for semithin sections and 3-5 months for thin sections. After the exposure time, the slides were developed with Kodak D19 and fixed in Agfa fixer.

From the L4-coated slides, thin sections were recuperated by stripping and floating the supporting collodium membrane on distilled water. The sections were picked up with copper grids, lightly stained with uranyl acetate and lead citrate and observed with a Jeol CX100 electron microscope operating at 60-80 Kv.

The autoradiographical quantification was done by counting the labelled cells in the different regions of the apical prescaling epidermis and in the forming scales of the regenerating tail (Fig. 1). The percentage of labelled cells (%L) was calculated over 150-200 counted cells. The cells were counted only in the basal germinative layer of the epidermis (Germinative %L, Fig. 1a) or in all the layers of the epidermis (Global %L, Fig. 1b).

The %L of the distal and proximal sides of the regenerating scales, was obtained by adding the numbers of unlabelled and labelled cells of at least 3-7 consequent scales in each animal (in order to reach at least 150-200 counted cells).

Results

Four-five hours post-injection

The epidermis located by the apex of the regenerating tail, both in *Anolis* and *Lampropholis*, appeared pluristratified (7-10 cell layers) with the external layers more or less keratinized (Figs. 1, 2). The cells in the external and intermediate layers were more or less flattened and were unlabelled. Labelled and dividing cells were only present in the germinative or the basal layer of the new epidermis, as was confirmed with the electron microscope (Figs. 2-4). As reported in Fig. 1a,b, the highest %L was found in the basal layer of the epidermis behind the tip of the regenerating tail (mean of Germinative %L 42.8; mean of Global %L 11.8). The cells of the basal layer in the apical prescaling epidermis were in contact with an irregularly differentiated basement membrane and were often organized in a pseudo-stratified manner. In this layer most of mitosis also occurred. The tip of the regenerating tail (apical cup, Fig. 1a,b) showed scarce or no basal labelled cells. Lateral to the epidermal tip, in the crescent epidermis (Fig. 1a,b), labelled cells were more numerous (mean of Germinative %L 15.9; mean of Global %L 6.6). In these distal thinner epidermis (5-7 layers of epidermal cells), there was no sign of scale formation till 0.5-2 mm from the tail tip.

Under the apical epidermis, mesenchimo-fibroblasts and many small clotted or lacunar capillaries were observed. Extravasated blood cells were commonly seen in the loose connective tissue underlying the apical epidermis, 1-2 mm from the tail tip.

The formation of scales in a regenerating tail 21 days old began at about 0.5-2 mm from the tail tip, and varied according to the length of the new tail at that stage (3-6 mm) and also from animal to animal.

While the labelled cells in the apical non-scaled epidermis were located more or less uniformly along the basal germinative layer (beside the apical cup, Fig. 1), in the prescaling epidermis it was observed that labelled cells were grouped to form alternated areas with unlabelled epidermal cells (Figs. 5, 6). These labelled spots of epithelial cells foreshadowed the formation of scales. In fact, in more proximal regions of the regenerating tail, the epidermis gradually folded and sank into the dermis forming invaginations or epidermal papillae. These latter progressively deepened and lengthened in a disto-proximal direction (Fig. 1c). Most of the labelled cells were concentrated in these invaginations (mean of Germinative %L 31.0; mean of Global %L 12.8) which gave a wave-like outline to the basement membrane separating the epidermis from the

