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Scale morphogenesis during embryonic development in the lizard *Anolis lineatopus*

LORENZO ALIBARDI

Dipartimento di Biologia Evoluzionistica e Sperimentale, University of Bologna, Italy

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ABSTRACT

The development of scales was analysed in embryos of the Jamaican Iguanid lizard *Anolis lineatopus* after the injection of cell proliferation markers [^{3}H]thymidine and 5-bromodeoxyuridine. Embryos were fixed at successive postinjection periods, from 2 h up to 13 d and sections of developing skin were studied by autoradiography and immunocytochemistry. The epidermis during the initial stages of morphogenesis (flat epidermis and symmetric scale stages) expands chiefly by the tangential proliferation of the basal layer. The superficial periderm took part in its own laminar expansion. The rates of proliferation in the epidermis and dermis were similar during the flat epidermal stage, but dermal proliferation decreased under the wave-like epidermis of symmetric scales. No specific localisation of proliferating cells was visible either in the epidermis and or the dermis at these 2 early stages. Scale asymmetry is brought about by cell multiplication and hypertrophy of the cells of the future outer surface of the scale that behaves as an epithelial placode capable of producing β-keratinised cells. The growth of the outer surface determines the asymmetry of the scale. Conversely, the future inner side and hinge region showed a lower degree of cell proliferation and cells remained cuboidal or became flat. Also the proliferation of the dermis under the scaling epidermis was significantly diminished. During the asymmetric scale stage, labelled cells were more common in the hypertrophied basal epithelium from 2 h until 4 d postinjection, during which time a few cells moved into the upper keratinizing layers. At 6–13 d postinjection, labelled cells were commonly seen in the upper β-keratinising layers.

Key words: Reptiles; cell proliferation; embryo; epidermis; dermis.

INTRODUCTION

One of the specific characteristics of reptiles is the presence of a scaly epidermis covering the body which, in different orders, can be reinforced by dermal ossification (Maderson, 1985; Landmann, 1986). Scales in reptiles are composed of several types of epidermal proteins called α and $\beta(\phi)$ keratins (Landmann, 1986; Wyld & Brush, 1979, 1983).

The formation of scales in squamate reptiles (lizards and snakes) during development begins as an undulation of the epidermal surface which produces symmetric dermoepidermal elevations, termed epidermal papillae (Maderson, 1965, 1985; Liu & Maneely, 1969; Dhouailly, 1975). At this stage, the epidermis consists of at least 2 cell layers, a superficial flat periderm and a basal cuboidal germinal layer. During development, new flat layers are produced from the germinal layer of the developing outer surface of the scales and accumulate under the peridermis, forming the first 'epidermal generation', which is shed only after birth (Dhouailly & Maderson, 1984).

Despite the biological significance of reptilian scales as one of the class characteristics, previous studies on the histology and ultrastructure of the skin (Maderson, 1965; Dohuailly & Maderson, 1984) have only partially given a dynamic interpretation of the morphogenesis of scales. In particular, there is still no information on the pattern of cell proliferation and migration responsible for shaping an initially flat epidermis into overlapping scales. Early studies (cited in Maderson, 1985), based on the observation of mitotic divisions in the skin, did not provide definitive

Correspondence to Dr L. Alibardi, Istituto di Anatomia Comparata, Dipartimento di Biologia Evoluzionistica e Sperimentale, via Selmi 3, University of Bologna, 40126 Bologna, Italy.

information. More specific and quantifiable methods are required. To this purpose the present study employed the cell proliferation markers [^{3}H]thymidine (^{3}H-TdR) and 5-bromodeoxyuridine (5 BrdU). The latter also served as a comparison for the labelling pattern obtained with the classical radioactive tracer ^{3}H-TdR.

MATERIALS AND METHODS

Eggs of the common Jamaican lizard *Anolis lineatopus* were collected around Mona campus, University of the West Indies, Kingston, Jamaica, and brought to the United States. The eggs were incubated at 24–28 °C in humidified soil or in synthetic litter-like material (vermiculite) in glass containers covered by aluminium foil or cellophane (where the relative humidity was 70–90 %).

The collected eggs weighed between 0.250 and 0.450 g each and embryos examined were between the generalised embryological stages 34–39 (Dufaure & Hubert, 1961; Hubert, 1985). 35 eggs were injected with 30–40 µl of [^{3}H]thymidine (^{3}H-TdR) disolved in sterile Ringer's solution, at a dosage of 6–10 µCi/g body weight (methyl-[H^3]thymidine, specific activity 70–90 Ci/mM, Amersham). Seven embryos were collected 2 h after injection, 4 after 10 h, 5 after 1 d, 10 after 4 d, 9 after 6 d, and 7 at 13 d postinjection.

Table. *Distribution of the labelling index (percentage of labelled cells) in embryonic skin* from 2 h to 4 d after injection of 3H-TdR*

PI time		2 h	10 h	1 d	2 d	4 d
FBE						
% PE		15.6±2.34 (6-3)	14.9±3.35 (8-2)	15.7±3.23 (12-3)	14.3±2.92 (11-3)	—
% LP		3.8±1.44 (5-3)	8.4±1.36 (5-2)	13.9±4.69 (9-2)	7.2±2.81 (6-3)	35.8±6.82 (8-3)
% EP		7.0±3.61 (7-3)	13.0±3.05 (8-2)	21.5±5.15 (12-3)	14.9±3.64 (12-3)	47.4±17.19 (14-3)
% DE		11.7±3.40 (7-3)	17.9±2.51 (8-2)	28.2±7.28 (12-3)	16.1±3.55 (12-3)	48.4±13.50 (14-3)
SSA						
% PE		7.8±1.59 (4-3)	—	16.8±3.24 (9-3)	15.0±4.36 (5-3)	—
% LP		1.1 (1)	—	4.0±1.27 (5-3)	5.7±2.24 (6-3)	39.5±9.99 (5-3)
% EP		7.7±1.38 (4-3)	—	27.5±4.94 (10-3)	19.9±6.97 (8-3)	61.5±10.63 (5-3)
% DE		4.1±1.40 (4-3)	—	9.9±2.23 (9-3)	12.2±4.40 (8-3)	53.3±19.82 (5-3)
ASA						
% PE		—	—	13.5±4.06 (4-2)	—	—
% LP		—	—	—	5.7±2.71 (4-2)	3.1±1.33 (3-3)
% EP	OS	11.4±1.73 (5-2)	—	34.3±7.61 (4-2)	23.1±6.48 (10-2)	36.6±13.04 (5-3)
	IS	5.9±2.59 (5-2)	—	21.9±1.28 (4-2)	10.6±5.80 (10-2)	23.8±13.03 (5-3)
% DE		2.5±0.49 (5-2)	—	11.4±4.30 (4-2)	8.64±3.40 (10-2)	17.6±6.10 (5-3)
KAS						
% LP		—	—	—	0 (4-2)	—
% EP	OS	4.9±1.19 (4-2)	—	—	11.5±3.61 (3-2)	45.5±14.43 (3-2)
	IS	1.6±1.44 (4-2)			6.6±3.20 (3-2)	12.2±4.82 (3-2)
% DE		3.4±0.20 (4-2)	—	—	7.5±0.59 (3-2)	12.7±3.19 (3-2)

* Values are expressed as the means±S.E. Values in brackets represent the number of samples (X) and the number of animals counted (Y) (X-Y). PI time, postinjection time; FBE, flat bilayered epidermis; SSA, symmetric scale anlagen; ASA, asymmetric scale anlagen; KAS, β-keratinized asymmetric scale; % PE, percentage peridermal cells; % LP, percentage labelled peridermal cells; % EP, percentage labelled epidermis; % DE, percentage labelled dermis; OS, outer scale surface; IS, inner scale surface.

