JOURNAL OF MORPHOLOGY 179:203–228 (1984)

Ultrastructural Observations on the Embryonic Development of the Integument of *Lacerta muralis* (Lacertilia, Reptilia)

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ABSTRACT Integumentary development on the dorsal and ventral aspects of the body of 14, 21, 26, 33, and 40-day incubated embryos of the European Wall Lizard (*Lacerta muralis*) is described. While the earliest stages of epidermal differentiation resemble those reported for other tetrapods, precocious differentiation of dermal collagen more resembles that of anamniotes than that of birds and mammals. Anchoring complexes comprising cellular components, anchor filaments, and collagen are described, and their possible relationship to the formation of scale anlagen is discussed. The first embryonic epidermal generation differentiates beneath the periderm; most features of its histogenesis resemble those that have been described for the epidermis of adult squamates, but certain previously ignored organelles, including possible earlier β-keratin precursors, are reported. Different strategies regarding in ovo peridermal loss and posthatching shedding behavior are described and discussed in light of presently available data concerning control of cell differentiation in the squamate epidermis.

The cytological changes in the epidermis of adult squamates associated with periodic skin shedding have been studied extensively at light and electron microscopic levels during the past 20 years [see references in Maderson and Chiu ('81); Landmann et al, ('81)].

In the century preceding Lange's ('31) review, information on the embryonic development of the squamate integument derived from isolated observations in the German literature. The first systematic histological study based on observations of staged embryos of *Lacerta vivipara, Anguis fragilis, Vipera berus*, and *Natrix natrix* was provided by Maderson ('65) and several aspects were confirmed and extended by Dhouailly's unpublished data on *Lacerta muralis*, which appeared in Sengels's ('76) monograph.

As Kerbert (1876–1877) and Maurer (1895) emphasized, the initial stages of integumentary histogenesis are similar in all vertebrates, but inter- and intraspecific differences become evident during the last two-thirds of embryonic/fetal life as the adult phenotype emerges (Maderson, '65; Sengel, '76). Maderson ('72a) suggested that the generic term "reptilian scale" was inappropriate in view of the diverse patterns of distribution of keratinous proteins in extant species, a contention that has been reinforced by accumulating knowledge of protein molecular structure (Wyld and Brush, '79). A similar problem has emerged with respect to "avian scales" (Sawyer, '83). Maderson and Sawyer ('79) reported that the embryogenesis of crocodilian scales resembled that of squamate scales (Maderson, '65; Sengel, '76) rather than that of avian scutate scales (Sawyer, '83). The adult phenotype of the latter, however resembles that of crocodiles (Maderson, '72b), thereby raising a minor problem of "developmental homology."

The "epithelial–mesenchymal" interactions responsible for the emergence of structural heterogeneity during embryogenesis are currently topics of major interest (Sawyer and Fallon, '83). Our knowledge of the underlying dynamics with regard to the amniote integument derives primarily from studies of avian material, supported to some degree by data from mammalian models, but there are few

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data concerning reptiles (Dhouailly, '75, '77; Sengel, '76). Studies of adult mammalian integument, and to a lesser extent that of birds, suggest a degree of similarity between tissue homeostatic and post-trauma responses to certain embryonic phenomena (Goss, '69, '78). Because of the inherent difficulties in obtaining reptilian embryonic material, it would be operationally useful if we could confirm that comparable similarities obtain with reptilian integument, a system for which approximately the same amount of information exists concerning the dynamics of embryonic development (Dhouailly, '75, '77) as for integumentary wound healing and regeneration (Maderson and Roth, '72; Maderson et al., '78a,b).

Here we provide ultrastructural data on integumentary embryogenesis in *Lacerta muralis* and discuss them first on a comparative basis with respect to birds and mammals; second, with respect to possible morphogenetic mechanisms involved in scale anlage formation; and, finally, the structural and functional implications of the in ovo entry in the unique cycling mode of epidermal cell behavior.

MATERIALS AND METHODS

Embryos of the European Wall Lizard (*Lacerta muralis*) reported here came from eggs incubated in a terrarium in the Grenoble laboratory at 26°C, wherein hatching occurs in 46 days. A table of development for this species has been published (Dhouailly and Saxod, '74).

Pieces of skin were dissected from the dorsal and ventral body of 14-, 21-, 26-, 33-, and 40-day incubated embryos. The samples were fixed for 1 hr in 2% glutaraldehyde solution buffered at pH 7.4 with Michaelis buffer, postfixed in 1% osmium tetroxide solution in the same buffer for 1 hr, and dehydrated in alcohol. They were embedded in Epon 812. Semithin sections, approximately 1.0 μm in thickness, were stained with methylene blue and azur.

Thin sections were cut with a diamond knife and double-stained with lead citrate and uranyl acetate prior to examination in a Siemens Elmiskop 1A electron microscope operated at 80 kV.

RESULTS

Introductory comments

Lacerta muralis has a completely scaled integument, but the exact form of the units varies over the body, as does the degree of overlapping. Ventral scales are smooth, wider transversely than they are long, but overlapping to the same degree as the caudal scales that are smaller and are longer than they are wide; dorsal scales are polygonal and show little overlap. All scales have identifiable outer and inner surfaces and hinge regions, but the degree of development of the last two named regions is reduced in less overlapping units (Maderson, '64). Because the precise locations of the inner surfaces and hinge regions cannot be discerned until the first embryonic epidermal generation differentiates, the term "interscale" region is used without prejudice in describing early integumentary differentiation; it is not homologous with the interfollicular tissues between primordia of the more widely studied avian (feathers) and mammalian (hairs) appendages (Maderson, '72a,b, '84a).

Maderson ('65) and Dhouailly ('75) have confirmed Maurer's (1895) observation that scale morphogenesis and primary epidermal histogenesis are heterochronous over the body surface; this is important with respect to the presentation of results. In *L. muralis*, scale morphogenesis is first evident externally when a series of transverse furrows appear on the ventral surface at day 15 of incubation; symmetrical, ventral anlagen are individualized at 22 days, and they become asymmetrical by 25 days. Caudal scale primordia are recognizable at 17 days and asymmetrical units are present at 25 days. On the dorsum, scale morphogenesis begins at 20 days, symmetrical anlagen are present by 25–26 days, and asymmetrization is evident at 30 days. By 35 days of incubation, integumentary differentiation over the entire embryo is synchronized when "perfect embryonic scales" appear (Maderson, '65).

In describing integumentary histogenesis in four phases, we have neglected the heterochrony discussed above because most of our observations are based on dorsal tissues with occasional references to the venter. However, in precisely identifying the electron micrographs, the reader will find that "younger" tissues may sometimes seem more advanced in development than "older" tissues; this simplification is justified by the fact that age alone is an inappropriate means of identifying morphogenetic and histogenic events which may vary even in siblings incubated at the same temperature. Descriptions of later epidermal differentiation cover only the

tissues of the outer scale surfaces, following custom in accounts of the adult system [see references in Landmann et al. ('81); Maderson ('84a); Maderson and Chiu ('81)]; the tissues of the inner surfaces and hinge regions show only quantitative differences.

Early integumentary differentiation (Figs. 1–3)

Prior to the onset of scale morphogenesis, the integument of the early embryo consists of a two-layered epidermis lying above a presumptive dermis (Fig. 1). Electron micrographs confirm the cuboidal nature of the basal cells seen with the light microscope, but reveal the extreme flatness of the peridermal cells (Fig. 2). The dermo-epidermal junction is characterized by a well-developed basement membrane (Figs. 2,3) and abundant collagen fibers arranged in an orthogonal, laminated pattern, penetrated by conspicuous processes from the tightly packed subjacent fibroblasts (Fig. 3). One or two layers of the deepest fibroblasts have a distinct epithelial-like organization (Fig.2).

Scale anlage formation and asymmetrization (Figs. 4–13)

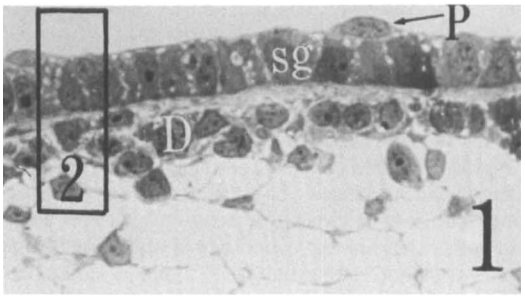
When a scale anlage first appears, it is a symmetrical elevation from the body surface with the epidermis being the same thickness everywhere and with a dermis whose only conspicuous light microscopic feature is the presence of collagen bundles (Fig. 4). The epidermis differs from the preanlage condition only in that two, or occasionally three, layers of cells lie above the cuboidal basal cells.

The electron microscope reveals that the dermis is extremely complex; while its precise structure differs depending on the location on the anlage (cf. Figs. 5 and 11), everywhere there are three distinct regions: (1) an outermost region with many cells, (2) an intermediate region with fewer cells, and (3) an innermost population of epithelial-like fibroblasts. The last-named region is so distinct from, and loosely attached to, the subintegumentary tissues, that portions of skin may be stripped easily from unfixed embryos.

In the interscale regions, where the dermal tissues are compact (Figs. 4,5), the superficial fibroblasts do not send processes toward the basement membrane of the epidermis, and they are surrounded by compact collagen bundles (Figs. 6,8). There are numerous, vertically directed "anchoring complexes" comprising cellular and extracellular elements running between the epidermis and the deep epithelial-like layer of the dermis (Figs. 5–10). The complexes contain inwardly directed processes from the superficial fibroblasts, and outwardly directed processes from similar cells in the intermediate region (Figs. 5,6). Epidermal cell protrusions through the basement membranes (Fig. 9) may be regular components of the anchoring complexes. Two types of extracellar fibril are seen; some show the distinct periodicity of type I collagen (Figs. 6–8), while the others are narrower and uniformly electron opaque (Figs. 7,8,10).

Toward the core of the scale anlage (Figs. 4,11), the dermal tissues are less compact. Immediately beneath the basement membrane, numerous cell processes run toward the epidermis from the superficial fibroblasts, and collagen fibers are sparsely distributed between them. The outermost cellular component contains four to five layers of fibroblasts and accounts for about 50% of the volume of the dermal tissues (Fig. 11) in contrast to the comparable component in the interscale region where the two to three layers of fibroblasts account for only about 25% of the volume of the dermal tissues (Fig. 5). Anchoring complexes are less abundant in the core of the anlage. In the intermediate region, which together with the innermost epithelial-like fibroblasts accounts for the other 50% of the volume of the dermal tissues, there are few fibroblast bodies; but there are many narrow cell processes running at various orientations between the conspicuous, orthogonally arranged collagen layers. Figure 11 shows that the organization of the collagen network is different in the core than toward the interscale region. A large cell, interpreted as a lysosome-releasing macrophage (Fig. 11, M), lies in a region where the collagen fibrils seem to have been broken down; we are uncertain whether the discontinuity of the epithelial-like population (Fig. 11, X) also results from lysosome activity, or whether it is a preparative artifact. This is the only location where such a discontinuity has been seen.