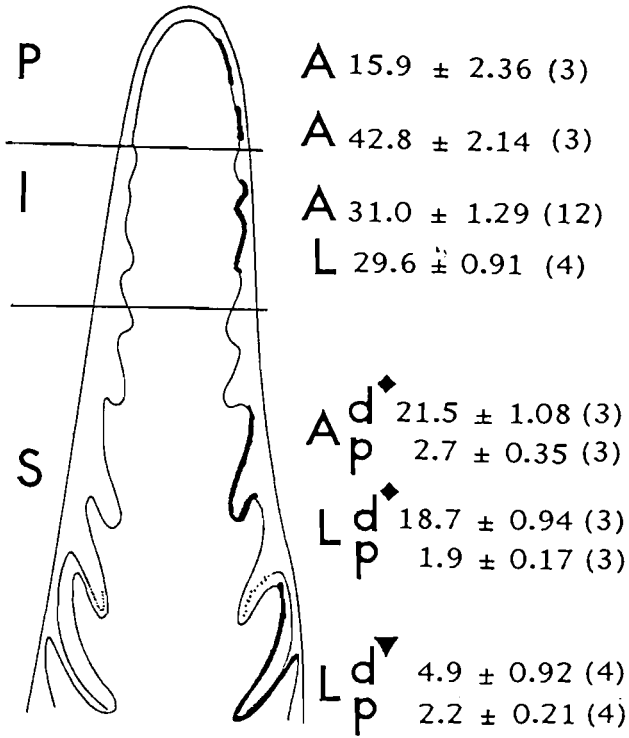


Fig.1a

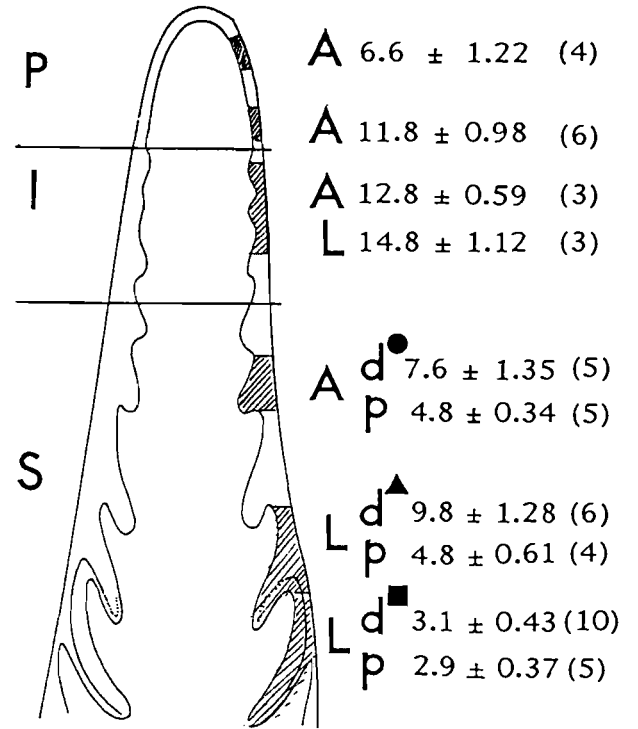


Fig.1b

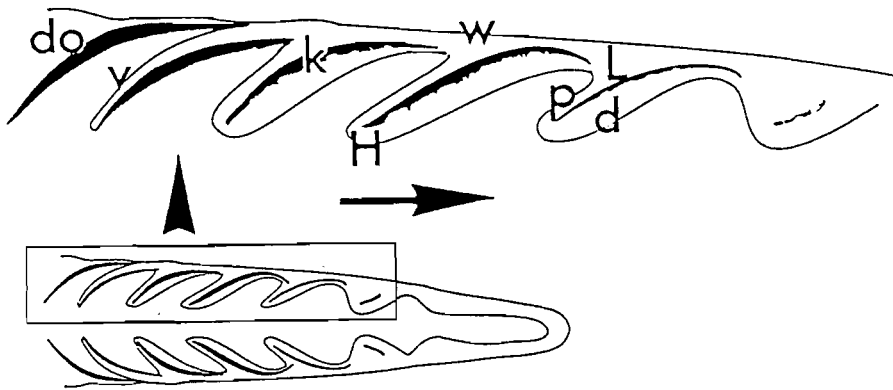
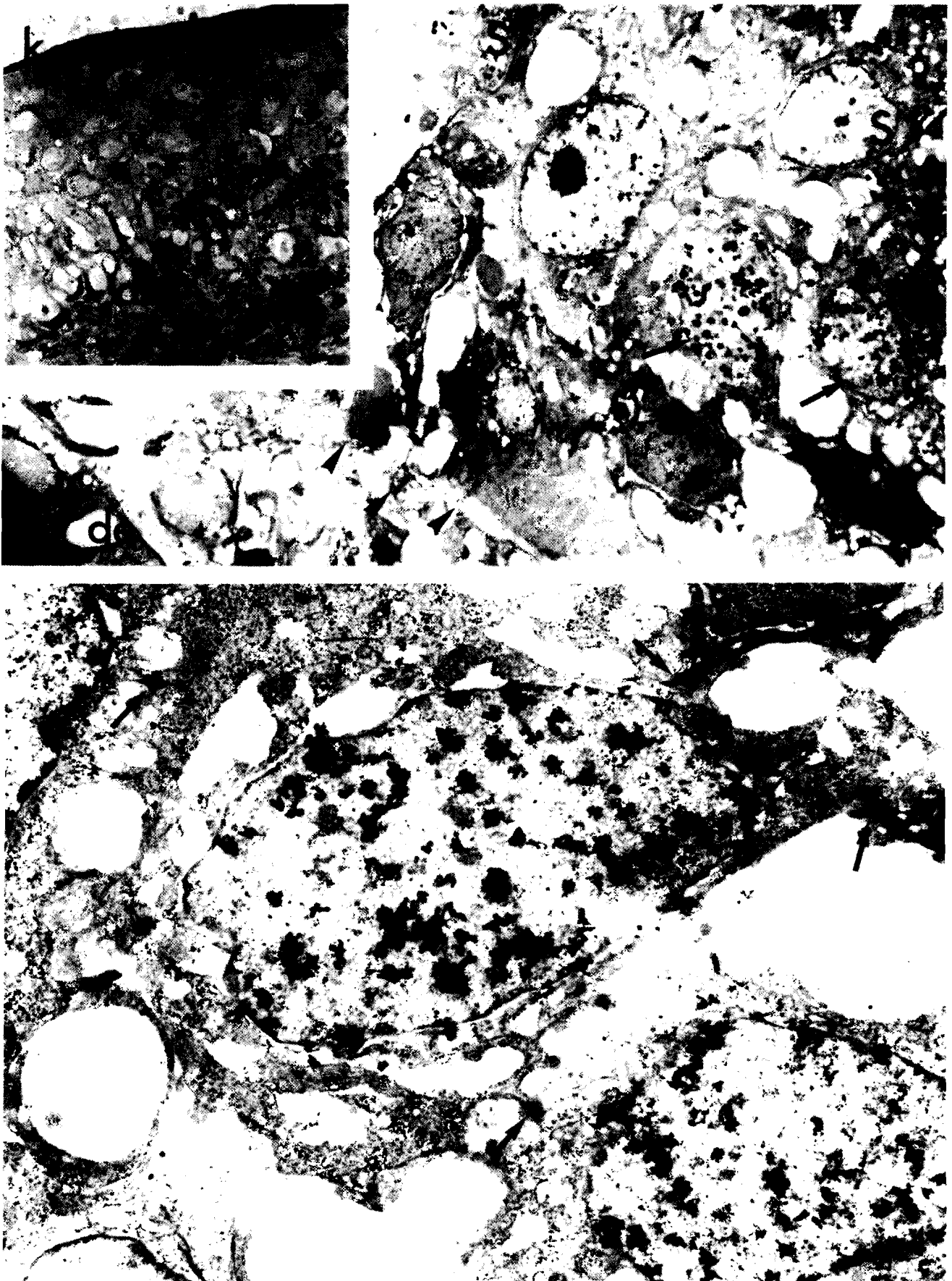


Fig.1c

Fig. 1. Schematic drawings of scale formation in the regenerating lizard tail. P, prescaling apical epidermis. I, intermediate scale anlage region. S, proximal scaling epidermis. ●, distal side made up of 15-20 cells. ▲, distal side made up of 30-40 cells. ■, distal side made up of 70-80 cells. ◆, distal side made up of 20-40 cells. ▼, distal side made up of 50-70 cells. **Fig. 1a** indicates the %L in the basal layer (black curves), (as mean \pm standard error; numbers in parentheses indicate the number of samples). L, *Lampropholis*. A, *Anolis*. **Fig. 1b** indicates the %L referring to the whole epidermis layers (striped regions). **Fig. 1c** shows the general sequence of scale morphogenesis from the tail tip backward (rectangle). Legends: w, wound. L, lacunar tissue. p, proximal side. d, distal side. H, hinge region. do, dorsal side. v, ventral side. k, keratinized layer. The arrow points to the tail tip.

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Fig. 2. *Lampropholis*. Pluristratified epidermis (e) of the apical regenerating tail. In the basal layer two labelled cells are seen (arrows). de, dermis; K, outer lightly keratinized layer. x 700

Fig. 3. *Anolis*. Electron microscopic view of two labelled cells (arrows) near the basal layer of the apical epidermis. Arrowheads on the incomplete basement membrane. S, cells of the stratum spinosum; de, dermal fibroblast. x 3,100

Fig. 4. *Anolis*. Close-up of labelled vacuolated epidermal cells, showing desmosomes (arrows). A few tonofilaments are seen in the cytoplasm. x 11,500

underlying dermis (Figs. 1, 6-8).

Mitoses were observed with relatively high frequency in these epidermal papillae and many dermal fibroblasts were oriented more or less perpendicularly to the irregular basement membrane (Figs. 5, 9-11). Also, these fibroblasts often appeared more concentrated under the invaginated epidermal papillae than in the interpapillar regions where blood vessels were numerous (Fig. 10).

The process of invagination and deepening of the epidermal papillae continued in a progressive fashion in a disto-proximal direction in the following days (Fig. 8). From the bottom part of the invagination (forming a hinge region), two columns (or sides) of epithelial cells reached the epithelial surface. The distal side was oriented by the tail tip while the proximal side was oriented by the animal body (Fig. 1c).

When the two sides were made up by 10-15 cells they had a similar number of labelled cells and dividing cells (Figs. 7, 8, 10), but with more cells, the labelled and dividing cells were more frequent in the distal than in the proximal side.