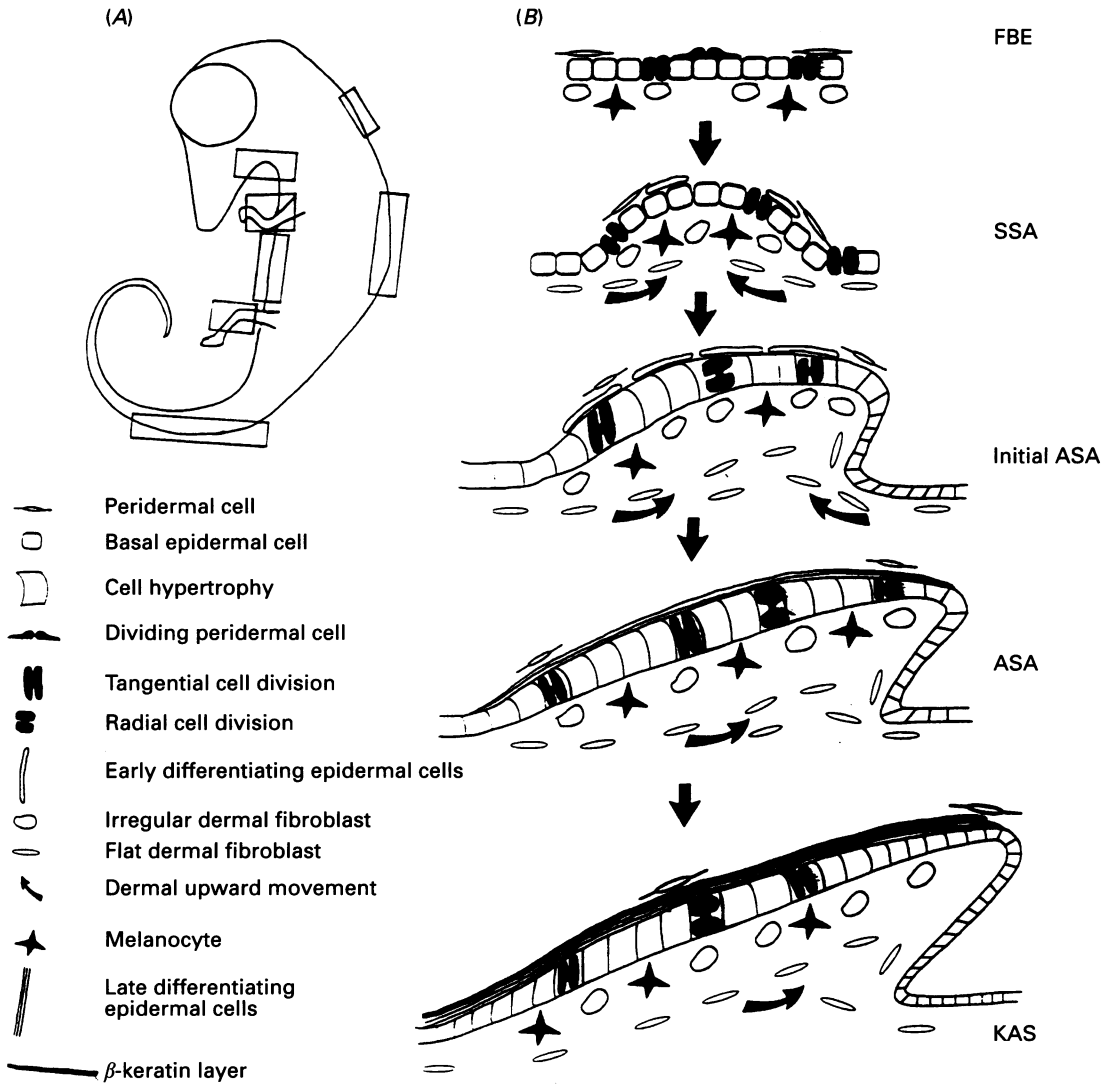

Fig. 1. (*A*) Schematic drawing of an advanced lizard embryo showing areas where most of the observations and quantification was performed. (*B*) shows schematically the principal stages of scale morphogenesis. FBE, flat bilayer epidermis; SSA, symmetric scale anlagen; ASA, asymmetric scale anlagen; KAS, β-keratinizing asymmetric scale.

Despite the fact that different embryonic stages (34–39) were collected in each group, the embryos showed all the representative phases of scale morphogenesis, as defined in the present study (see below). The embryos were fixed for 6–12 h in 10% neutral formaldehyde in Ringer's solution and embedded in JB-4 resin (Polyscience, Inc.). Other embryos were collected at 2 h (3 embryos), 2 d (6 embryos) and 6 d (3 embryos) postinjection, and fixed for 5–8 h in 2.5% glutaraldehyde in Ringer's solution and embedded in Araldite.

The embryos were sectioned with glass knives using a rotary microtome or an ultramicrotome. The sections were coated with nuclear emulsion K5 (Ilford), exposed for 6 and 12 w, developed and lightly stained with 0.5% toluidine blue.

Thirty-four other eggs, weight as above, were injected with 5-bromo2′-deoxyuridine (5 BrdU, Sigma) disolved in sterile Ringer's solution (8–10 mg/ml), in order to administer a dose of about 40–50 mg/kg body weight (20–40 µl/egg). Six embryos were collected and fixed 2 h after injection, 5 after 2 d, 3 after 4 d, 8 after 7 d, and 4 at 13 d postinjection. They were fixed in Carnoy's solution for 5–8 h and embedded in JB-4 resin.

After sectioning, the 5 BrdU was revealed, without treatment with pepsin, by a monoclonal anti-BrdU (Sigma or Bekton-Dikinson) and an HRP-conjugated antimouse IgG secondary antibody (Silenus) diluted 1:50–1:100 (Schutte et al. 1987). In 5 BrdU control sections the primary antibody was omitted. Sections were lightly counterstained with toluidine blue.

Quantification was undertaken for [^{3}H]thymidine treated embryos only. The percentage of labelled cells (labelling index) in different parts of the epidermis and dermis of various areas of the embryo (Fig. 1) was