As asymmetrization of the central anlage proceeds (Figs. 12,13), two features are apparent. First, the dermal tissues maintain much the same morphology as has been described for the symmetrical anlage. Second, the epidermis remains essentially homogeneous over the entire anlage at the beginning of asymmetrization (Fig. 12); regional differences become apparent later with the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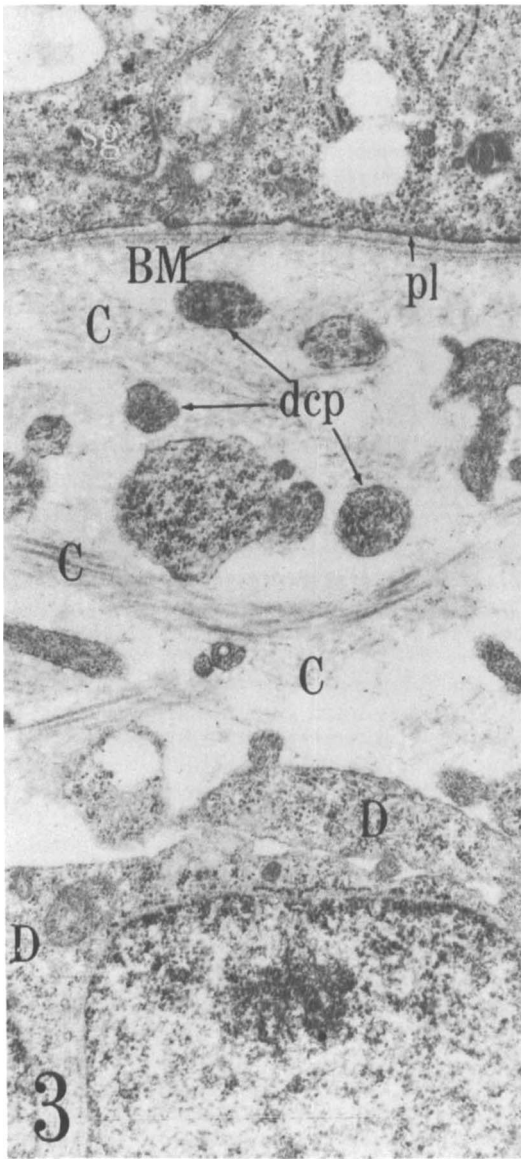
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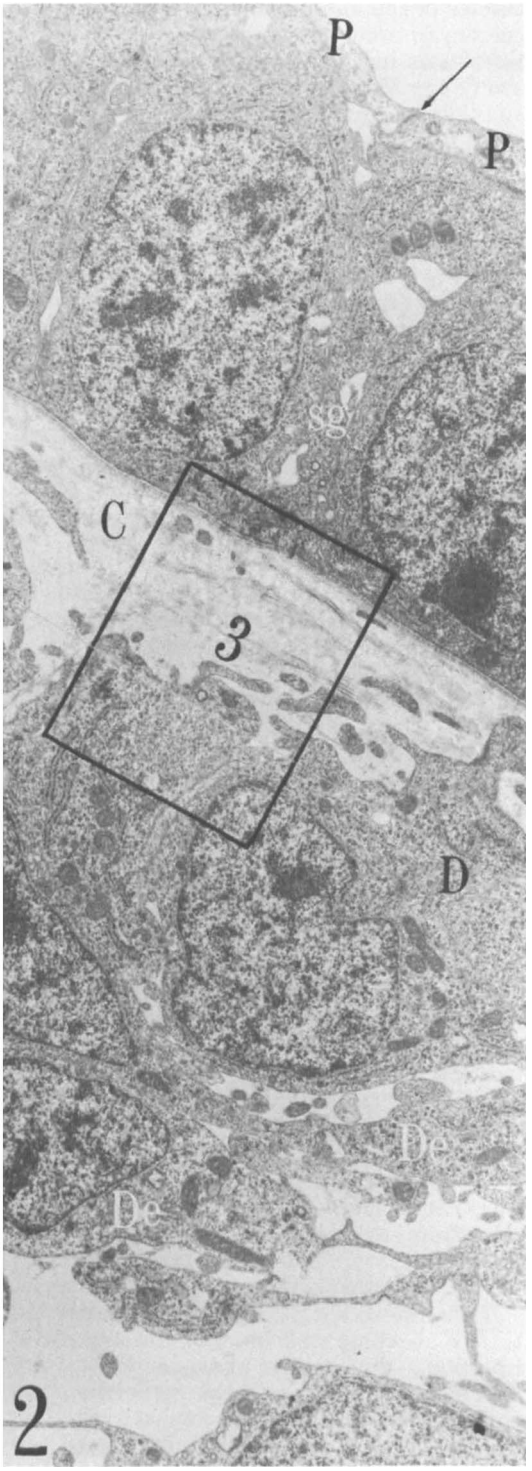
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Fig. 1. Longitudinal semithin section through dorsal skin of a 14-day embryo. The epidermis consists of cuboidal cells, the stratum germinativum (sg), above which lie flattened periderm cells (P), the nuclei of which are sparsely distributed, but whose cytoplasmic processes contact each other (see Fig.2). There is a region of dense, noncellular material between the stratum germinativum and the cells of the presumptive dermis (D). The numbered rectangle(s), here and elsewhere, refer to figures of the same number. ×720.

Fig. 2. Thin section of region indicated in Figure 1. Note the extremely flattened peridermal cells (P) meeting (arrow) on the surface of the stratum germinativum (sg). The dense material of the dermo–epidermal junction contains some striated collagen (C) arranged in an orthogonal plywood fashion. The fibroblasts of the innermost dermis (De) form an epithelial-like structure. ×8000.

Fig. 3. Higher magnification view of dermo–epidermal junction in region outlined in Figure 2. Note the clearly defined innermost plasma membrane (pl) of the germinal cells (sg) abutting the complex basement membrane (BM). Between the basement membrane and the dermal fibroblasts (D) may be seen the collagen bundles (C) of the orthogonal complex and numberous fibroblast processes (dcp), both cut at various angles. ×20,000.

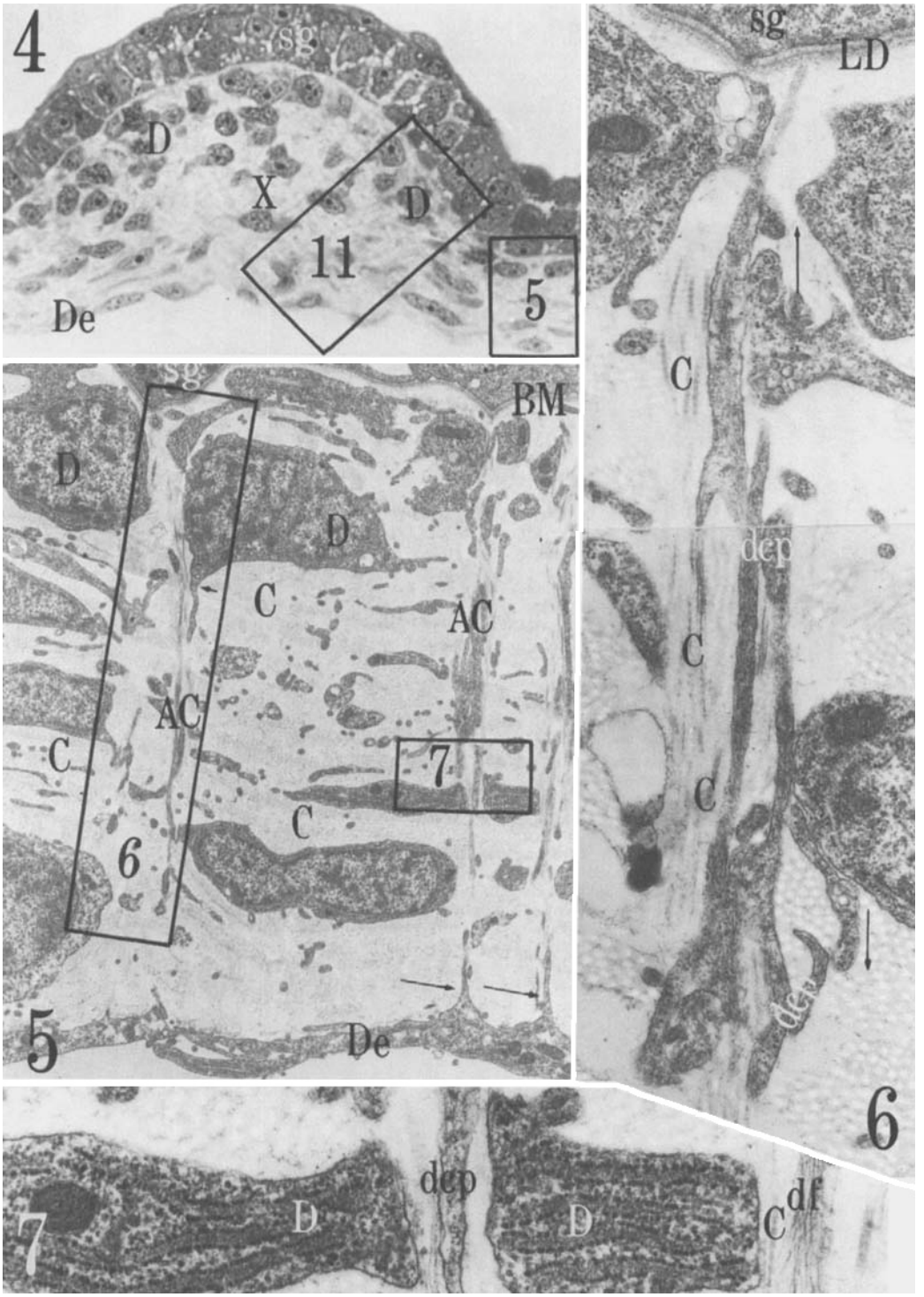
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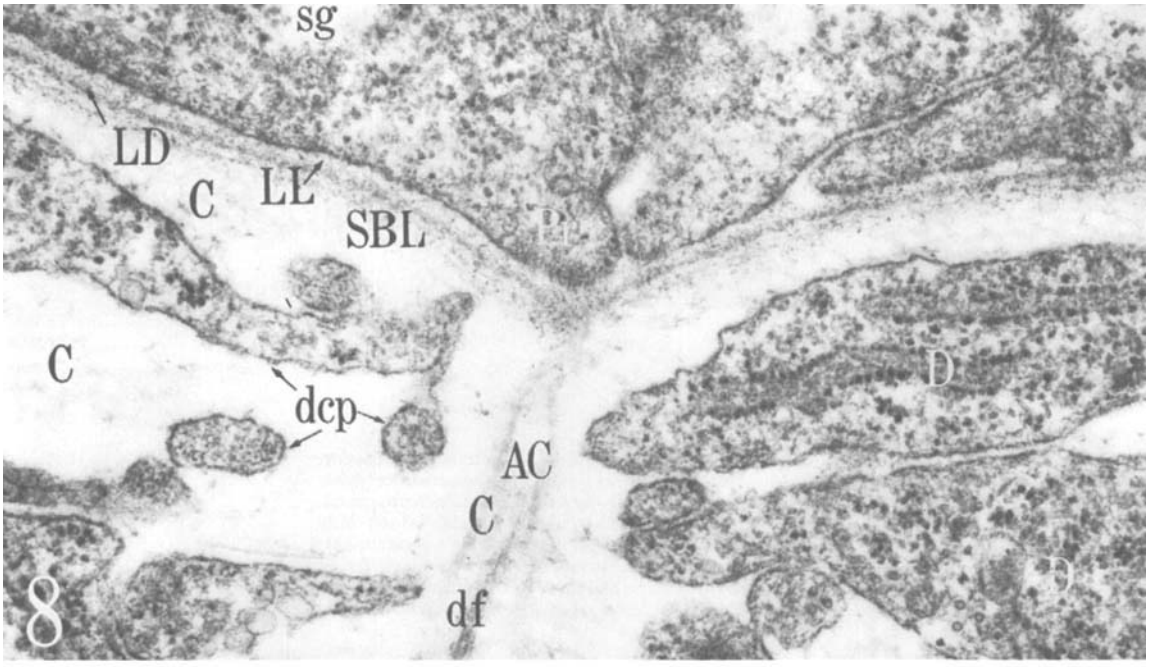
Fig. 4. Semithin longitudinal section through the dorsal skin of a 26-day embryo showing a symmetrical scale anlage. The epidermis is the same thickness throughout, consisting of two to three layers of cells (which may represent a multilayered periderm) above a stratum germinativum (sg). While there are more fibroblasts (D) in the core of the anlage (X) than in the interscale region (Fig. 5), there are no visible localized condensations. ×720.