Later the invagination appeared to progressively lose the initial symmetry and tilted backward (Figs. 12, 13). Also, in this 10-20-columnar cell stage, the cells of the distal side appeared cuboidal or polygonal and larger than the smaller flat cells of the proximal side; this contrast appeared particularly evident in the bottom part of the invagination (the presumptive hinge region of the forming scale, Figs. 14, 15).

During the following stages of deepening of the epidermal invaginations, the proximal side became flatter and flatter, the cells progressively stretched and

few of them appeared labelled.

On the contrary, the distal side of the developing scale kept for most of the stages of development a higher %L and number of dividing cells than the proximal side; as a result the distal side lengthened more than the proximal side and the epidermal impocketings tilted further backward. The Germinative %L ranged between 7.0 and 23.5, depending on the degree of scale development (%L was higher in younger scales during rapid scale lengthening than in later stages, see representative values in Fig. 1a,b). Labelled cells were often seen close to the hinge region and in the apical region of the forming scale.

The distal side showed the complete sequence of differentiating layers of the normal epidermis during the shedding cycle (Maderson, 1985; Landmann, 1986), with a hypertrophic germinative layer, and intermediate and outer keratinized α and β layers (Fig. 15).

In the distal side of the forming scale the number of keratinizing strata was higher moving from the hinge region up toward the scale tip. The germinative layer contained mostly clear, and some darker cells of thinner diameter.

In the proximal side, clear flat cells were seen in the stratified narrow layers over the basal layer. These paler stretched cells were in contact with the more stratified layers produced by the distal side (Figs. 14, 15).

One or more blood vessels, clothed or of sinusoidal aspect, were always seen on both sides of the scale. Furthermore, melanocytes were essentially localized under the epidermis of the distal side of the developing scale and were rarely seen under the proximal side

Fig. 5. *Anolis*. Group of labelled cells (arrows) in the pre-scaling epidermis. Arrowheads point to areas without labelled cells. de, dermis with fusiform fibroblasts; m, regenerating muscles. x 320

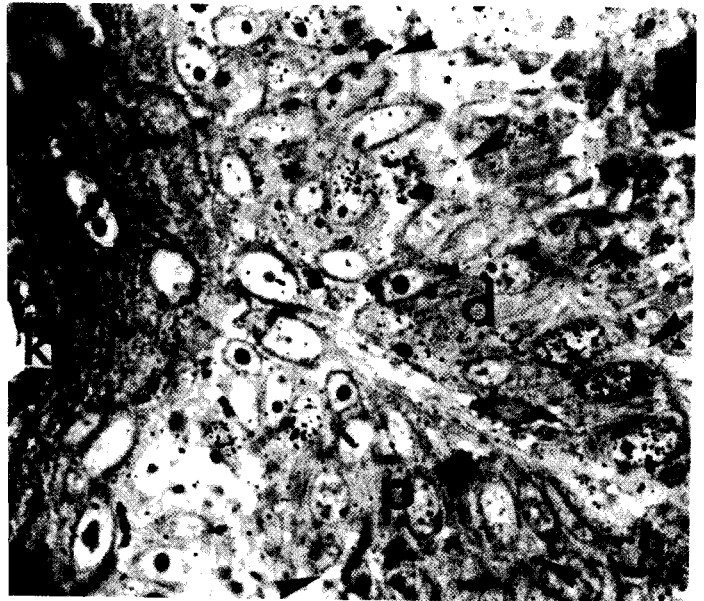
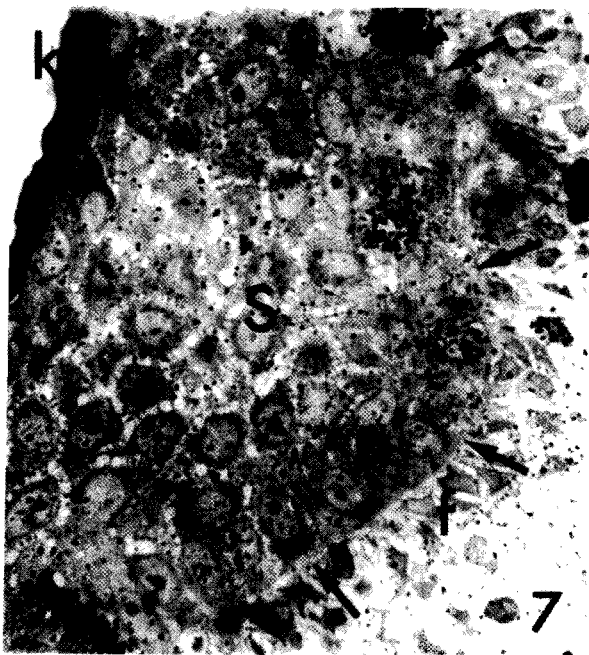
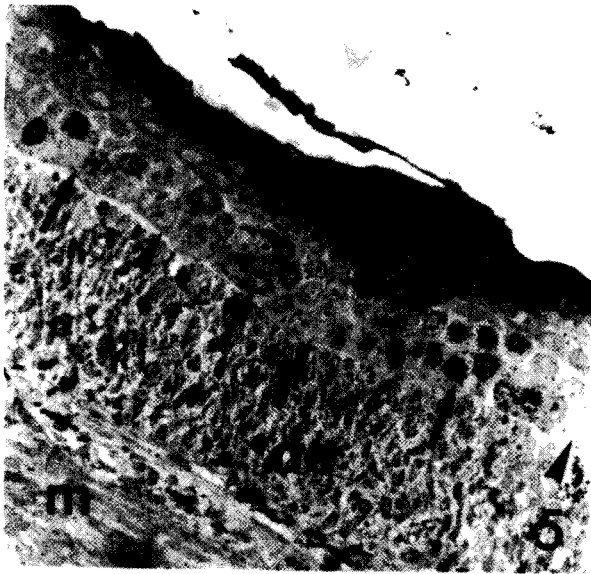
Fig. 6. *Anolis*. Cross-sectioned apical prescaling epidermis. Labelled cells (arrows) tend to concentrate in spots along the epidermis. Arrowheads point to non-labelled areas. v, cross sectioned dermal blood plexus. x 320