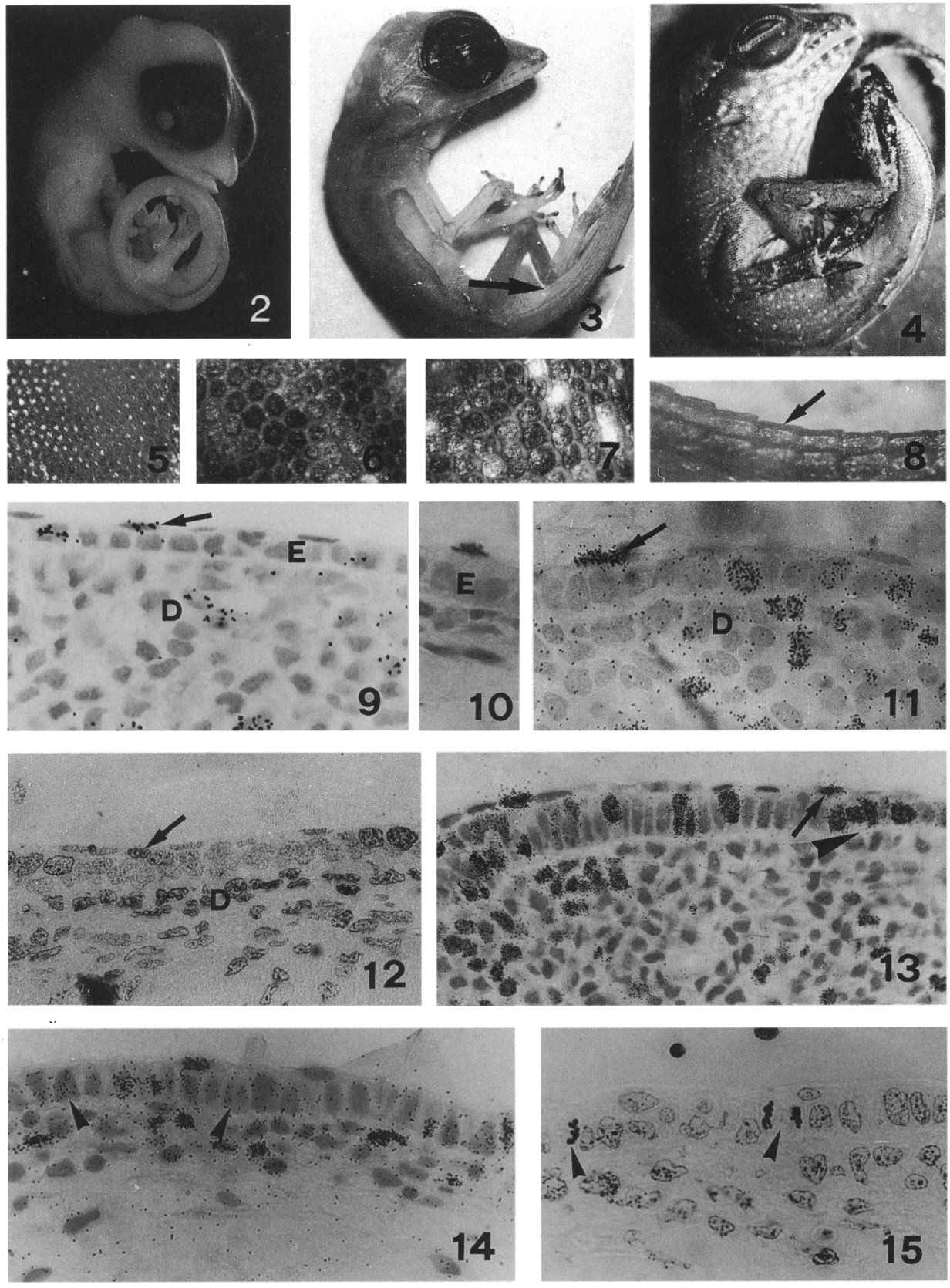

Fig. 2. Embryo of *A. lineatopus* at approximately stage 35. The skin is smooth and almost unpigmented. ×7.7.

Fig. 3. Embryo at more advances stage (37–38). Note pigmentation over most of the body and scale formation particularly in the tail (arrow). ×7.3.

obtained at successive postinjection times (Table, Fig. 1). The counting of peridermal cells was performed over most or all of the surface of each embryo in order to count a sufficiently large number of cells (> 100). The reported values for the epidermis and dermis were obtained by identifying labelled cells out of 200–300 counted cells (labelled and unlabelled). The study of 5 BrdU treated tissues was only qualitative, e.g. determining the pattern of distribution and the time of appearance of labelled cells in the different epithelial layers above the germinal layer.

RESULTS

Skin development and scale morphogenesis

The developing skin of *Anolis lineatopus* passes through a series of stages that transform a soft and flat monocellular ectodermal epithelium into a folded, pluristratified and cornified epithelium. Skin morphogenesis occurs rapidly in late fetal life, from stages 34–35 to stages 39–40, the latter just preceding hatching (Hubert, 1985; Figs 2–8).

Although few regional differences in the pattern of scale morphogenesis exist (e.g. the scales of the tail or limbs, or the lamellae of the specialised finger pads, in comparison with the remaining less overlapping body scales), in the present study we have considered 4 main stages of scale development (Fig. 1).

Stage 1: flat bilayered epidermis (*FBE*) (stages 3–4 according to Maderson, 1965). In this phase of development the epidermis showed a peripheral flat peridermis above a cuboidal or polygonal basal layer (the peridermal/basal epidermis ratio was 1:6–1:8). The dermis appeared as an irregular mesenchyme, often continuous with the underlying tissue; sometimes the lowermost cells assumed a flat, parallel configuration. Mitoses (mostly tangential, i.e. with mitotic spindles parallel to the epidermal surface) were commonly observed in the epidermis and occasionally also in the periderm.

Stage 2: symmetric scale anlagen (*SSA*) (stages 5–6, Maderson, 1965). The flat epidermis took on a wave-like configuration which produced a series of small or broad elevations (epidermal papillae) made of epidermis and a loose core dermis. This was typically seen in the ventral and dorsal skin which produced small scales with little overlap. In the regions of the embryo where very long scales are formed, such as in the tail, limbs or the specialised lamellae of the finger pads, the epidermis became wave-like in a serrated pattern. Mitoses were commonly observed in a random distribution along the epidermis. The orientation of the spindles was mostly tangential but radial division was also observed (i.e. oriented perpendicular to the epidermal surface). One, and occasionally 2, irregular layers of flat-ovoidal epidermal cells were seen between the basal layer and the periderm.

The core dermis appeared irregular and loose but, in symmetric scales sectioned along their medial axis, the orientation of cells and dermal extracellular fibrils suggested that the superficial dermis had followed the upward movement of the epidermis. The deep dermis consisted of flat cells with a parallel orientation with respect to the superficial epidermis, but in the scale core dermis these flat fibroblasts were oriented towards the epidermal elevation.

Stage 3: asymmetric scale anlagen (*ASA*) (stages 6–7, Maderson, 1965). The basal cells of one side (the more rostral or proximal) of the initially symmetric elevation became taller and hypertrophic with respect to the other side, where cells were cuboidal or flat. This basal hypertrophic layer of the outer surface (BLOS) is reminiscent of an epidermal placode with a tilted orientation, and gives origin to the outer surface

Fig. 4. Embryo at prehatching stage (39) showing the skin pattern with white dots and streaks. Scales are well differentiated in all body regions. × 7.0.

Fig. 5. Appearance of skin at the stage of epidermal papillae formation (bright spots) in the trunk. × 58.5.

Fig. 6. Later stage of skin development showing symmetric scales (tubercle-like) in the neck. × 59.

Fig. 7. Well differentiated slightly asymmetric scales on the trunk. × 58.5.

Fig. 8. Asymmetric scales in the tail region (arrow indicates expanded outer surface). × 58.

Fig. 9. Flat bilayered epidermis of forelimb (E) 2 h after ^{3}H-TdR injection. A few scattered grains are seen over an epidermal basal cell and a peridermal cell (arrow), and over few fibroblasts in the dermis (D). × 830.

Fig. 10. Dividing peridermal cell in trunk epidermis. × 820.

Fig. 11. ^{3}H-TdR labelled epidermis and dermis (D) of trunk 10 h after injection. Note increase of silver grains over the nuclei. × 730.