Fig. 5. High power view of interscale region from Figure 4. Beneath the germinal cells (sg) there is a continous basement membrane (BM). The more superficial fibroblasts (D) are stellate with many processes, while the innermost ones are epithelial-like (De) with upward-directed processes (arrows). Anchoring complexes (AC) run through the orthogonally arranged collagen bundles (C) from the basement membrane to the innermost dermis. ×6,400.

Fig. 6. A detail of an anchoring complex similar to that outlined in Figure 5. It consists of dermal cell processes (dcp) which may run upward (↑) or downward (↓), striated collagen (C), and thinner unstriated elements (see Fig. 7). ×20,000.

Fig. 7. Further detail of two anchoring complexes from Figure 5. The cell processes (dcp) and striated collagen fibers (C) are paralleled by bundles of homogeneous, unstriated filaments (df). Large portions of fibroblast bodies (D) here, and in Figures 5 and 6, seem to bear a topographical relationship to the anchoring complexes in the vertical plane of the dermis. ×32,000.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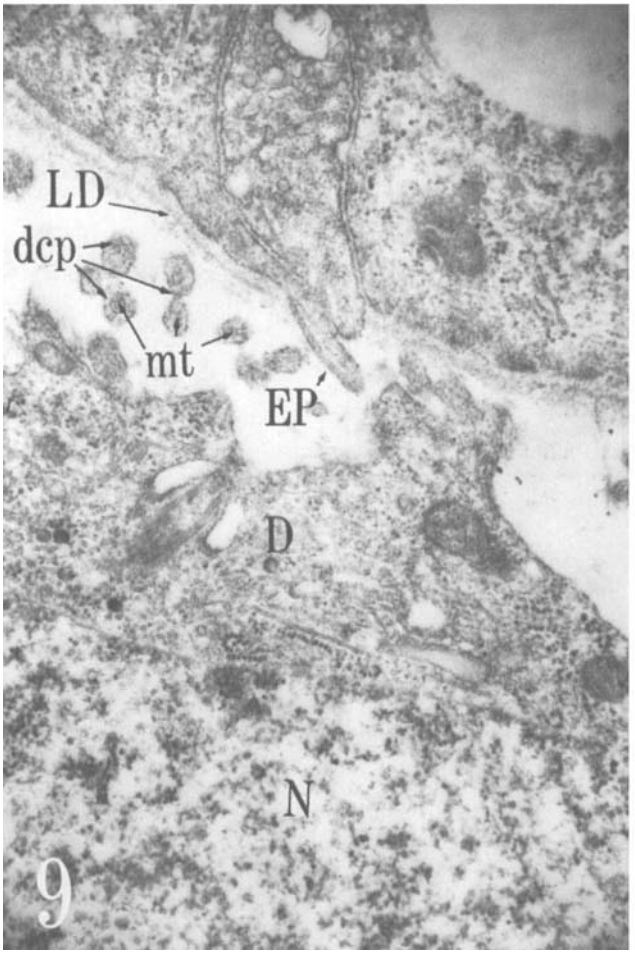
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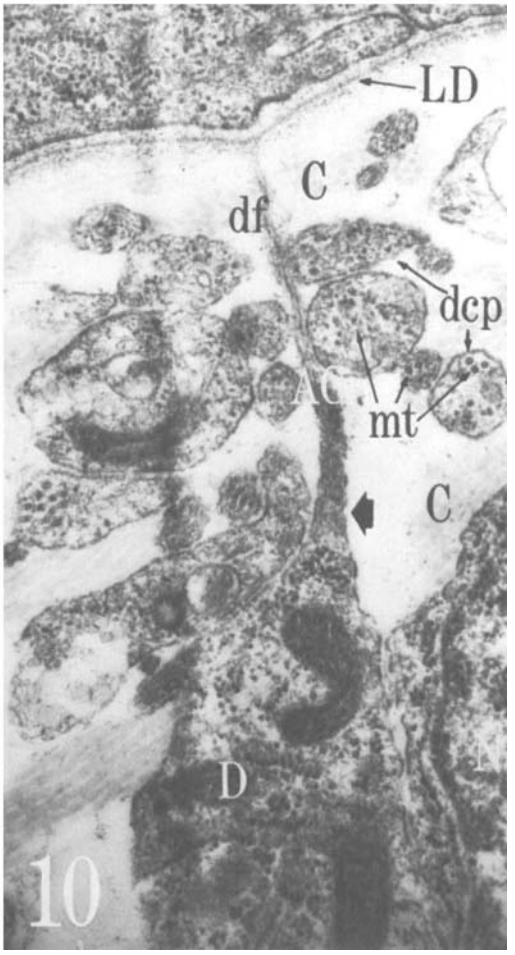
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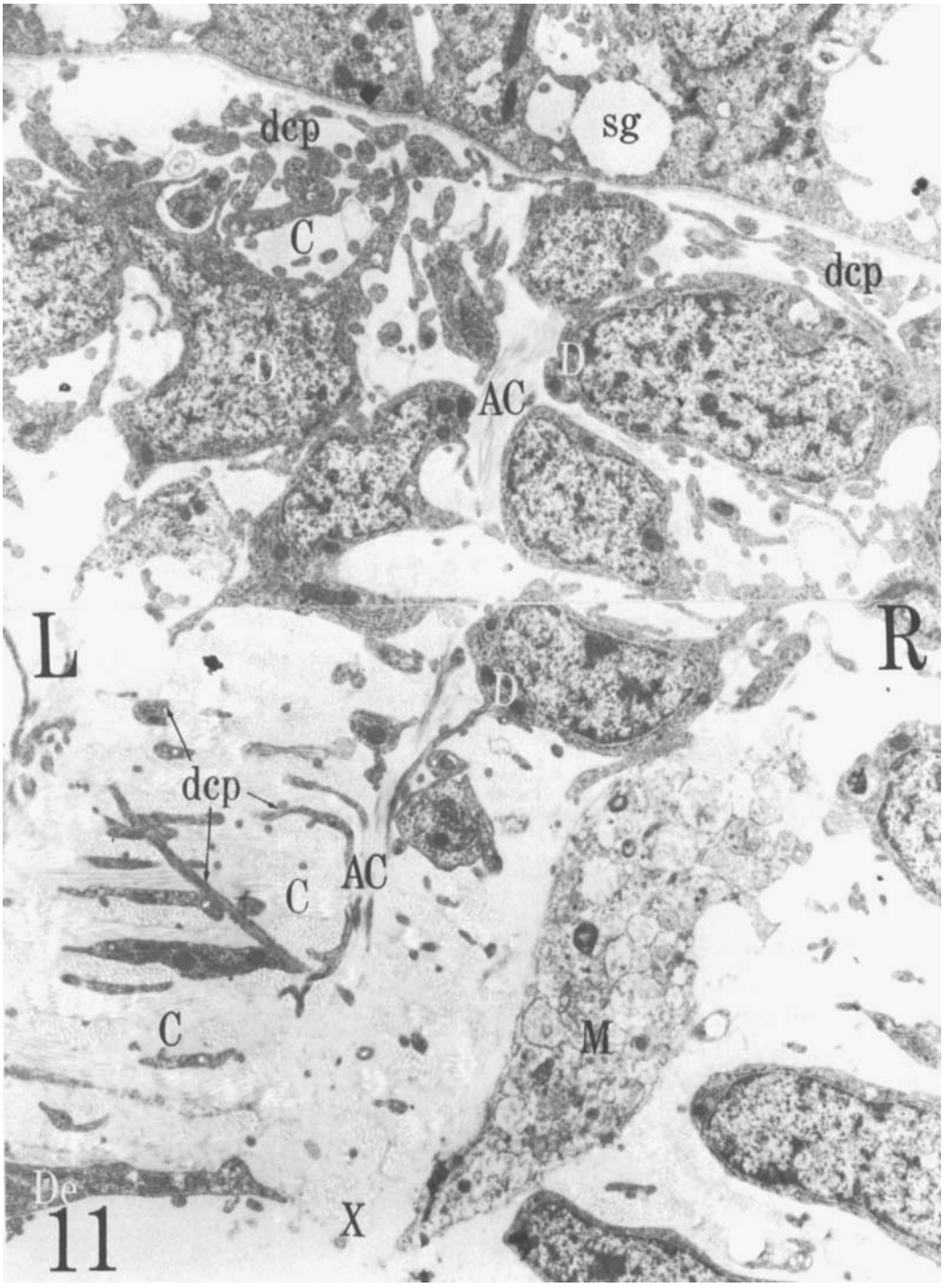
Figs. 8–10. Dermo–epidermal junctions in interscale regions.