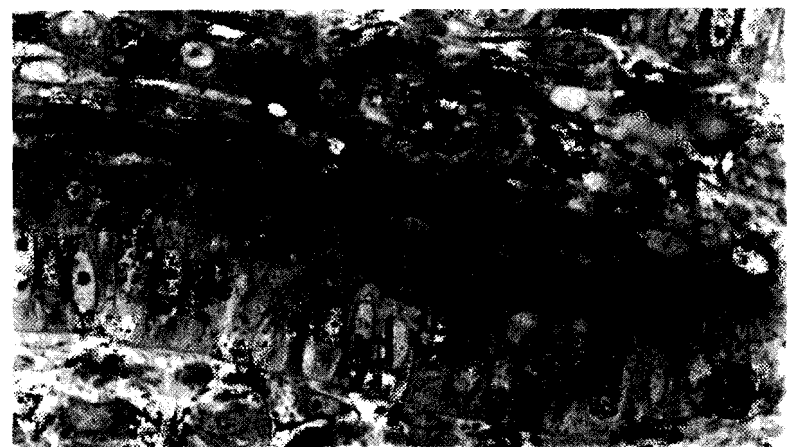
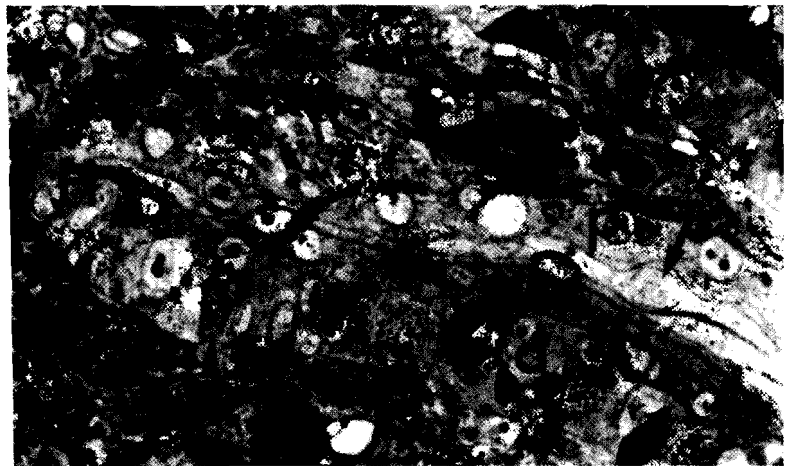
Fig. 7. *Anolis*. Beginning of invaginating epidermis (arrows) where some labelled cells are visible. Note the row of fibroblasts (f) just below the basal layer. S, spinosus cells; K, keratinized thin layer. x 850

Fig. 8. *Lampropholis*. Epidermal invagination showing labelled cells in both proximal (p) and distal (d) sides. Arrowheads outline the basement membrane; K, superficial keratinizing wound layer. x 800

Fig. 9. *Lampropholis*. Basal layer of apical non scaled epidermis showing mitotic cells (arrows). Two weakly-labelled cells are also seen (arrowheads). S, spinosus layers. x 1,100

Fig. 10. *Lampropholis*. Two mitotic cells (arrows) in the proximal side (p) and in the distal side (d) of a deepening epidermal papilla. The two sides are roughly similar at this stage. The arrowhead points to a blood capillary. x 800





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Fig. 11. *Lamphropolis*. Electron microscopic view of mitotic cell in the distal side of a forming scale. A few tonofibrils are seen in the cytoplasm of basal epidermis. Arrowheads point to the basement membrane. x 4,900

Fig. 12. *Anolis*. Two deepening epidermal papillae in the scaling region (the distal direction is upperward). Labelled cells (arrowheads) are essentially localized in the distal side (d). In the shorter proximal side (p) no labelled cells are seen. de, dermis; arrows point to a blood vessel. x 320

Fig. 13. *Lamphropolis*. Sequence of advanced proximo-distal scale formation by epidermal papillae (e) downgrowth migration into the dermis (de). K, keratinized wound epidermis. H, forming hinge region. P, proximal side. d, distal side. x 300

Fig. 14. *Lamphropolis*. Hinge region (H) of deepening scale at stage 2-3. Note most labelled cells (arrowheads) in the distal side (d) and only one in the proximal side (p). From the hinge region rightward the number of flat cell layers increases. On the proximal side flat pale cells are often seen (arrows). B, interscale blood vessel. x 800

Fig. 15. *Lamphropolis*. Hinge region (H) of more developed scale at stage 3-4. Most of the labelled cells are in the distal side (d). o, possible Oberhautchen layer, β , pre- β layer; arrows on pale flat cells from the proximal side (p). x 830.

(Fig. 16).

Two-four days post-injection

Both in non-scaled apical epidermis as well as in the scaling epidermis, labelled cells were found in the basal and in the intermediate (spinosus) layers of the epidermis above (Figs. 16-20).

Due to cell division and upward migration, the intensity of the labelled nuclei was diminished in the basal layer with respect to 4-5 hours post-injection. Labelled cells of the spinosus layers were often more labelled than the basal cells.

However, no labelled cells were seen in the forming flat keratinizing layer (presumptive or mature- β layer) of the regenerating scales. This was seen both in the distal and proximal sides of the forming scale at any stage of their development, in early as well as in the more developed scales (Fig. 21).

Also, in the apical epidermis, near the regenerating tail tip, the basal cells appeared both in the basal and spinosus layer (Fig. 19).

In the initial epidermis invaginations (forming papillae), the labelling was also present in the spinosus

layer of the epidermal papillae.

Six-eight days post-injection

Labelled cells were commonly seen both in the apical epidermis and in the forming scales. Rare labelled cells were seen in the germinative layer. Though some labelled cells were still located in the layer just above the germinative layer (close to the hinge region which represents the youngest part of the forming scale), most of the labelled cells were seen in the intermediate and upper pre-keratinized (pre- β) layers.

In the apical epidermis, where no scales were yet seen, labelled cells were often seen to contain less trace granules than in earlier stages. These cells were located in the intermediate layers, 3-4 cell layers above the germinative layer, and even in the upper flat pre-keratinized cell layer (Figs. 22-25).

Labelled cells were also seen in the pre-keratinizing layers of the developing distal side of the new scale (Fig. 24, pre- β , located 3-4 layers above the germinative, during stage 4 according to Maderson, 1985). Labelled cells were less frequently seen in the thin layer just over the germinative and in pre-keratinizing layers by the tip

Fig. 16. *Anolis*. Forming scale two days post-injection. Stratified labelled cells (arrows) are seen throughout the distal side (d). No labelled cells are in the flat pre- β layer (β) nor in the proximal side (p) of the scale. Note the melanophores (arrows) mostly under the distal side of the scale. B, blood vessel. S, spinosus lacunar layer of the outer interscaling epidermis. x 400