Fig. 12. Heavily labelled nuclei 1 d after 5BrdU injection. D, dermis. Arrow indicates a labelled suprabasal cell. × 745.

Fig. 13. Heavily labelled cells in limb skin 1 d after ^{3}H-TdR injection. The arrow indicates a labelled peridermal cell and the arrowhead labelled basal cells. × 650.

Fig. 14. Ventral epidermis 2 d after ^{3}H-TdR injection. Note some cells with fewer grains (arrowheads) than others. × 670.

Fig. 15. Ventral skin 8 d after 5BrdU showing positive clumps of labelling in most of the cells. Two tangential mitoses (arrowheads) are seen. × 800.

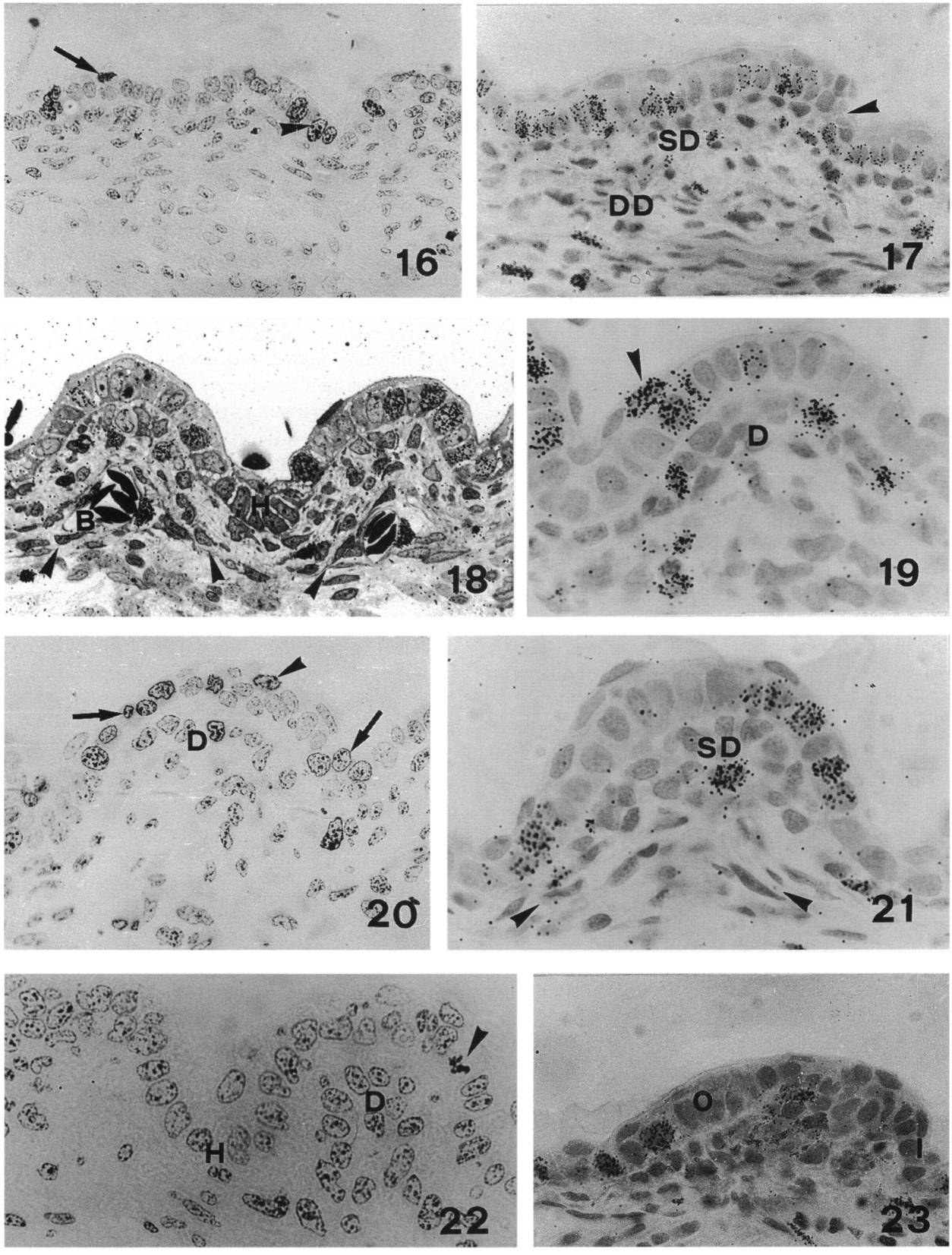

Fig. 16. Undulating ventral skin 1 d after 5BrdU injection. The arrow indicates a labelled peridermal cell and the arrowhead a labelled basal cell. × 745.

Fig. 17. Asymmetric hump in forearm epidermis 10 h after injection of ^{3}H-TdR. Cells on the longer side of the elevation are taller than on the shorter side (arrowhead). SD, mesenchyme-like superficial dermis. DD, flat fibroblasts in the deep dermis. × 650.

of the scale. Depending on the body region, 1–3 irregular cell layers were seen over the hypertrophied basal cell layer and under the superficial peridermis. Mitoses, mostly tangential, were still common, particularly in the BLOS. Some mitoses were radial, to provide cells for the upward migration. The dermis was divided into an irregular, loose superficial portion and a more compact, deep laminar portion. Dermal cells under the BLOS were irregular and close to the basal membrane. Melanosomes were localised mainly under the BLOS. Dermal cells under the inner side showed a fusiform shape, were smaller than fibroblasts under the BLOS, and were oriented parallel to the inner basement membrane.

Stage 4: β-keratinising asymmetric scale (KAS) (stage 8, Maderson, 1965). The BLOS lengthened by tangential cell divisions which, in long scales, were less frequent than in earlier stages. Radial divisions were sometimes seen in the basal layer. Two to 6 layers of flat keratinising cells were seen above the basal layer but under the peridermis. A flat layer was present under the periderm which in some preparations showed a thin saw-like edge (the forming 'Oberhäutchen') and 2 or 3 darker layers of differentiating β-keratinising cells. The dermis under the BLOS remained loose and was composed of larger fibroblasts than were observed either in the deep dermis or under the inner surface of the scale.

At the tip of many scales (ventral, thoracic, caudal and dorsal), a small dome-like protrusion, representing a sensory organ (Maderson, 1971), was produced.

Labelling variation with time and cell stratification

The labelling index sometimes differed for the same stage (FBE, SSA, ASA, KAS) in scales of different body regions (Fig. 1*A*). Therefore, the values given in the Table are means + S.E. in order to show the general trend of the labelling index during these stages.

Stage 1: flat bilayered epidermis (FBE). Two hours after injection of ^{3}H-TdR, cells labelled with a few grains were seen in the basal layer (Table). Occasional labelled or even dividing peridermal cells were also noted (Figs 9, 10). At 10 h postinjection, more basal and periderm cells were labelled with an increased intensity of labelling per nucleus (Fig. 11, Table).

Although not quantified, the 5 BdrU labelling followed a similar pattern (Fig. 12). The labelling index for dermal fibroblasts or mesenchyme was even higher than in the epidermis and it increased from 2 h to 4 d postinjection (Table).