Fig. 8. Junction in the interscale region from the dorsal skin of a 26-day embryo. ×40,000.

Fig. 9. Junction in the interscale region from the ventral skin of a 21-day embryo. ×32,000.

Fig. 10. Junction in the interscale region from the ventral skin of a 26-day embryo. ×32,000. Various features in these figures (Figs 8–10) show a close relationship between the epidermis and the dermal anchoring complexes. In Figure 8, opposite a ventrally directed protrusion (Pr) from a germinal cell (sg), the continuous basement membrane forms a wide V at the point of which lies the outermost tip of an anchoring complex (AC). In Figure 9, a germinal cell process (EP) penetrates the lamina densa of the basement membrane (LD) pointing toward a dermal fibroblast (D). In Figure 10, the lamina densa (LD) is continuous beneath the germinal cells (sg) but the tip of an anchoring complex (AC) lies very close, and at the broad arrow, a dermal cell process is cut obliquely so that dermal filaments appear to grow out of the cell. All figures show sections through dermal cell processes (dcp), cut at various angles, many containing microtubules (mt) and microfilaments. Some of these superficial dermal cell processes (see also Fig. 11) (and perhaps the epidermal process in Figure 9) appear to be associated with the anchoring complexes. C, collagen; df, dermal filaments; LL, lamina lucida; N, nucleus; SBL, subbasal laminal area.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first cytological indications of generation histogenesis (Fig. 13).

The histogenesis of the first embryonic epidermal generation (Figs. 13–19)

As asymmetrization proceeds, the epidermis becomes more complex (Figs. 13–15). Two layers of flattened peridermal cells may be discerned, the outermost of which bear conspicuous microvilli and zonulae occludentes (Figs. 14,15). Between the basal and peridermal cells lie three layers of suprabasal cells (sb_1–sb_3, Fig. 14) that are distinguishable from the periderm cells by two features. First, they are not flattened and they contain rounded nuclei. Second, in common with the basal cells, their cytoplasm contains conspicuous concentrated and diffuse masses of granular material (X, Figs. 14,15). The individual granules are approximately as electron dense as glycogen granules, but less dark than ribosomes, and are considerably larger than either. The identity of these granules will be discussed later.

Asymmetrization produces a "perfect embryonic scale" (Fig. 16) (Maderson, '65) which has an epidermal structure comparable to a mid-late stage four of the adult shedding cycle (see Discussion), differing only in the presence of an outermost periderm. The number of cells comprising the periderm cannot be discerned with the light microscope (Fig. 16). In Figures 17 and 18, two layers of periderm cells are seen, the outermost being extremely flattened with persistent microvilli, the innermost being characterized by arabesque whorls of electron-dense material cut in various planes (Fig. 18). The four layers of suprabasal cells between the basal layer and the periderm (sb_1–sb_4, Fig. 17) possess bundles of 30 Å filaments localized primarily in the peripheral regions of the cells, which are cut in both transverse and logitudinal planes (Fig. 19). Melanosomes are visible in the cytoplasm of all subperidermal cells (Fig. 17). The characteristic bundles of 30 Å filaments and the columnar, chromophobic appearance of the basal cells (Figs. 16, 17) permit the identification of the suprabasal cells as the first Oberhäutchen (sb_1, Figs. 17,18) and the presumptive β-cells (sb_{2-4}, Figs. 17,18). The granular material previously seen (Figs. 14,15) is absent and, in contrast to the cytology of adult epidermal cells (Maderson et al., '72), very few bundles of 70 Å filaments can be discerned.

The dermis has attained its adult form and has melanophores at the margin between the superficial and deep components (Fig. 16).

Epidermal structure in the late embryo (Figs. 20–24)

A few days before the embryo hatches, its epidermal structure is equivalent to a late stage five of the adult cycle except that instead of an outer epidermal generation there is a periderm that may consist of several layers of cells (Figs. 20,22).

Between the periderm and the basal layer, three quite distinct tissues comprise an incomplete first embryonic epidermal generation. From without inwards these are: the mature syncytial β-layer (with its Oberhäutchen) in which no filaments may be discerned, but which contains numerous melanosomes (Figs. 21,22a,23); seven to eight layers of cells with characteristic lamellar inclusions that constitute the mature mesos layer (Figs. 21,23,24) as described by Landmann et al. ('81); two layers of presumptive α-cells containing some bundles of 70 Å filaments, although here, and in the basal cells, such bundles are not as common as they are in adult material (Figs. 21,23). An unusual feature of the presumptive α-cells is the sporadic occurrence of what appear to be bundles of 30 Å filaments (X, Fig. 23) on the basis of their similarity to those seen in Figure 19.

Fig. 11. High power view of the lateral region of the core of a symmetrical scale anlage similar to that outlined in Figure 4. The superficial dermal cells (D) are stellate with many processes (dcp) while the innermost cells have an epithelial organization (De) as in the interscale region (Fig. 5). Anchoring complexes (AC) are less frequent than in the interscale region (cf. Figs. 5–10). Instead of the continuous, vertical, orthogonal organization of collagen bundles seen in the interscale region (Fig. 5), here the superficial dermis contains many cells between the processes of which are sparse collagen fibers (C). Throughout the dermis, dermal cell processes (dcp) also show an interwoven, criss-crossed organization. Dermal organization is conspicuously different on the right (R) and left (L) of the figure; the large cell in the center is identified as a macrophage (M) × 8,000.

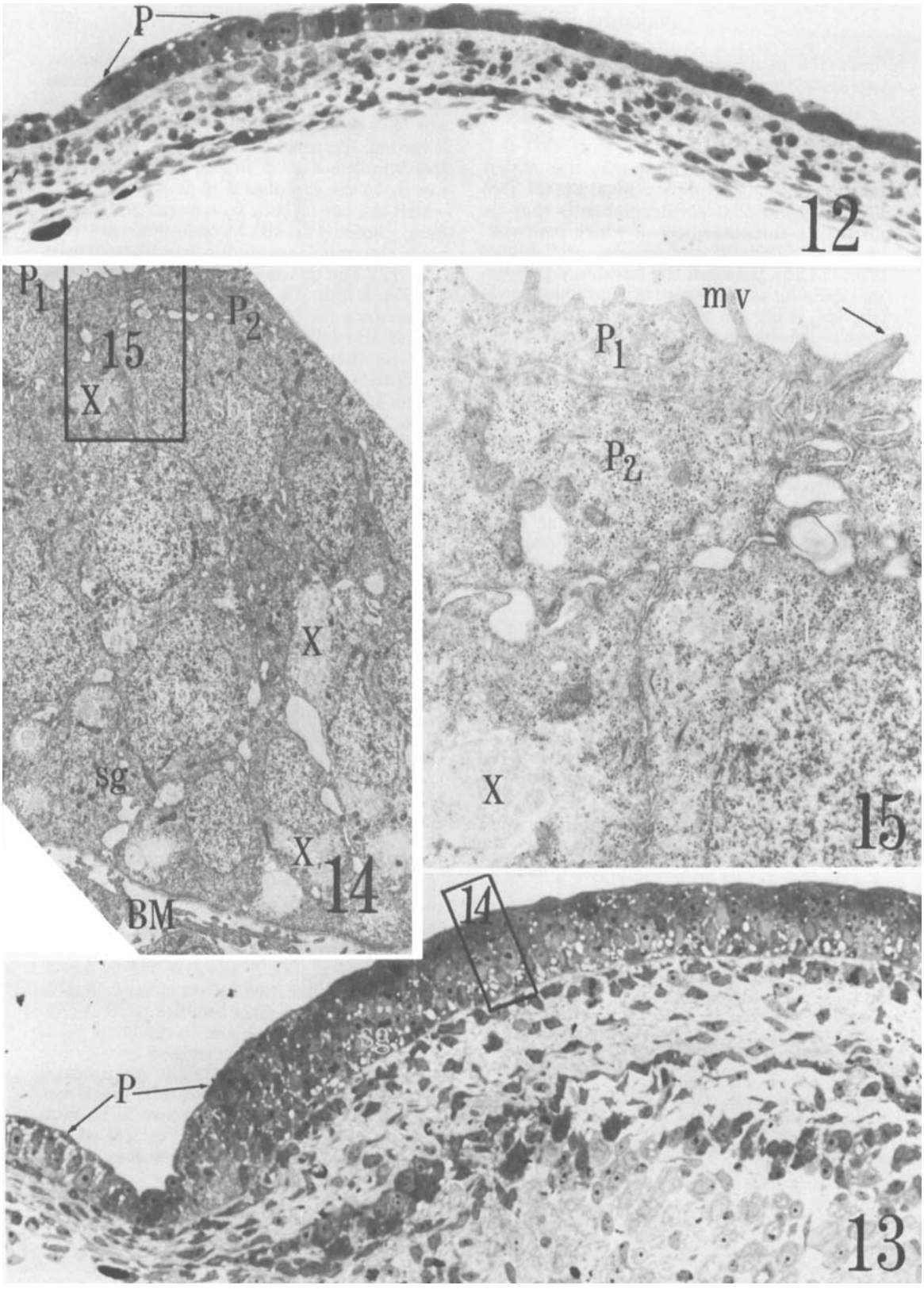
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Fig. 12. Longitudinal semithin section of 21-day ventral skin; the anlage is becoming asymmetric with the presumptive posterior margin on the left. The epidermis is only two-layered, a periderm (P) lying atop the germinal cells (cf. dorsal scale anlage in Fig. 4). ×450.

Fig. 13. Longitudinal semithin section through apex of a 26-day ventral scale anlage. The stratified epidermis has a well developed periderm (P) and germinal layer (sg). ×720.