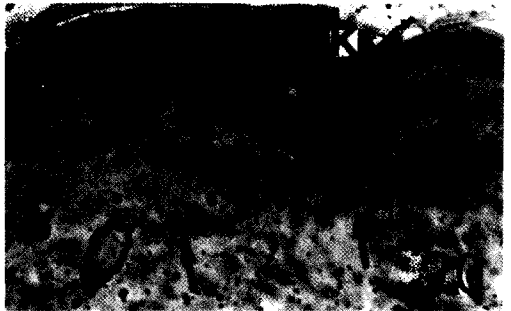
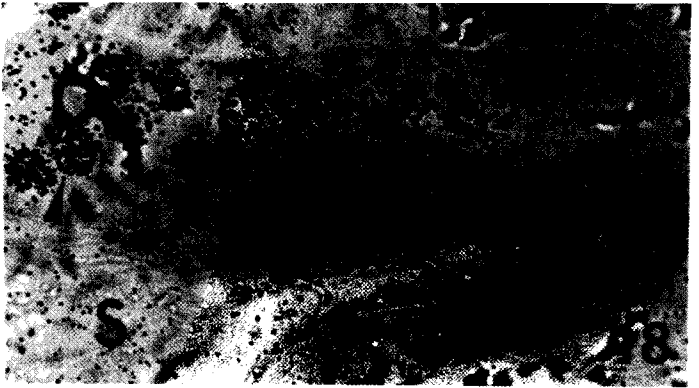
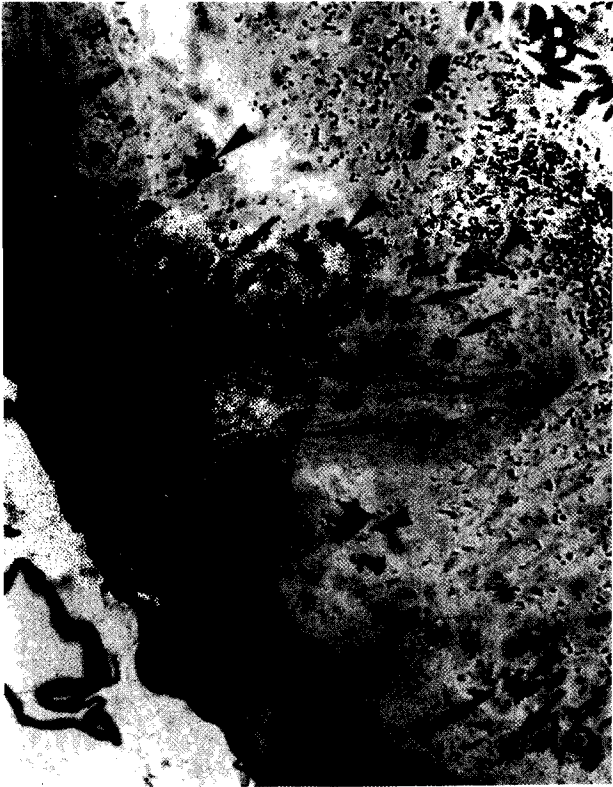
Fig. 17. *Anolis*. Prescaling apical epidermis two days post-injection. Five strongly labelled cells (arrowheads) are located in the intermediate (spinosus) layer, just above the germinative layer, this latter still showing fewer labelled cells (arrows). K, keratinized «wound» epidermis. de, dermis. x 800

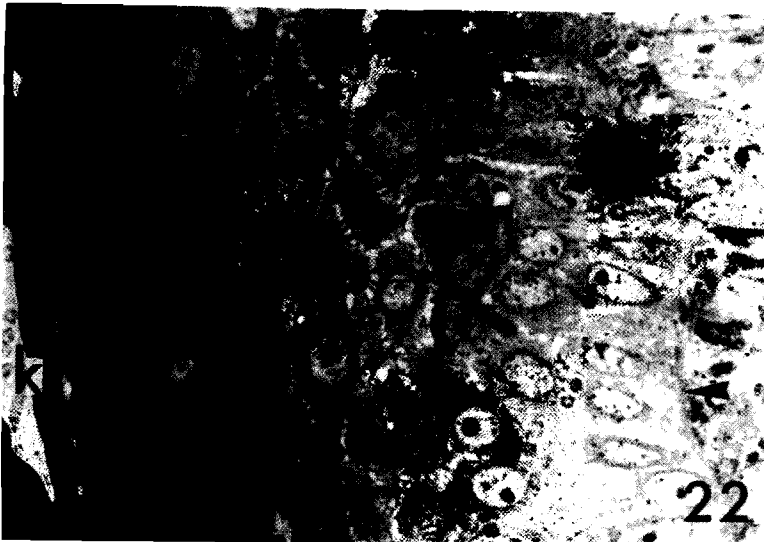
Fig. 18. *Anolis*. High concentration of labelled cells throughout the living part of the distal side (d) in a forming scale two days post-injection. Most labelled cells are in the intermediate layer (arrowheads) more than in the basal layer (ba). Only one labelled cell is seen in the proximal side (p) and no labelled cells are in pre- β and the putative Oberhautchen (o) layer. S, spinosus lacunar cells of the outer interscaling epidermis. x 800

Fig. 19. *Anolis*. Electron microscopic view of a labelled cell (arrowheads) in the intermediate spinosus layer two days post-injection. Stretched intercellular spaces (i) among epidermal cells are joined by desmosomes (arrows). t, tonofilaments; M, melanosome arrms. x 13,400

Fig. 20. *Anolis*. Labelled cells (arrowheads) in the spinosus layer of pre-scaling epidermis after four days post-injection. No labelled cells are in the uppermost keratinized layer (K). Arrows point to the basal epidermal layer. x 760

Fig. 21. *Lamphropolis*. Proximo-distal sequence (upward) of the splitting lines (arrowheads) from the hinge region (H) of forming scales into new scales. The distal side (d) of the epidermal papillae becomes the dorsal outer part of the new scale (s). The proximal side (p) forms the ventral inner part of the scale (v). x 200





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Fig. 22. *Lampropholis*. Prescaling apical epidermis six days post-injection (the distal direction is downward). Two labelled cells (arrows) are in the spinosus layer, 4-5 cell layers above the basal layer (arrowheads). m, epidermal melanocyte. K, upper keratinized wound epidermis. x 830

Fig. 23. *Lampropholis*. Prescaling wound epidermis at six days post-injection (the distal direction is leftwards). Two stretched labelled cells (arrows) are seen under the keratinized layer, at 6-7 cell layers from the basal stratum. S, spinosus cells; m, intra-epidermal melanocyte. x 850

Fig. 24. *Lampropholis*. Hinge region (H), with epidermis of the distal side (d) at stage 4, six days post-injection. Labelled cells (arrows) are observed in the forming pre- β -layer (β) and in the underlying differentiating layer. In the proximal side (p) a labelled cell (arrow) is seen in contact with the pale flat cells. Arrowhead points to an interscale capillary. x 850

Fig. 25. *Lampropholis*. Hinge region (H) with well keratinized (K) epidermis at stage 5-6, six days post-injection. In the distal side (d) labelled cells (arrows) are visible under the cornified layer and in the underlying differentiating (pre- α) layer. Two labelled cells (arrows) are also seen in the flat proximal side (p). Arrowheads on melanocytes under the basal layer. x 850

Fig. 26. *Lampropholis*. Area by the tip of a well keratinized (k) scale, six days post-injection. In the distal (dorsal) side of the scale (d) labelled cells (arrows) are located in the differentiating α -layer. In the proximal side (p), near the scale tip, two flat labelled cells (arrows) are incorporated into the keratinized layer. The arrowhead points to the tip of the next scale. x 800

of the new scale (Fig. 26). No labelled cells were seen in the germinative layer at this stage and only occasional labelled cells were located in the maturing layer just above the germinative layer or in the scale tip. Also, the flat proximal side of the forming scale contained some labelled cells (Figs. 24-27).

After 6-8 days, labelled flat cells in the proximal side were seen in the basal and upper narrow layers facing the β -cells of the distal side (Fig. 28). The latter labelled cells sometimes showed a diminished number of trace granules.

Twelve-fourteen and twenty days post-injection

Occasional labelled cells were seen after this period both in the apical non-scaled epidermis of the regenerating tail as well as in more proximal regions where more or less differentiated scales were present.