At 1 d postinjection (both tracers) the labelling per nucleus in the epidermis was even more intense and the labelling index increased (Fig. 13). Substantial ^{3}H-TdR and 5 BdrU labelling was also seen 2 and 4 d postinjection (see Table) despite variability in cell labelling created by the rapid cell proliferation (Fig. 14). At 4 d postinjection many cells were still strongly labelled (the labelling index was the highest), but most of the remaining cells showed different degrees of labelling. Mitotic figures were commonly seen in the basal layer of the epidermis. At 8–13 d postinjection, the ^{3}H-TdR labelling was diluted to background level amongst most basal and peridermal cells. Eight to 13 d after injection of 5 BrdU, most cell nuclei still showed some labelled clumps of chromatin (Fig. 15).

Stage 2: symmetric scale anlagen (SSA). As shown in the Table, the labelling index (and the 5 BdrU labelling) increased (both as labelling index and nuclear intensity) in the epidermal papillae from 2 h to 4 d postinjection. Labelling dilution in later stages made counting uncertain and therefore no further determination of the labelling index was undertaken after 4 d postinjection.

Many randomly distributed mitoses with a tangential or radial orientation were also commonly seen at this stage. Variations in dimensions were randomly distributed along basal cells, but never regularly spaced to produce the epidermal papillae. At 2 h to 4 d postinjection, most labelled cells were seen in the basal layer and several were seen in the overlying flat irregular periderm (Figs 16–19). The distribution of labelled cells was irregular and no localised cell

Fig. 18. Symmetric scale stage of ventral epidermis 10 h postinjection showing randomly distributed labelled cells. B, central capillary; H, hinge region. Arrowheads point to flat dermal fibroblasts oriented towards the core of the papilla. ×690.

Fig. 19. Symmetric scale on trunk 1 d postinjection showing a labelled cell (arrowhead) located over the basal layer. ×870.

Fig. 20. Symmetric ventral scale 1 d after 5BrdU injection showing a random distribution of labelled (arrows) cells. A labelled suprabasal cell (arrowhead) and few labelled dermal cells are also seen. ×740.

Fig. 21. Medially sectioned symmetric scale on trunk 1 d after ^{3}H-TdR injection. The superficial dermis (SD) contains irregular mesenchyme-like cells and the deep dermis is made up of fusiform cells with axes oriented toward the core dermis (arrowheads). ×870.

Fig. 22. Symmetric scale 8 d after 5BrdU injection. Positive chromatin clumps are still seen over many epidermal and dermal (D) cells. The arrowhead points to a tangential mitosis. H, hinge region. ×680.

Fig. 23. Onset of asymmetry in limb scale at 10 h postinjection. Two labelled cells are seen in the left lower part of the BLOS of the forming outer scale surface (O). I, developing inner scale surface. ×690.

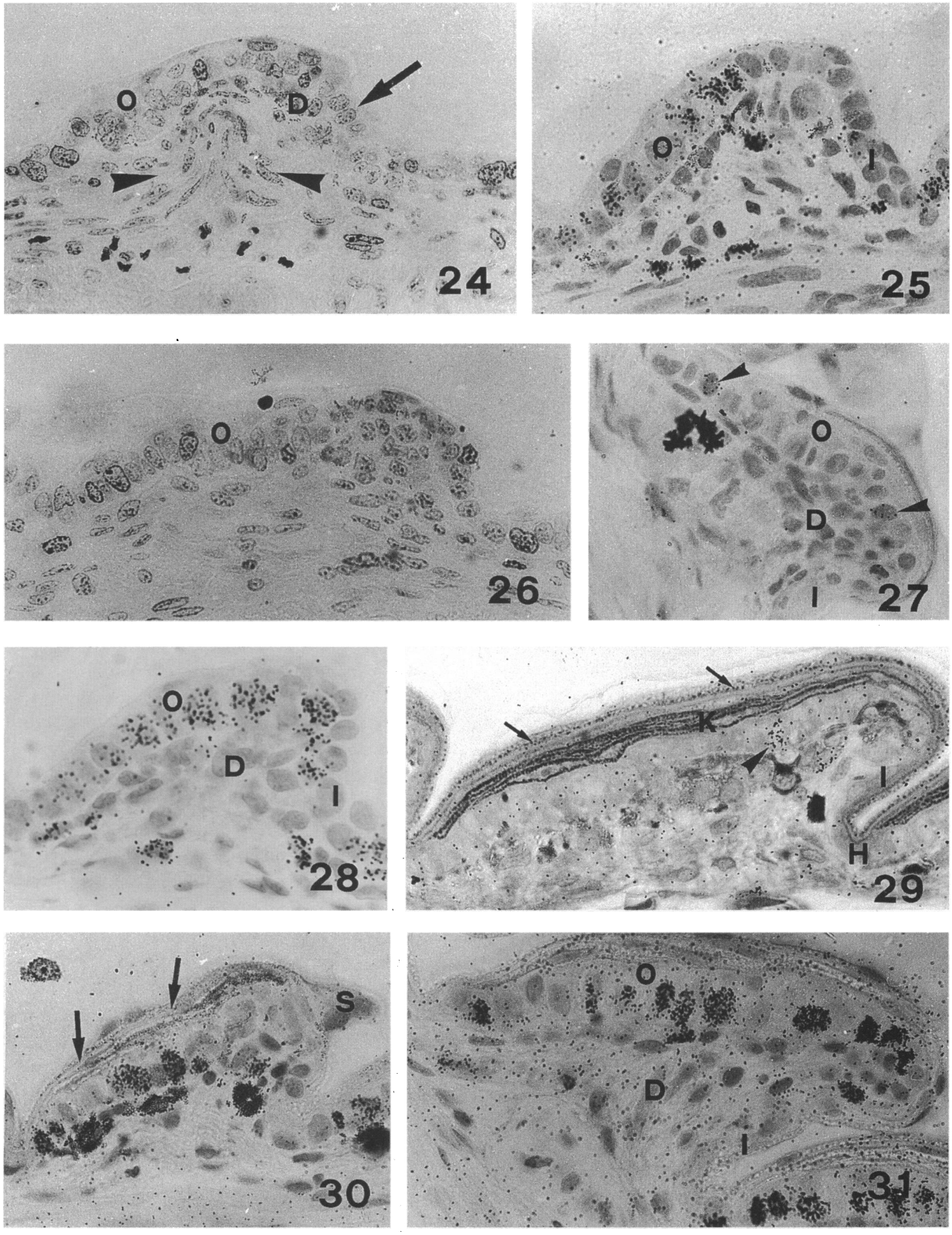

Fig. 24. Onset of asymmetry in forelimb epidermis 1 d after injection of 5BrdU. Labelled (darker) cells are seen in the developing outer side (O). The arrow points to the future inner side and the arrowheads indicate the upward orientation of flat fibroblasts. D, dermis. × 740.

Fig. 25. Onset of scale asymmetry 1 d after injection of ^{3}H-TdR showing labelled and taller cells on the developing outer scale surface (O) while the inner (I) shows cuboid cells. × 730.

Fig. 26. Longer BLOS with labelled cells of the developing outer surface (O) of a forelimb scale 1 d after 5BrdU injection. A labelled cell (dark nucleus) is also visible on the developing inner side. × 725.

proliferation was observed either in the epidermis or the dermis (Figs 20, 21). Additionally, the labelling index in the dermis increased from 2 h to 2–4 d after injection and no dermal condensation was seen. However (see Table) the index was lower in the dermis than in the corresponding epidermis, and sometimes this difference was quite prominent.

At 8–13 d after injection of ^{3}H-TdR, most of the cells in the epidermis and dermis appeared unlabelled (grain distribution was low and similar to background). Despite dilution, nuclear clumps of 5 BdrU positive chromatin were still seen in most of the nuclei of the epidermis and dermis at 8–13 d postinjection (Fig. 22).