Fig. 14. Thin section of portion of epidermis comparable to that outlined in Figure 13. There are two layers of periderm cells (P_1 and P_2) and three layers of suprabasal cells (sb_{1-3}) lying above a stratum germinativum (sg). Note the well-developed basement membrane (BM). ×3400.

Fig. 15. Higher magnification of region of outer epidermis outlined in Figure 14. The outermost peridermal cells (P_1) bear conspicuous microvilli (mv), two of which are joined by what appears to be a tight junction (arrow). The second layer of peridermal cells (P_2) is distinguished from the suprabasal cell (sb_1) because the latter contains concentrated and diffuse masses of a granular material (X). Note that these individual granules here and in Figure 14 are noticeably less electron-dense and larger than ribosomes. ×20,000.

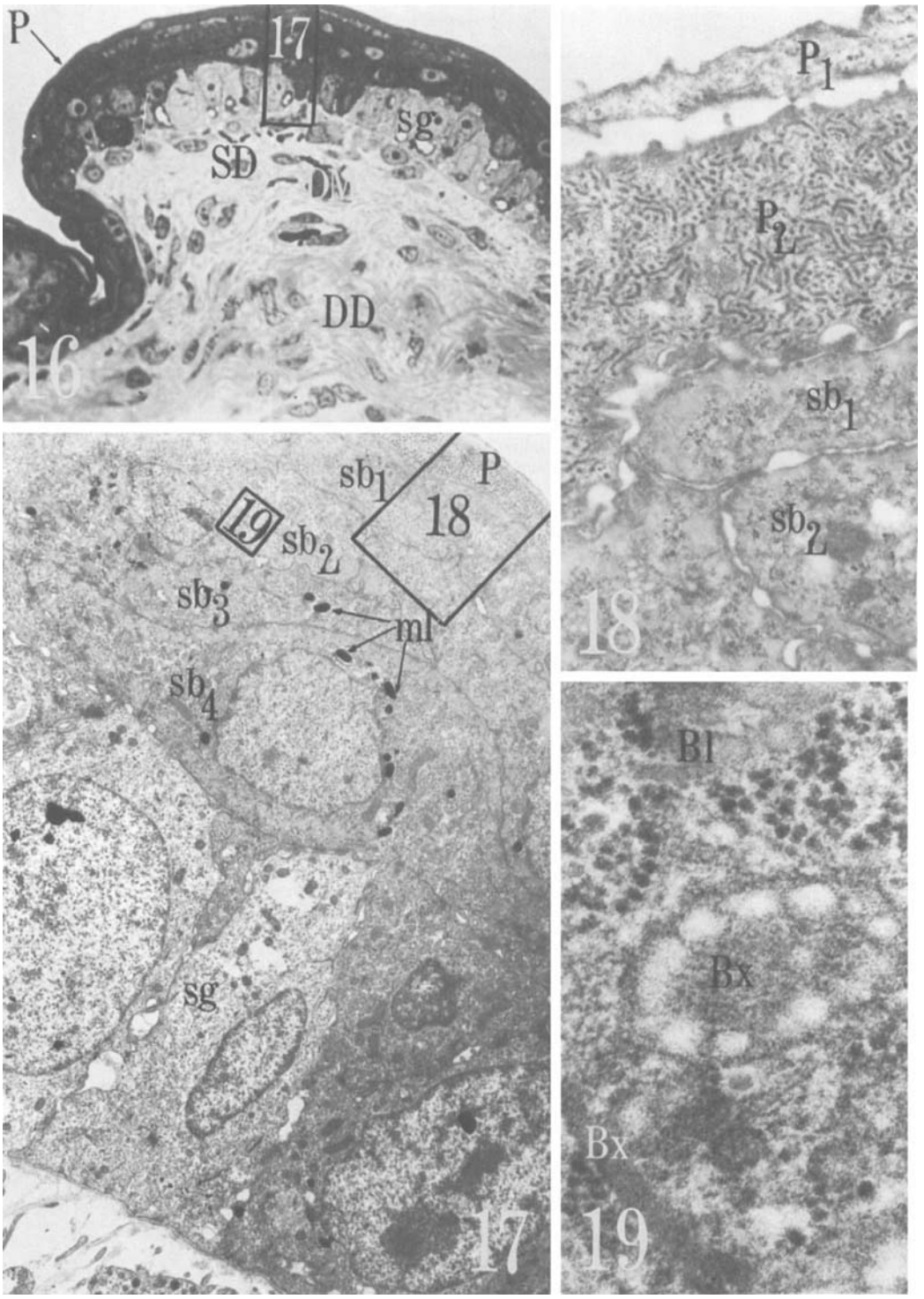
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Fig. 16. Longitudinal semithin section through a dorsal "perfect embryonic scale" (Maderson, '65) from a 33-day embryo. Between the periderm (P) and the stratum germinativum (sg), lie other epidermal cells showing the histology of a mid-late stage 4 of the adult renewal phase. The superficial dermis (SD) contains much looser collagen bundles than the deep dermis (DD) and a dermal melanophore (DM) can be seen at the junction between them. ×720.

Fig. 17. Thin section of epidermis in region outlined in Figure 16. Two layers of flat peridermal cells (P) can be seen; between the innermost layer and the stratum germinativum (sg) can be seen four layers of differentiating cells (sb_{1-4}). Note the mature melanosomes (ml) and the relative electron lucency of two of the four basal cells. ×5,400.

Fig. 18. High power view of region of outer epidermis as outlined in Figure 17, showing two layers of peridermal cells (P_1,P_2) and underlying suprabasal cells. The innermost layer of periderm cells (P_2) contains "arabesque whorls" of electron-dense material cut in various planes but lying at regularly spaced intervals. The subjacent suprabasal cells are the first embryonic Oberhäutchen (sb_1) above two layers of presumptive β-cells (sb_2, sb_3), all identified by bundles of 30 Å filaments in the peripheral cytoplasm; see also Figure 19. ×17,000.

Fig. 19. High power view of bundles of 30 Å filaments (β-keratin precursors) in suprabasal cells, cut longitudinally (B1) and transversely (Bx). ×92,000.

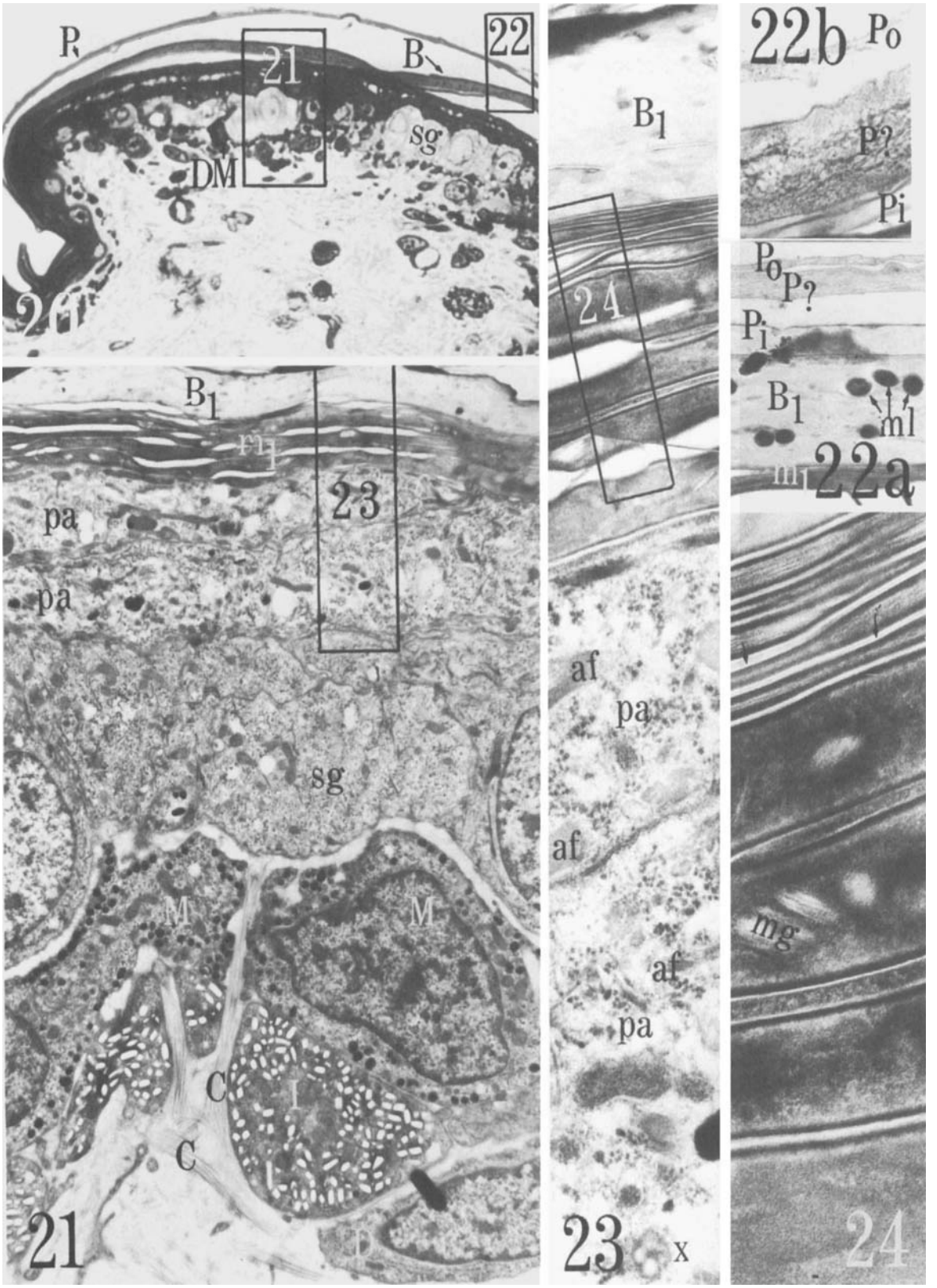
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Fig. 20. Semithin longitudinal section through a dorsal scale of a 40-day embryo showing, beneath the periderm (P), an histology equivalent to that of the inner epidermal generation at late stage 5 of the adult renewal phase. Note the dermal melanophores (DM). ×790.

Fig. 21. Thin section of the first (incomplete) epidermal generation in a region similar to that outlined in Figure 20. The syncitial β-layer (B_1) lies above a mature mesos layer (m_1) and two strata of presumptive α-cells (pa), all having derived from the germinal layer (sg). Note the dermal melanophore (DM) and adjacent iridophore (I) surrounded by collagen bundles (C), and a single fibroblast (D). ×5,500.