In general, the labelling was quite diluted over the nuclei and labelled cells were observed essentially in the apical prekeratinized layers of the outer shedding layer that covered the forming scales; the so called «wound epithelium and lacunar layer» (Maderson et al., 1978; Figs. 1, 29, 30).

Rare labelled cells appeared near the keratinized layers, at the tip of the new scales, whilst rare labelled cells were seen in some layers of the deeper intermediate

and hinge regions.

After twenty days post-injection, there was almost no trace of radioactivity in the skin.

Later, up to 50 days of tail regeneration in the present study, regenerated scales continued to lengthen in their dorsal outer side, so that they became less and less overlapped (Figs. 21, 31), finally resulting in the hinge region and a short portion of the scale being covered by the proximal new scales.

Discussion

Cell multiplication and morphogenesis of regenerating scales

The present autoradiographical analysis throws new light on the mechanism of scale morphogenesis in the regenerating tail of lizards (see Figs. 1, 32a). In fact, both in *Anolis* and in *Lampropholis*, the downgrowth of epidermal papillae into the dermis seems initially to derive from a more or less localized cell multiplication in alternated spots along the regenerating apical epidermis.

The high Germinative %L in these areas with respect to the epidermis in the tail tip (apical cup, see Fig. 1) suggests that the epidermis grows faster in these regions and that a folding process may take place. These results

Fig. 27. *Lampropholis*. Medial part of regenerating scale at stage 6. After 8 days post-injection, labelled cells are visible in the forming α -layer (α) under the thick β -layer. Note the hypertrophy of the pale basal layer of the distal side (d) and the underlying melanocytes. The arrow points to a labelled cell in the proximal side. x 900

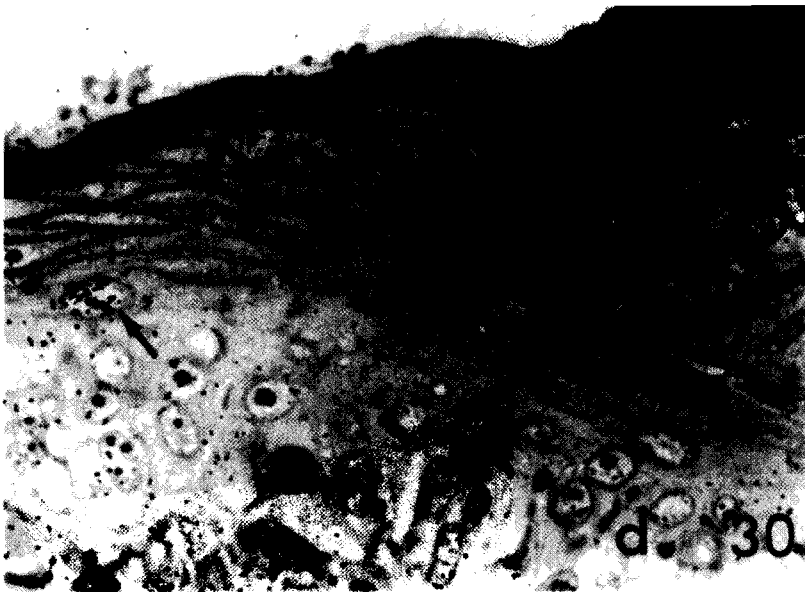
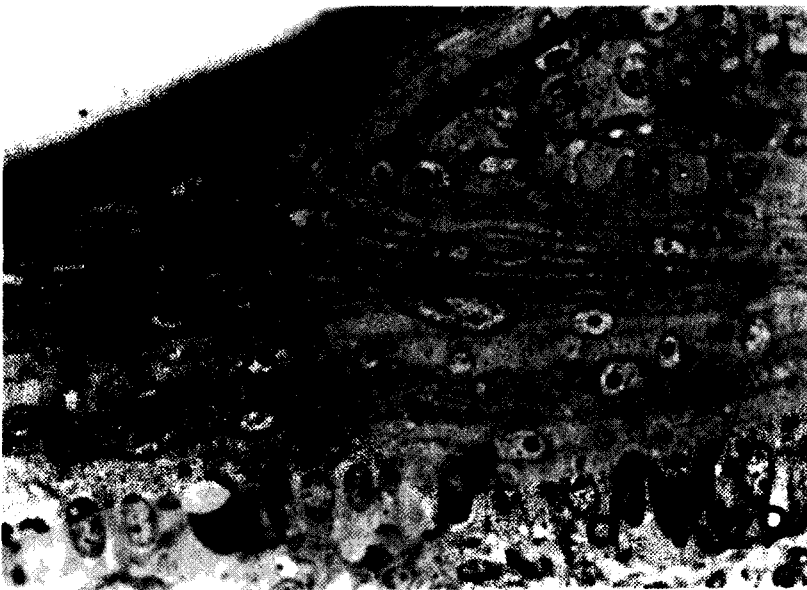
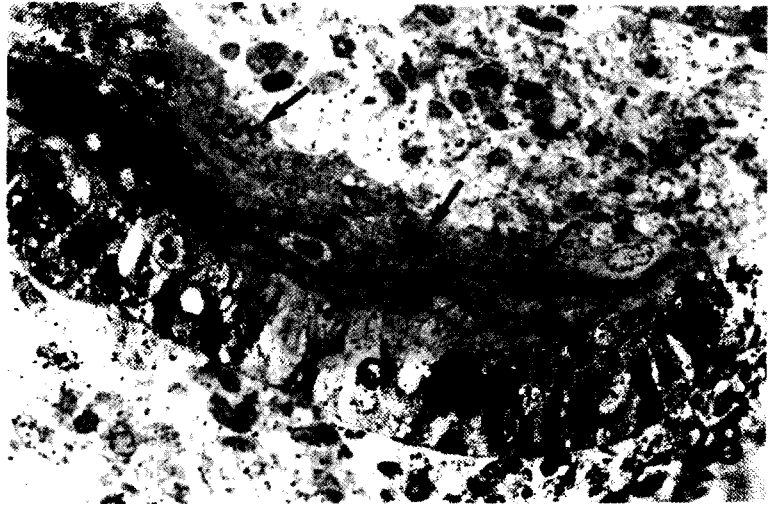
Fig. 28. *Lampropholis*. Hinge region with a distal side at stage 2-3. In this section after 8 days post-injection, three labelled cells are visible in flat layers of the proximal side and one (arrowhead) has reached the forming β -layer of the distal side (d). x 800

Fig. 29. *Lampropholis*. Apical interscale region (p indicates the forming proximal side of a scale, d points to the distal side of the other scale). After 12 days post-injection, weakly-labelled cells are visible in the «lacunar tissue» (S) under the external «wound epidermis». x 830

Fig. 30. *Lampropholis*. Another interscale region showing four labelled cells (arrows) in the spinosus «lacunar tissue» (S), 12 days post-injection. d points to the distal side of a scale. x 830

Fig. 31. *Lampropholis*. General view of a new scale in the proximal region of a regenerating tail after 50 days of regeneration. The superficial well-keratinized dorsal side (s) of the scale has lengthened much more than the ventral side (v). Arrowheads points to the shedding «wound epithelium». H, hinge regions. de, dermis. m, muscles. x 170