Stages 3 and 4: asymmetric scale anlagen and β-keratinising asymmetric scales (ASA and KAS). Generally, the BLOS of most of the scales showed more labelled and mitotic cells than the flatter inner side of the developing scale (Figs 23–26). The labelling index for cells in the BLOS, as compared with that in the inner surface (see Table) showed that the difference between the 2 scale surfaces was often distinct. A few faintly labelled cells were seen 2 h after injection of ^{3}H-TdR, but the labelling became clearly visible 10 h to 1 d postinjection (Figs 27, 28).

The location of labelled cells in the BLOS was apparently random and, at 2–10 h postinjection, labelled cells were often seen in the zone close to the hinge region. After 1–4 d, labelled cells were seen at random in the BLOS.

During ASA at 2–4 d postinjection, labelled cells were limited to the BLOS and occasional cells were seen in the upper differentiating layer.

At the time of Oberhäutchen and β-keratin layer differentiation (KAS), the labelling index clearly decreased in the epidermis. No labelled peridermal cells above the β-keratinising layer were seen at 2 h to 4 d postinjection (Fig. 29). At this stage, the periderm appeared bistratified and contained granules in the lower layer (Fig. 29).

The labelling index in the BLOS at the ASA and KAS stages, also increased from 2 h to 4 d postinjection, although the intensity of labelling varied in different cells (Figs 30–33, Table). Six to 8 days after injection, labelled cells with various degrees of labelling were still seen in the BLOS.

During KAS, at 2–4 d postinjection, a few labelled cells were seen above the germinal layer. Conversely, many labelled differentiating cells, both during ASA and KAS, were frequently seen 6–8 d after injection (Figs 34–38), often appearing as presumptive 'Oberhäutchen' or β-keratin differentiating cells. Eight to 13 d after injection, very flat and labelled keratinised cells were seen even in the outermost layers (Figs 37, 39). Although the labelling index of the dermis increased from 2 h up to 4 d postinjection, it was always lower than that observed in the corresponding epidermis, particularly when compared with the BLOS (Table). This difference was pronounced during the phase of scale growth, when the BLOS was composed of 15–30 hypertrophied cells (depending on the body region in which the scale was studied). Labelled dermal cells were often seen near the tip of the growing scale 4–8 d postinjection.

DISCUSSION

The present study complements previous information on the morphogenesis of scales in lizards (Maderson, 1965, 1985; Liu & Maneely, 1969; Dhouailly, 1975). Despite local differences, this study has shown that a general pattern of cell proliferation is recognisable for all areas of the embryo. In general, the pattern of proliferation as revealed by ^{3}H-TdR and 5 BdrU was similar. However, once incorporated, 5 BrdU diluted less efficiently than ^{3}H-TdR, and positive chromatin clumps were still visible at 8–13 d postinjection, while ^{3}H-TdR labelling was diluted to background level during that same period. The possibility exists that the immunodetection of 5 BdrU was more sensitive than ^{3}H-TdR autoradiography.

Cell kinetics of stages 1 (FBL) and 2 (SSA)

The present study has shown that the periderm has a low but significant proliferative activity (Sengel, 1976).

Fig. 27. Asymmetric ventral scale showing 2 weakly labelled cells (arrowheads) in the BLOS of the outer side (O) 2 h after ^{3}H-TdR injection. D, dermis; I, inner side. × 745.

Fig. 28. Asymmetric scale 1 d postinjection showing many ^{3}H-TdR labelled cells on the outer side (O). Under the inner side (I) 2 dermal cells are labelled. D, dermis. × 870.

Fig. 29. Asymmetric β-keratinised tail scale 1 d after ^{3}H-TdR injection showing a labelled cell (arrowhead) in the BLOS. Arrows point to the granulated layer extending along the inner side (I) and hinge region (H). × 740.

Fig. 30. Dorsal symmetric scale showing labelled cells in the BLOS 2 d after injection. Dark melanocytes are present under the BLOS. Arrows point to flat differentiating layers not yet showing β-keratin formation. S, sensory organ at scale tip. × 740.

Fig. 31. Prekeratinised ventral scale 2 d postinjection showing many labelled cells in the BLOS of the outer surface (O) and 1 labelled cell in the inner side (I). D, dermis. × 745.

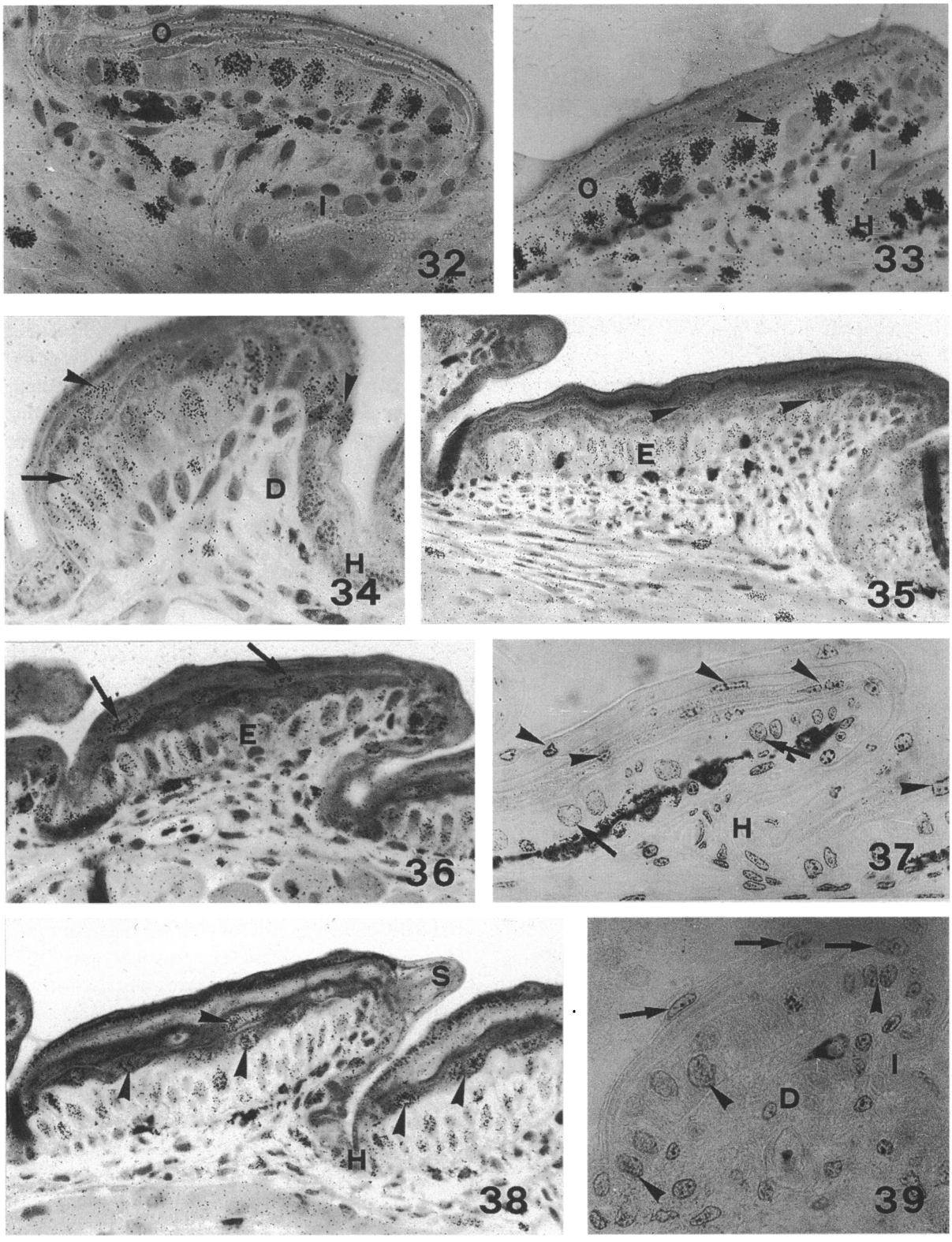

Fig. 32. Prekeratinized ventral scale 4 d postinjection showing most labelled cells limited to the BLOS of the outer surface (O). One small labelled cell is seen in the inner side (I). ×680.