Fig. 22. High power views of the mature periderm as shown in Figure 20. In Figure 22a, the innermost layer (Pi) is seen only as a thin electron-dense line lying above the mature β - (B_1) and mesos (m_1) layers. The outermost peridermal layer (Po) is recognizable as being one-cell thick, but the rest of the periderm may be multicellular (P?); ml, melanosomes. ×17,600. In Figure 22b, outermost (Po) and innermost (Pi) layers are again recognizable, but the number of cells between them (P?) is unclear. ×35,000.

Fig. 23. Higher power view of outer part of the first embryonic epidermal generation in region outlined in Figure 21. At this magnification, 30 Å filaments cannot be discerned in the syncitial mature β-layer (B_1). Seven to eight strata of mature mesos cells can be seen (see Fig. 24), and bundles of 70 Å filaments (af) are visible in the presumptive α-cells. For further comment on bundle-like structure (x), see text. ×35,000.

Fig. 24. Lipid-containing intracellular mesos granules (mg) and extracellular material (arrows) from region outlined in Figure 23. Note the very thickened plasma membranes. ×110,000.

DISCUSSION

The early histogenesis of the amniote integument

Previous opinions that the earliest stages of amniote integumentary histogenesis are basically similar are confirmed. The primary ectodermal epithelium representing the presumptive epidermis, consisting of attenuated cells above a population of stellate mesenchymal cells [embryonic stage one, Maderson ('65)] is not described here. During embryonic stages two and three (Maderson, '65), the epidermal cells become cuboidal, a basement membrane is laid down, flattened peridermal cells cover the body surface, and a presumptive dermis becomes recognizable (Figs. 1–3). The present study provides two pieces of information not detectable with the light microscope. First, the outermost peridermal cells bear microvilli (Figs, 14,15), which may indicate the existence of a percutaneous absorption mechanism permitting entry of amniotic fluid into the embryo (see review, Holbrook and Smith, '81). Second, the dermis differentiates precociously with respect to epidermal differentiation as judged by the very early appearance of a complex orthogonal, laminated arrangement of collagen bundles; such a feature has been reported previously only in anamniotes and non-appendage-bearing regions of avian integument (Démarchez et al., '81, p. 216).

The collagen organization is enhanced by a complex array of associated dermal cell processes and other extracellular filamentous materials (Figs. 2, 3, 5–11). As the symmetrical scale anlagen form, we emphasize the predominant location of "anchoring complexes" in what we here term the "interscale regions" which probably represent the presumptive hinge regions (? plus the inner scale surfaces) of the adult scales (Maderson, '64). The anchoring complexes comprise three, or perhaps four, elements—dermal (Figs. 5–8,10) and possibly also epidermal processes (Fig. 9), collagen fibers (Figs. 6–8,10), and thinner, more electron-dense, homogeneous filaments (Figs. 7,8,10), which strongly resemble those present in the anchor filaments figured by Haake and Sawyer ('82) and demonstrated by them to contain, among other components, fibronectin. All the constituent elements of our anchoring complexes are figured and described by Démarchez et al. ('81) and Haake and Sawyer ('82). However, here they are conspicuously concentrated in the interscale regions, intimately related to the basal lamina (Figs. 6,9,10) and the dermal fibroblast bodies (Figs 5,7), and connect the epidermis to the deep epithelial-like dermal fibroblasts. For these reasons, we feel that they might be actively involved in anlage morphogenesis as will be discussed next.

Scale anlage formation and asymmetrization

Older German observations on the putative occurrence of localized epidermal or dermal proliferation in squamate scale morphogenesis are contradictory and inadequately documented (Maderson, '65, '84a). In the embryogenesis of a scaled integument (sensu Maderson, '72a), there must be mechanism(s) responsible for the conversion of a linear system into a contiguous series of symmetrical anlagen and their subsequent asymmetrization. Ignoring for the present that an earlier, prepatterning process must be involved, several basic mechanisms could underly such conformational changes. (1) The epidermis could be passively evaginated by mechanical pressures emanating from the mesenchyme. (2) Intrinsic changes in epidermal cell behavior, such as differential adhesion, microtubule–microfilament interactions, localized proliferation, or migration, could produce placodes that deform above a passive mesenchyme. (3) The contraction of regularly spaced "units" joining the epidermis to the deep dermis could produce the deformation. (4) Combinations of any two, or all three, of the aforementioned could produce the effect.

Each of the two most intensively studied integumentary deformations—the primordia of avian feathers and scutate scales show conspicuous anatomical features that imply that mechanisms (1) and/or (2) are involved. Feathers are first seen as ectodermal placodes and subjacent mesenchymal condensations form soon after (Sengel, '76), whereas scutate scales have complex ectodermal placodes (Tanaka and Kato, '83a,b). As will be discussed below, the mechanisms involved in feather and scutate scale anlage formation are certainly more complex than as stated in (1) and (2) above. Because scale morphogenesis in reptiles does not involve epidermal placodes or mesenchymal condensations (Dhouailly, '75; Maderson, '65; Maderson and Sawyer, '79), mechanisms (1) and (2) and, by extropolation, (4), seem less probable than mechanism (3), which involves predictable morphological features.

If, in a previously homogeneous system (Fig. 25A), a prepatterning mechanism produced regularly spaced units capable of contraction (Fig. 25B), their contraction would give the system a spaced, lentoid configuration. Not only does this not occur, but subsequent asymmetrization would necessitate a second morphogenetic mechanism. Let us assume that, simultaneous with the initial establishment of the contractile elements, changes occurred in the tissues comprising the presumptive anlage cores (Fig. 25C), making these tissues differentially resistant to mechanical forces operating in the vertical plane of the skin. Thus, upon contraction, not only would symmetrical anlagen appear (Fig. 25D), but the first step in asymmetrization (Fig. 25E) would have been taken. With these assumptions in mind, four sets of morphological features can be predicted if mechanism (3) is the basis for reptilian scale formation. First, the entire epidermis should remain homogeneous until definitive overlapping scales become recognizable. Second, the dermis should be initially homogeneous. Third, regularly spaced contractile units should appear simultaneously with changes in the anlage core tissues. Fourth, the changes occurring in the anlage core tissues should facilitate subsequent asymmetrization.

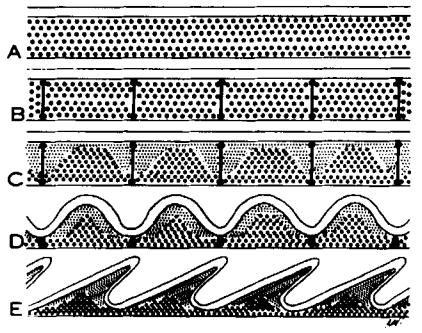


Fig. 25. Schematic representation of a mechanism for converting a linear, homogeneous embryonic integument into a contiguous series of overlapping scales. (A) An intially homogeneous epidermis (fine stippling) lies atop a homogeneous dermis (large dots). (B) A prepatterning mechanism produces regularly spaced, potentially contractile units (double-headed arrows) joining the epidermis to the deep dermis. (C) A prepatterning mechanism produces contractile units like those indicated in (B), but simultaneously, the dermis becomes heterogeneous. (D) Symmetrical scale anlagen form by contraction of the spaced units facilitated by remodelling of the cellular and extracellular components of the dermis (fine stipple). (E) The remodeled dermis now facilitates asymmetrization of the scale analagen, an event possibly involving differential growth of the epidermis of the outer scale surface and, while the deep dermis (medium stipple) remains virtually unchanged, the scale elongates as new superficial dermis is laid down (fine stipple). For further discussion see text.

Figures 1, 5, and 12 show that the epidermis remains homogeneous throughout the integument from the preanlage condition through the onset of asymmetrization. Basal cell proliferation increases the number of layers of suprabasal cells from one (Fig. 2), to two to three (Fig. 5), to five (Fig. 14) during these phases of development. Not until a "perfect embryonic scale" (Fig. 16) forms can regional heterogeneity of the epidermis be recognized.

The preanlage dermis (Figs. 1–3) is homogeneous throughout with a conspicuous array of orthogonal, laminate patterns of collagen fibers between the basement membrane and the deepest epithelial-like layer of fibroblasts (Fig. 2).

With the appearance of symmetrical anlagen, the dermal tissues become conspicuously heterogeneous (Figs. 4–11). Located primarily in the interscale regions are the anchoring complexes discuss under early histogenesis. These complexes seem to join the lamina densa (perhaps even epidermal cells, see Fig. 9) to the deep epitheloid fibroblasts; which are organized in a pattern not described for other integumentary systems. The distance between the lamina densa of the basement membrane and the innermost plasma membrane of the deep epitheloid fibroblasts is approximately 25% greater in Figure 5 than in Figure 2, a fact apparently associated with an increased flattening of the dermal cells and multiplication of the layers of orthogonally arranged collagen bundles. Static morphological data alone cannot prove that these anchoring complexes are "contractile"; tests involving drug action in vitro are required. However, the abundant microtubules in the fibroblast processes (Figs. 9,10) and associated microfilaments (not marked), and the close association between cell bodies and vertically oriented collagen and possibly fibronectin (Fig. 7), suggest a skeletal function (Jacobson and Ebendal, '78). Figures 4 and 11 show that the core tissues of the scale anlage become heterogeneous from the preanlage condition. More cells are present, but there is no evidence of localized condensation and/or proliferation. The relative absence of orthogonal collagen networks beneath the epidermis and the greatly in-

creased numbers of stellate mesenchymal cells (Fig. 11) is strikingly different from the preanlage appearance (Fig. 2). The deeper portions of the core (X, Fig. 4 and L, Fig. 11) resemble the interscale regions (Fig. 5), although anchoring complexes are less numerous. However, the deep central collagen mass seems to have been isolated from that of the interscale region (Fig. 11) These data suggest that remodeling of mesenchymal tissues facilitates scale morphogenesis and further studies might reveal activites involving extracellular and cell surface proteins similar to those that have been documented in avian feather condensations (Kitamura, '80, '81; Knapp and Sawyer, '83; Mauger et al., '82).

When asymmetrization begins, the histology of the dermis is uniform across the body of the anlage (Fig. 13), but in the overlapping scale (Fig. 16), the superficial dermis, especially toward the posterior scale tip, contains fewer cells and less collagen than the deep dermis. Thus, the deep dermis is seen as a strand of tissue directed toward the scale tip (Maderson, '65, his Fig. 7a), and a similar histology is seen during scale neogenesis in the adult (Maderson et al., '78a). Asymmetrization must involve differential growth of the epidermis of the outer scale surface. Although this has not been studied in embryonic material, Lillywhite and Maderson ('68) showed that distal migration of daughter cells occurred during foot-pad histogenesis and *Anolis carolinensis*, and argued that differentiation of this complex structure must involve dermal remodeling.