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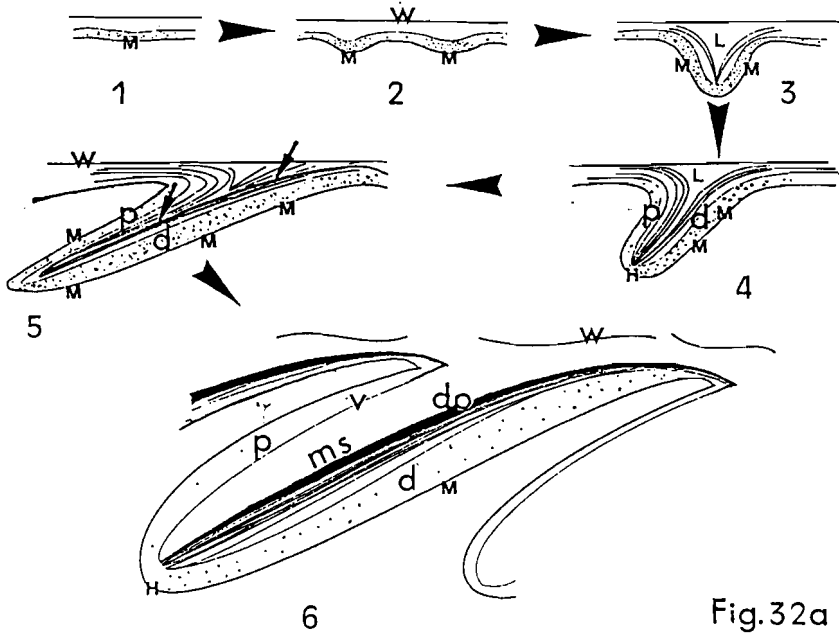


Fig.32a

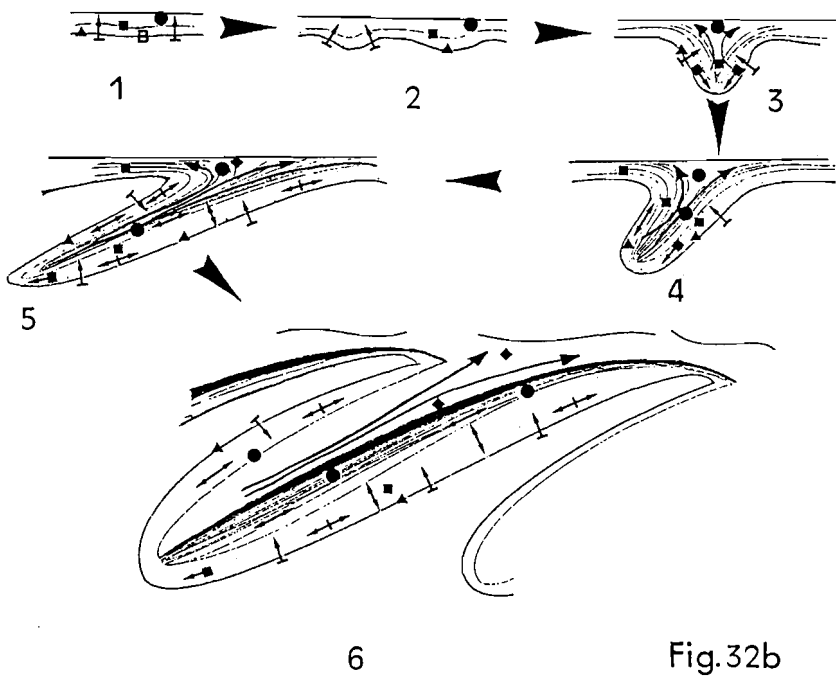


Fig.32b

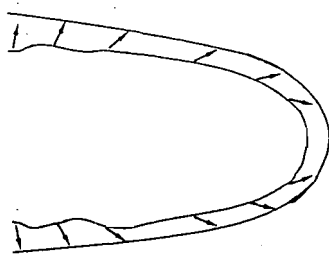


Fig.32c

Fig. 32. Schematic drawing of cell proliferation (a) and movement (b) during scale morphogenesis and at the apex of the regenerating tail (c). Fig. 32a features 6 arbitrary stages of scale morphogenesis and dots represent areas where labelled cells (and mitosis, M) are more frequent. Arrows point to the forming β -layer where the medial splitting will take place. In Fig. 32b the long curved arrows indicate the direction of cell migration while the symbols (\blacktriangle , \blacksquare , \bullet , \blacklozenge) refer to the periods to reach that level. In Fig. 32c the movements (arrows) of epidermal cells in the apical region of the regenerating tail are indicated. Legends: B, basal layer. d, distal side. p, proximal side. do, dorsal side. v, ventral side. H, hinge region. W, wound epidermis. L, lacunar tissue. ms, medial splitting. \blacktriangle , 4-5 hours. \blacksquare , 2-4 days. \bullet , 6-8 days. \blacklozenge , 12-14 days.

- \longleftrightarrow : intercalar cell division.
- \uparrow : upward cell division.
- \downarrow : downward cell division.
- \updownarrow : cell hypertrophy.
- \longleftrightarrow : cell flattening.

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accord well with those of Cox (1969), who found a Labelling Index of 5-15 in the epidermis of the regenerating tail in *Anolis*.

The morphogenetic mechanism that determines the downgrowth of the regenerating epidermis appears to be different from that of the normal development of scales, where an epidermal outgrowth is produced (Maderon, 1965; Bryant and Bellairs, 1967; Dhouailly and Maderon, 1984).

In their study Liù and Maneelly (1969) hypothesized that the soft dermis of the regenerating tail was a more permissive environment to epidermal invagination than the outer harder cornified wound epithelium (which is strengthened by cell junctions).

The specific localization of dermal melanocytes essentially under the distal side of the forming scale, gives some indications that the dermis may play a role in this process, but further study is required.

After the initial invagination of the linear epidermal surface of the apical epidermis, the distal side of the invaginations keeps most of the dividing cells, whilst in the proximal side cell proliferation decreases.

Though the mechanism that is responsible for the latter phenomenon is unknown, this results in some morphogenetic effects which are completely different from those in hair development (see Bryant and Bellairs, 1967).

First, the distal side will lengthen much more than the proximal side because of the intercalary cell multiplication. As a result of this process, the distal side keeps growing for a long time and will extend itself in order to form the future dorsal outer side of the regenerated scale. This is shown by the greater %L still present in the distal side of proximal scales in more advanced stages of development (Fig. 1a,b).

Secondly, the asymmetrical growth of the two sides will result in a backward tilting movement of the axes of the forming scale, so that the orientation will set the hinge region more proximal than the growing tip of the new scale (Figs 1, 13, 21, 32). Therefore the new scales will be initially superimposed and later, after the splitting in the middle of the scales, the continuous growth of the distal dorsal part will extend the new scale (Figs. 21, 31).