Fig. 33. Tail scale showing heavily labelled cells in the BLOS on the outer surface (O) 4 d after ^{3}H-TdR injection. The arrow head indicates a labelled nucleus located over above the basal layer. I, inner side. H, hinge region. ×590.

Fig. 34. Ventral scale 7 d after ^{3}H-TdR injection showing labelling dilution in the BLOS (arrow) and labelled cells in the differentiating flat layers above it (arrowhead). D, dermis. H, hinge region. ×620.

Fig. 35. KAS of tail 7 d after injection of ^{3}H-TdR. Note labelling dilution among cells of the BLOS (E) and in cells of the overlying differentiating β-layer (arrowheads). ×310.

This was noted particularly in the early stages (FBL, SSA) but was reduced or undetectable during late stages (ASA, KAS). The lack of labelling in the peridermis when a β-keratin layer is forming may be due to a decrement of periderm proliferation, anticipating the coming shedding (Sengel, 1976; Maderson, 1985). On the other hand, it may also be possible that the formation of the β-keratinised layer and its stratification act as a barrier to the diffusion of the injected ^{3}H-TdR and 5 BdrU from the basal layer to the outer periderm (Landmann, 1979). A similar effect of uneven tissue labelling was reported by Sawyer (1972*b*).

Periderm proliferation, though much lower than in the basal epidermis (the periderm:basal epidermis ratio was 1:6–1:8), allows the covering epithelium to keep pace with the rapidly growing basal germinal epithelium. The proliferation of the peridermis, however, might also produce a thin stratification as previously observed in *Lacerta* (Dhouailly & Maderson, 1974).

Another point arising from the present study is that the ^{3}H-TdR uptake (and probably 5 BrdU) increases steadily from 2 h up to 1–2 d postinjection, both as the number of labelled cells (quantified as the labelling index in the Table) and the number of grains per nucleus (not quantified but evident from the observations). This suggests that, in the isolated embryonic environment, the tracers remain available for uptake for at least a day, possibly longer.

Two to 4 days following the injection, the rapid cell division produced an evident 'labelling dilution effect' (at least with the dosages employed here).This fact was also evident at lager stages (3, ASA and 4, KAS), despite the decrement in the labelling index (Table). The dilution of the tracers to background levels was reached at 6–8 and 13 d postinjection.

The observation (not quantified) that the mitotic spindles were preferentially tangential, i.e. oriented parallel to the surface of the epidermis, indicates that cell proliferation in these early stages is most closely related to the surface expansion of the epidermis.

No epidermal placode in the FBE precedes the formation of scales (Maderson, 1965; Maderson & Sawyer, 1979). Although irregularities of cell height in the basal epidermal layer were sometimes observed, they did not appear regularity spaced in order to determine the formation of papillae (Liu & Maneely 1969).

Once the epidermis has formed elevations or 'humps', 2 epidermal domains can be recognised along the wave-like epidermis, namely the 'hump domain' (most of the future scale domain) and the 'interhump domain' (the future hinge regions and part of the inner scale surface). The hump domains represent by far the largest part of the epidermal surface as compared with the interhump domains. Therefore, even without localised cell proliferation in the hump domains, these units are destined to expand more than the interhump units where a lesser number of cells is present.

The analysis of labelling distribution in the Table indicates that also in the 2nd stage (SSA), cell proliferation is mostly directed towards expanding the epithelial surface where little stratification occurs.

In the dermis it appears that in the initial morphogenetic stage (FBL), dermal fibroblasts proliferate sufficiently to keep pace with the epidermal proliferation. During stage 2 (SSA), although the labelling index of the dermis was still high, it was generally lower than in the epidermis. This proliferative decrement in the dermis with respect to the epidermis may be responsible for the appearance of the wave-shaped contour of the epidermis and upward displacement of the superficial fibroblasts, which appeared stretched. The movement of the superficial core dermal cells towards the epidermis may be due to the pre-existing links between dermal cells and the epidermal basement membrane, as appeared to be the case during scale regeneration (Alibardi, 1994*b*).

A different rate of proliferation between epidermis and dermis (heterochrony) was also observed during skin regeneration in lizards (Alibardi, 1994*a*, *b*). This process may be responsible for the invagination of the regenerating epithelial sheet instead of the typical evagination seen during scale embryogenesis. The

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Fig. 36. KAS of forelimb 7 d postinjection showing labelled cells in the BLOS (E), in the dark β-layer and in the paler presumptive Oberhäutchen (arrows). × 390.

Fig. 37. KAS of tail 8 d after 5BrdU injection showing labelled cells (arrowheads) in all keratinized layers and the periderm. Weakly labelled cells are still seen in the BLOS (arrows), under which there are numerous melanocytes. × 640.

Fig. 38. KAS of forelimb 7 d after injection of ^{3}H-TdR. Labelled cells are visible in the dark β-layer (arrowheads). H, hinge region; S, apical sensory organ. × 420.

Fig. 39. Ventral scale 8 d after 5BrdU injection showing labelled cells (arrows) in the more external layers, periderm and some still in the basal layer (arrowheads). D, dermis; I, inner side. × 760.

explanation for the different mechanisms between skin development and regeneration remains unclear, but it is probably related to the different localisation of anchoring complexes in the 2 processes (Dhouailly & Maderson, 1984; Alibardi, 1994*b*).

Cell kinetics of stages 3 and 4

The asymmetry of the scale anlagen and the growth of the future outer surface appear to be determined by 2 mechanisms, namely differential cell hypertrophy and cell proliferation. Cell hypertrophy and increase of cell height in the formation of the BLOS give this layer the appearance of an epidermal placode with an oblique orientation. At the onset of scale asymmetry, 2–6 cells represent the beginning of the BLOS, and are probably located in the centre of the epidermal papilla. This process, if visualised in 3 dimensions, suggests that from a few hypertrophic core cells a laminar outer scale surface is shaped. The BLOS stage strongly resembles the 'definitive scale-ridge' in the chick (Sawyer 1972*a*, *b*; Sawyer et al. 1974). Although the mechanism of placode formation in lizards is unknown, the epidermis in the BLOS acquires new potentials for differentiation, since the hypertrophy of basal cells is associated with the production of a hard layer of β-keratin (Maderson, 1985; Landmann, 1986). In fact, for other placodes (lens, neural, olfactory, otic, and for the feathers and scutate scales in birds), the formation of a columnar epithelium produces new potentialities for differentiation in the ectoderm cells. A similar 'regionalisation' also takes place in the 'distal side' of the regenerating scales of lizards, which forms the β-keratinised outer surface of the scale (Alibardi, 1994*a*, *b*, 1995). Without such regionalisation, probably all epidermis would be alike. In the reptilian tilted placode, in contrast to other placodes, there is no lag of ^{3}H-TdR uptake during the initial hours after injection, but a further study reducing the labelling period would give additional information about cell movement within the BLOS (Sawyer 1972*b*; Sengel, 1976; Tanaka & Kato, 1983).