The entry of the embryonic squamate epidermis into the adult cycle: Structural and functional implications

The present data permit two questions to be addressed. First, how does the embryonic epidermis enter the adult shedding cycle? Second, why do some neonate squamates shed once or twice soon after hatching or birth?

The entry of the embryonic epidermis of *L. muralis* into the typical, adult cycling mode is accompanied by ultrastructural features essentially similar to those that have been described for the adult system (Maderson et., '72) with two differences. First, at no time do the basal or suprabasal cells contain conspicuous bundles of 70 Å filaments so typical of immature amniote epidermal cells (loc. cit.); this must be interpreted as an embryonic feature of uncertain significance. Second, the first embryonic Oberhäutchen differentiates beneath a specialized innermost layer of periderm cells (Figs. 14,15,17,18), not a clear layer of an outer epidermal generation as in the adult system (Maderson et al., '72; Maderson, '84a).

Few data are available concerning the reptilian periderm by comparison with what is known concerning this tissue in mammals and birds (Holbrook and Smith, '81; Sawyer et al., 74; Sengel, '76). In embryos of *L. muralis*, periderm structure is difficult to discern with the light microscope unlike the situation in mammals and birds (loc. cit.). Ultrastructural examination reveals three important features. First, the tissure consists of a minimum of two layers of cells (Figs. 14, 15,17,18) but more in some locations (Fig. 22). Second, cells in the outermost layer(s) are extremely flat, but we have been unable to discern the size of cytoplasmic filaments. Third, the innermost layer of the periderm is characterized by arabesque whorls of electron-dense materials quite dissimilar from periderm inclusions which have been described in mammals and birds (loc. cit.), and, while this layer does cornify completely in the late embryo (Fig. 22), we do not know what role these materials play in differentiation, nor the nature of the mature structural protein. There is a general similarity between the stratified squamous periderm of the late embryo and its subjacent first embryonic generation and post-trauma epidermis reentering the normal cycle beneath a wound epithelium (Maderson et al. '78a). Because Oberhäutchen and β-cell differentiation involve the activation of transcription/translation processes to form "β-keratins," a major question is the nature of the "trigger" responsible (Chiu and Maderson, '75; Maderson, '84a). In vitro studies of adult lizard integument (Flaxman et al., '68), supported by xenoplastic recombinations between embryonic lizard and avian material (Dhouailly, unpublished data), suggest that the capacity for cyclic cell production and differentiation is intrinsic to the epidermis. The mechanism may involve a feedback loop emanating from the more superficial corneous tissues (Flaxman et al., '68; Flaxman, '72). The present study confirms previous histological conclusions (Dhouailly, '75; Maderson, '65) that the first β-layer (with its Oberhäutchen), differentiates beneath a periderm, which varies quantitatively. The circumstances under which β-protein synthesis can be triggered—

in the embryo, in normal, and in post-trauma adult integument—involve a variety of milieux for emergent daughter cells. Thus, further speculation on the nature of the triggering mechanism must await ultrastructural study of post-trauma epidermal restoration and further investigations of the possible roles of patterns of basal cell DNA synthesis and mitotic activity (Maderson, 84a).

Figure 26 combines embryonic data with data on the adult cycle [see references in Landmann et al. ('81); Maderson and Chiu '81)]. The primary ectodermal epithelium [embryonic stage one, Maderson ('65)] differentiates into the condition shown in Figure 26A where a stratum basale, consisting of cuboidal cells lying on a basement membrane, is covered by a single layer of flat periderm cells (P_1) (Figs. 1,2). Further proliferation produces a multilayered periderm beneath which are some less flat cells (Fig. 26B, light stippling; Fig. 4). Figure 26C indicates the quantitative variability reported here for the periderm (Figs. 14,15,17,18). This does not seem to be a regionally specific character and may result from insensible desquamation into the amniotic fluid. In Figures 26C through G, an innermost layer of peridermal cells (labeled as "Pi"), later characterized by arabesque whorls of electron-dense inclusions (Figs. 17,18), is emphasized. In Figures 26C through E, a stratified squamous cell population, previously termed the "presumptive/embryonic stratum corneum" (Maderson, '65, pp. 280–283) lies between the periderm and the stratum basale. Its cells contain bundles of 30 Å filaments (Figs. 17–19) so that the layer lying beneath the periderm is identifiable as the first Oberhäutchen (Ob_1, Figs. 26C–E) and those beneath, presumptive β-cells (Figs. 17–19; Figs. 27D and E, B_1). These Oberhäutchen and presumptive β-cells mature as the β-layer of the first embryonic epidermal generation (Figs. 20–23; Figs. 26F–L, tissue marked by "β-s" as part of OG_1).

Previous transmission electron microscope studies of adult squamate epidermis emphasize that "presumptive" Oberhäutchen and β-cells possess initially only 70 Å filaments, so that they are identifiable only by their location and time of appearance. Subsequently, accumulations of 30 Å filaments (inferred to be the first morphologically identifiable precursors of β-keratin) are seen. In Figures 14 and 15 concentrated and diffuse masses of granular material appear in these cells *and* in the germinal cells. The individual granules are less electron dense and at least five times larger than ribosomes, and are larger and more electron dense than glycogen granules. We suggest that these are not simply embryonic specializations because similar (but unlabeled) inclusions are discernible in electron micrographs of presumptive Oberhäutchen and β-cells in adult lizard (Maderson et al., '72) and snake [Landmann ('79) and references therein; Contard, personal communication]. They are also visible in some of Matulionis' ('70) figures of developing chick down feathers and in various papers by Sawyer and his colleagues reporting on avian scutate scales [see references in Sawyer ('83)]. They may be more noticeable in embryonic material because of the relative paucity of bundles of 70 Å filaments. The inclusions are interpreted as even earlier morphological manifestations of β-keratin synthesis than the more typical peripheral bundles of 30 Å filaments (Figs. 17–19), although this interpretation will require future substantiation by high resolution immunohistochemical procedures (O'Guin et al., '82). The one to three layers of diagonally cross-hatched cells in Figures 26D–H represent those in Figures 20, 21, 23, and 24, which lack bundles of 30 Å filaments, have very thickened membranes, show characteristic lamellar inclusions and are extremely flattened. All of these features have been reported in adult tissues (Landmann, '79, '80; Maderson et al., '72) and permit the identification of these cells as the mesos layer of the first embryonic epidermal generation (m_1, Figs. 26D–H).

Although we have not followed β-layer maturation completely in embryonic material, we can identify Figure 16 as a mid-to-late stage four of the adult cycle, and Figure 20 as a stage five; these identifications are based on the columnar, chromophobic appearance of the basal layer (Figs. 16,17,20) shown schematically in Figure 26E. The electron-dense inclusions in the innermost peridermal cells (Figs. 17,18; 26E–G, P_i) must coalesce (? gradually or suddenly) because, in the late embryo, the cells are seen as a thin, electron-dense line, separated artifactually from the surface of the first mature β-layer (Fig. 22). Figure 22 permits the identification of a single outermost cell layer (Po), a single innermost layer (Pi) and between them a region of exceptionally flattened cells, which appear to