Thirdly, the cells in the proximal side, where a lower cell multiplication occurs, become flatter and probably stretch each other in order to keep pace with the rapid distension of the opposite distal side.

The proximal side (future inner-ventral side of the new scale) will result as a flat layer of pale stretched cells, as is typical for this region in snakes and lizards where a simplified cell stratification occurs (Roth and Jones, 1967). Though the ventral side of the scale shows a basal layer, this latter will generate layers of flat cells.

Conversely, the distal side appears thicker and the basal layer is typically made up of polygonal, often hypertrophied cells. Their multiplication produces the characteristic cell stratification of the normal epidermis that follows the typical sequence of the shedding cycle

(Landmann, 1979; Maderon, 1985).

During the intercalary lengthening of the dorsal side of the regenerating scale, a concomitant upward epidermal stratification occurs. This process leads to the formation of a β , (probably of a meso) and α -layers, lacunar and clear layers toward the axial region of the forming scale. The differentiation of an outermost Oberhautchen layer in the middle of the regenerating scale is a problem that will be solved only by means of electron microscopy.

These differentiating layers have been observed in the distal side, from the hinge region to the tip of the new scale. Conversely, this typical sequence is not seen over the flat cells of the basal layer of the proximal side over which other flat and long pale cells are stratified. The nature of these cells is not known at the moment but further studies with the electron microscope should clarify this problem.

Timing and epithelial cell migration

During epidermal and scale regeneration the following general consideration on the timing and direction of migrating epithelial cells are drawn from the present study (Fig. 32b,c).

By the tip of the regenerating tail, the proliferating epidermis mostly supplies cells that move distally and upwards, as represented in Fig. 32c. This has been suggested by the stretched appearance of normal or labelled cells in the prekeratinized layers, which are oriented in an oblique fashion after 6 days post-injection (Figs. 22, 23).

In more proximal regions (0.5 mm from the tail tip), the epidermal cells do not contribute so much to tip lengthening, and cell multiplication is mostly directed upwards to the epidermal surface (Fig. 32c). The intense cell multiplication in these areas determines the downgrowth movement leading to the formation of new scales.

In the distal and proximal sides of scales, cell multiplication in the basal layer is intercalary and some daughter cells remain in this layer whilst a few others migrate upwards to the medial axis of the forming scale and downwards, thus lowering the hinge region.

In fact, lower labelled cells in the basal layer were seen 2-4 and even 6 days post-injection.

In the proximal side, the cells directed toward the medial axis of the forming scale produce, in 6-8 days, a thin cornifying layer that faces the thicker keratinized layer of the opposite distal side.

In the distal side after 2-4-6-8-12-14 days post-injection, labelled cells appear localized according to the stage of the shedding cycle of the regenerating scale in which the ^3H -thymidine was injected.

As previously reported, in normal skin of the lizard *Anolis* (Flaxman, 1972; Flaxman and Maderon, 1973) and the snake *Constrictor* (Downing and Roth, 1974) after 1 and 24 hours from the administration of ^3H -thymidine, labelled cells were observed only in the basal layer, despite the stage of the shedding cycle in which

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the injection was done. This has been confirmed in the present study after 4-5 hours post injection.

Both in lizards and snakes, cells differentiate following the order of formation in the shedding cycle after the injection of ^3H -thymidine.

In normal epidermis, the migration of the labelled cells from the basal layer to the cornified upper layers does not take place before 3-5 days and the labelling index of the basal layer increases from less than 10% to 40-50% during this period (Flaxman, 1972; Flaxman and Maderson, 1973).

When the injection of ^3H -thymidine is done at the early-mid resting stage (stage 1 according to Maderson, 1985; Landmann, 1986) only the Oberhautchen, β and mesos layers of the outer epidermal generation are completed, but not the α -layers. Therefore, 3-4-5 days later labelled cells appeared in the forming α , clear and lacunar stages of the outer epidermal generation. These were the first labelled cells that appeared.

When the injection was delayed to the mid-late resting stage, labelled cells appeared in the clear layer of the outer epidermal generation and in the Oberhautchen and β layers of the inner epidermal generation.

Labelled cells in the mesos and α cells of the inner epidermal generation appeared when ^3H -thymidine was administered during the renewal phase (Flaxman, 1972).

In the present study a single pulse of ^3H -thymidine to an animal with scales in a proximo-distal gradient of development (Fig. 1a-c), would label cells of the distal side in different intrinsic situation of the shedding cycle. However, cell behaviour and kinetics should resemble renewal in normal epidermis.

Furthermore, over the first outer generation of a forming scale a provisional wound and lacunar epidermis are also present (Maderson and Roth, 1972), which constitute the shiny, soft, and unscaled epidermis of the young regenerating tail (Shah and Chakko, 1967; Maderson et al., 1978).

The present observations have shown that in the apical non-scaled epidermis and in the distal side of the forming scales (before the first shed of the wound and lacunar epidermis), epidermal cells have already moved from the basal layer into the living layer above in 2 days.

The labelled cells that still remain in the basal layer often show a reduction of radioactivity, indicating that part of the tracer has been incorporated in daughter cells that have moved one-two layers upwards.

After 4 days, labelled cells reached 3-4 cell layers above the germinative (spinous cells) and after 6-8 days post-injection they reached the cornifying outer layers, both in the apical, prescaling and scaling epidermis.

After 6-8 days post-injection or later, labelled cells tended to disappear in the apical epidermis of the tail tip.

In the prescaling and scaling epidermis after 6-8 days post-injection, labelled cells appeared as pre- β cells, pre-mesos or pre- α cells, according to the stage of scale differentiation present at the moment of the injection of the tracer.

Owing to the proximo-distal gradient of scale

morphogenesis, at the moment of ^3H -thymidine injection, the distal side of the regenerating scales were at stages 2-4, and some even during stage 5 of the shedding cycle (Bryant and Bellairs, 1967; Maderson, 1985).

Despite this, labelled cells reached the differentiating cornified layers (β or α) after 6-8 days while a few labelled cells were still observed in the basal layer, generally with a reduced number of trace granules with respect to 4-5 hours post-injection.

In the more proximal scales (stages 5-6) when the β layer was forming during the period of the injection, labelled cells appeared only in the underlying α forming layers (maybe also the mesos layer).

After 12-14 days post-injection rare labelled cells were still seen in the basal and intermediate differentiating layers (pre- β and pre- α), while weakly labelled flat cells were limited to the interscale lacunar and outermost wound layers before shedding. This suggests that these superficial cells have moved toward the surface from the deeper regions of the forming scale after being pushed out by the progressive formation of the underlying keratinized layers (Fig. 32b).

The low number of trace granules seen in these cells is probably due to the labelling dilution by some multiplication during the upward movement from the lower epidermal layers.

In the non-scaled epidermis some cell divisions were observed among spinous cells, up to three cell layers above the germinative stratum.

After 20 days post-injection the lack of radioactivity in more differentiated (proximal) scales was due to the disruption of the nuclei in the cells that reached the cornified layers and to the first shedding of the regenerating epidermis in the proximal regions of the regenerating tail, after 25-30 days of tail regeneration.

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