A greater degree of cell proliferation in the BLOS of the future outer surface with respect to cells in the inner surface also contributes to scale asymmetry. This proliferation is both tangential (for the expansion of the placode) and radial (for epithelial stratification and differentiation).

Since the outer surface of the scale occupies a greater length than the inner surface and hinge region, this qualitative observation was strengthened only after determining the labelling index following tracer administration. A greater number of mitoses in the BLOS was also noted in the developing tail scales of the geko *Hemidactylus bowringi* (Liu & Maneely 1969). Cells in the developing inner side of the scale probably flatten and stretch in order to keep pace with the faster growth of the outer side, as was reported during scale regeneration (Alibardi, 1994*a*, 1995).

Although the length of the cell cycle in embryonic or regenerating scales of lizards is unknown, the prominent localisation of labelled cells in the BLOS may be due to a shortening of the cell cycle in this region or to a lengthening of the cell cycle in the inner scale surface. This was previously shown by Sawyer (1972*b*) for chicken scutate scales.

The present study shows that the migration of differentiating epidermal cells towards the keratinizing layers follows that seen in normal and regenerating skin in Squamates (Flaxmann, 1972; Flaxmann & Maderson 1973; Downing & Roth 1974; Alibardi 1994*a*, *b*, 1995). The increased labelling in the BLOS from 2 h until 4 d after injection indicates that most of the proliferation takes place in the basal layer and that cells remain in the germinal layer during this time.

The first cells of the embryonic skin move into the layers above the germinal layer 2 d after injection of the tracers. However 4 d after injection more migrating cells were seen which, at 6–8 d postinjection, appeared as differentiating 'Oberhäutchen' or β-keratinizing cells (depending on when the injection was done during the first shedding cycle).

The low rate of dermal proliferation under the asymmetric scale further allows epidermal expansion into the dorsal scale surface. The mechanisms underlying the fall in the rate of proliferation in the embryonic or regenerating dermis (Alibardi, 1994*b*) are unknown.

The dermis under the BLOS retains mesenchymal features, showing large irregular cells when compared with the cells under the inner surface and hinge region of KAS. Whether these morphological differences lead to different types of dermoepidermal interactions (Sengel, 1976), or are correlated with the differentiation of an outer or inner scale surface, remains unclear.

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REFERENCES

ALIBARDI L (1994*a*) Fine autoradiographical study on scale morphogenesis in the regenerating tail of lizards. *Histology and Histopathology* **9**, 119–134.

ALIBARDI L (1994*b*) Dermal modification during tail regeneration of the lizard. *Histology and Histopathology* **9**, 733–745.

ALIBARDI L (1995) Electron microscopic analysis of the regenerating scales in lizard. *Bollettino di Zoologia* **62**, 109–120.

DHOUAILLY D (1975) Formation of cutaneous appendages in dermoepithelial recombinations between reptiles, birds and mammals. *Wilhem Roux Archiv für Entwicklungsmechanik* **177**, 323–340.

DHOUAILLY D, MADERSON PFA (1984) Ultrastructural observations on the embryonic development of the integument of *Lacerta muralis* (Lacertilia, Reptilia). *Journal of Morphology* **179**, 203–228.

DOWNING SW, ROTH SI (1974) The derivation of the cells of the epidermal strata of the boa constrictor (*Constrictor constrictor*). *Journal of Investigative Dermatology* **62**, 450–457.

DUFAURE JP, HUBERT J (1961) Table de developpement du lezard vivipare: Lacerta (Zootoca) vivipara Jacquin. *Archives d'Anatomie Microscopique et Morphologie Experimentelle* **50**, 309–327.

FLAXMANN BA (1972) Cell differentiation and its control in vertebrate epidermis. *American Zoologist* **12**, 13–25.

FLAXMANN BA, MADERSON PFA (1973) Relationship between pattern of cell migration from the germinative layer and changing patterns of differentiation in the lizard epidermis. *Journal of Experimental Zoology* **183**, 209–216.

HUBERT J (1985) Embryology of Squamata. In *Biology of Reptilia*, Dev. B, (ed Gans C, Billett F, Maderson PFA), vol. 15, pp. 1–34, New York: John Wiley & Sons.

LANDMANN L (1979) Keratin formation and barrier mechanisms in the epidermis of *Natrix natrix* (reptilia, Serpentes): an ultrastructural study. *Journal of Morphology* **162**, 93–126.

LANDMANN L (1986) The skin of reptiles. Epidermis and dermis. In *Biology of the Integument* (ed Bereiter-Hahn J, Matoltsy AG, Sylvia-Richards R), vol. 2 *Vertebrates*, pp. 150–193. Berlin: Springer Verlag.

LIU HC, MANEELY RB (1969) Observations on the development and regeneration of tail epidermis in *Hemidactylus bowringi* (Gray). *Acta Anatomica* **72**, 549–583.

MADERSON PFA (1965) The embryonic development of the squamate integument. *Acta Zoologica* **46**, 275–295.

MADERSON PFA (1971) The regeneration of caudal epidermal specializations in Lygodactylus picturatus keniensis (Gekkonidae, Lacertilia). *Journal of Morphology* **134**, 467–478.

MADERSON PFA (1985) Some developmental problems of the Reptilian integument. In *Biology of Reptilia* (ed Gans C, Billet F, Maderson PFA), vol. 14, dev. B, pp. 525–598. New York: John Wiley & Sons.

MADERSON PFA, SWAYER RH (1979) Scale embryogenesis in birds and reptiles. *Anatomical Record* **193**, 609.

SAWYER RH (1972*a*) Avian scale development. I. Histogenesis and morphogenesis of the epidermis and dermis during formation of scale ridge. *Journal of Experimental Zoology* **181**, 365–384.

SAWYER RH (1972*b*) Avian scale development. II. A study of cell proliferation. *Journal of Experimental Zoology* **181**, 385–408.

SAWYER RH, ABBOTT UK, FRY GN (1974) Avian scale development. III. Ultrastructure of the keratinizing cells of the outer and inner epidermal surfaces of the scale ridge. *Journal of Experimental Zoology* **190**, 57–70.

SCHUTTE B, REYNDEIRS MM, BOSMAN FT, BLIJHAM GH (1987) Effect of tissue fixation on anti-bromodeoxyuridine immunocytochemistry. *Journal of Histochemistry and Cytochemistry* **35**, 1343–1345.

SENGEL P (1976) *Morphogenesis of Skin*. Cambridge: Cambridge University Press.

TANAKA S, KATO Y (1983) Epigenesis in developing avian scale. II. cell proliferation in relation to morphogenesis and differentiation in the epidermis. *Journal of Experimental Zoology* **225**, 271–283.

WYLD JA, BRUSH AH (1979) The molecular heterogeneity and diversity of Reptilian keratins. *Journal of Molecular Evolution* **12**, 331–347.

WYLD JA, BRUSH AH (1983) Keratin diversity in the Reptilian epidermis. *Journal of Experimental Zoology* **225**, 387–396.