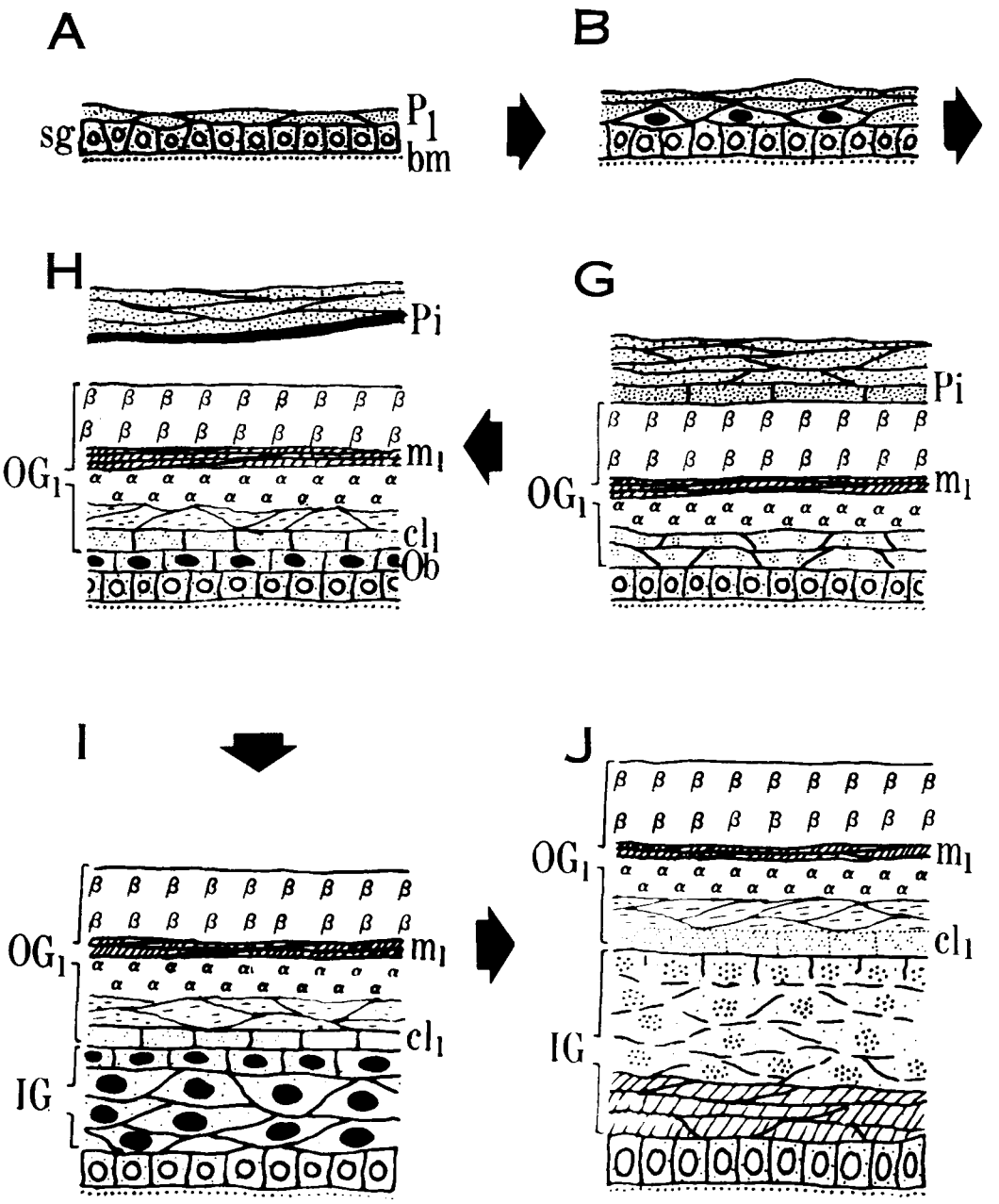
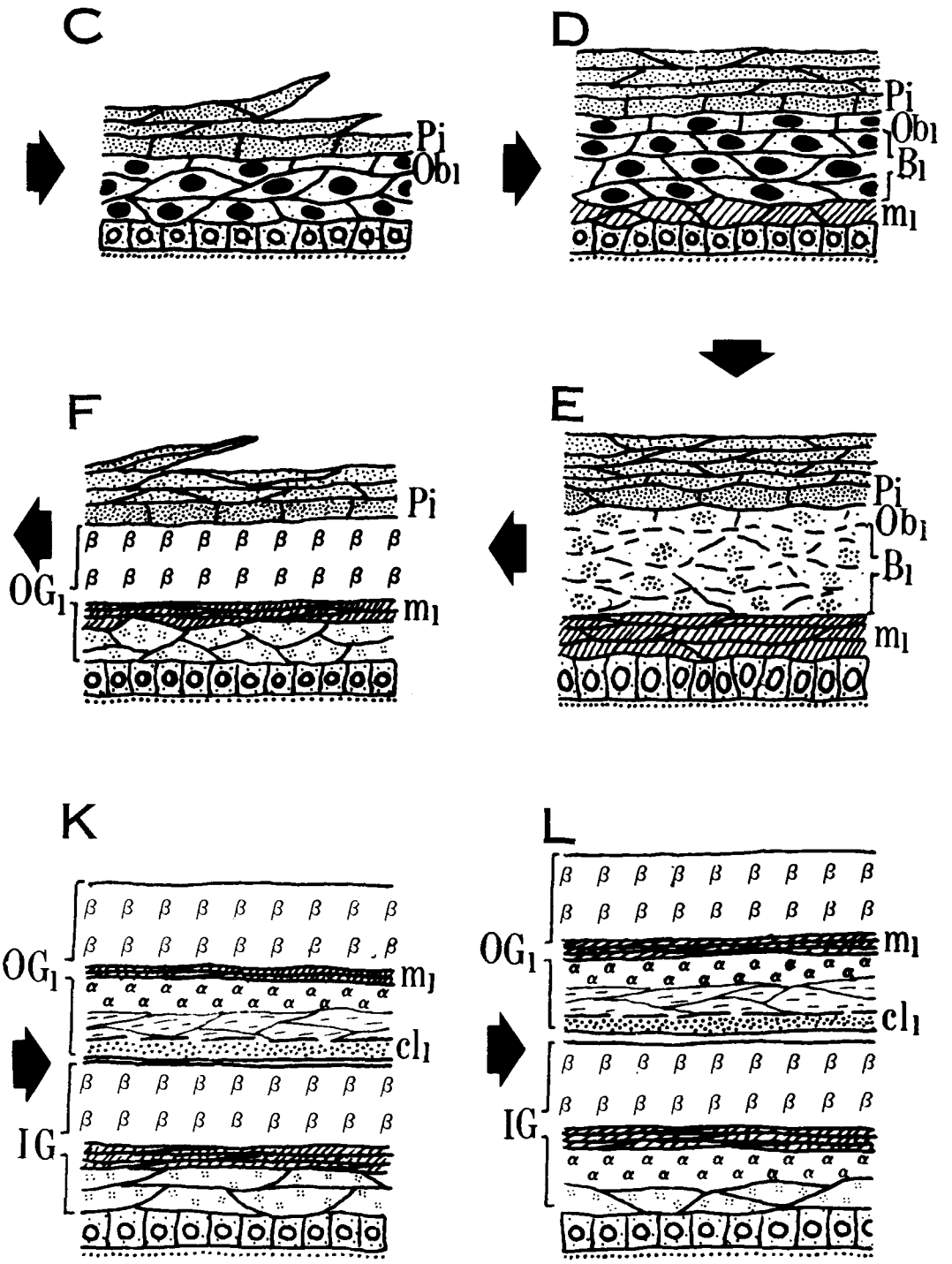


Fig. 26. Schematic representation of in ovo/utero squamate epidermal histogenesis leading to entry of the system into the adult cycling mode. For explanation see text.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be parakeratotic (P?); the tissue is shed from the body in ovo (Maderson, '65, p. 286). In ovo peridermal shedding is shown schematically in Figure 26H, a diagram that reflects other data concerning epidermal activity during late embryonic and posthatching natal life.

Maderson ('65, pp. 286–297) reported shedding in the third postnatal week in *Anguis fragilis*, but not in *L. vivipara*. Late snake embryos were described as showing what are now recognized as renewal phase histologies and reports of postnatal hatching in snakes were reviewed (Maderson, '65, p. 201); *some* newly born *Thamnophis sirtalis* shed within days (Maderson, unpublished), while newly hatched tokays (*Gekko gecko*) shed within hours of hatching (Maderson, '63). What is the significance, if any, of this variability in posthatching/natal shedding between and among species?

The study of the control of shedding frequency in squamate reptiles has long concentrated on endogenous hormones (Maderson and Chiu, '81; Maderson et al., '70; Maderson, '84a), but recent data imply that environmental humidity could be equally important (Maderson, '84b). The passive barrier to percutaneous water loss is the mesos layer (Landmann et al., '81; Lillywhite and Maderson, '82; Maderson, '84b; Roberts and Lillywhite, '83).

Some neonates, having shed the periderm in ovo (Fig. 26H), have an epidermal histology like that shown in Figure 26G. The incomplete outer epidermal generation (OG_1) has mature β - and mesos layers and an incomplete α-layer separated from the basal layer by two to three strata of suprabasal cells (quadrant stippled in Fig. 26G). Such is the equivalent of a "postshedding resting state" (Chiu and Maderson, '75) and the individual (or species) would subseqently enter the typical adult epidermal cycling mode. Figures 26H through K represent a different strategy. Near the time the periderm is shed in ovo, continued basal cell proliferation completes the first outer epidermal generation (OG_1) with an innermost clear layer (cl^1) lying immediately above the Oberhäutchen of a second epidermal generation (Ob_2, Fig. 26H): stage 2 of the normal adult shedding cycle (Chiu and Maderson, '75). Further proliferation produces an identifiable presumptive inner epidermal generation identifiable (IG Fig. 26I). Subsequently, these cells, and more resulting from continuing proliferation, constitute an incomplete second epidermal generation (IG, Figs. 26J,K). These events, beginning in the very late embryonic period, can permit shedding of the first epidermal generation (OG_1, Figs. 26I–L) any time within 2 weeks of free life, a period of time occupied by the renewal phase (Chiu and Maderson, '75). The only constraint on the exact time when a postnatal/hatching shed occurs is when the first clear layer (cl_1, Fig. 26K) matures and separates from the subjacent Oberhäutchen, the outermost component of the syncitial β-layer of the inner generation (Maderson, '66). Figures 26J through L represent the late renewal phase and when shedding occurs (Fig. 26L) the inner generation (IG) takes its place as the functional body surface; typical cycling continues throughout life. In either strategy, for 2 hr to 10–12 days of life, the neonate has as the functional body surface the first embryonic epidermal generation. Can these strategies be explained?

Because the length of the incubation/gestation period is temperature dependent, a precise neonate morphology is impossible to describe. Thus variablility in neonate shedding behavior might reflect merely our detailed knowledge of the temporal dynamics of epidermal cell activity. This would imply that all quantitative and qualitative parameters are determined precisely from the time epidermal histogenesis begins. Such an explanation can be criticized on several grounds. First, the relative paucity of 70 Å filaments in basal and suprabasal cells during embryonic differentiation is unlike the adult conditions. Second, while the differentiation of the first embryonic epidermal generation generally resembles that of the adult cycle, there is only the periderm above it. Thus, the *source* of the putative intraepidermal feedback loop controlling cell differentiation (Flaxman et al., '68; Flaxman, '72) differs from that in the adult, implying impossible qualitative differences in embryonic keratinization processes. Third, that data exist demonstrating inter- and intraspecific differences in neonate shedding behavior implies the existence of another factor(s) influencing epidermal activity.

The ambient humidity to which the neonate integument is exposed could be high, low, or intermediate, but, because we are dealing with microenvironmental factors, we would always expect variation around a statistical mean. From data showing the role of

the mesos layer in reducing percutaneous water loss, certain predictions can be made concerning its properties in the first embryonic generation.

If the neonate's microenvironment usually has a low humidity, selection would have produced an adequate first mesos layer and shedding would not be expected. However, if the microenvironment met most frequently by the neonates had a high humidity, high mortality would be expected if local weather conditions produced a temporary dry spell. This exigency could be met if the neonates could produce a new body covering with a more effective barrier within a short period; such a capacity has been demonstrated by manipulations of the environmental humidity of adult garter snakes (Maderson, 84b). Populations or species inhabiting environments where intermediate humidities were available to the neonates would show variable behaviors between individuals, because some will always be "excessively constructed" (Gans, '79; see discussion, Maderson, '84b).

Indubitably, there are other functional parameters that influence neonate shedding behavior, as indeed there appear to be in adults (Maderson, '84b). However, the importance of appropriate water balance of the neonate cannot be overemphasized. Following birth or hatching, while remnant yolk sacs can provide food, and the kidneys can cope with excretion, the neonate must control its water economy. The predictions made in the foregoing analysis are verifiable by further observation of neonate shedding behavior. They also provide a general explanation for the facts that individuals of this age group are rarely encountered in the field and that many species seem to have a high fecundity that balances the high infant mortalities so frequently observed in the laboratory (Maderson, personal observation).

ACKNOWLEDGMENTS

The authors are extremely grateful to Drs. B.A. Flaxman (Brown University), R.H. Sawyer (University of South Carolina), and P. Sengel (Université de Grenoble) for their comments on an early draft of this manuscript. Thanks are due to N. Tresallet, A.M. Boutineau, and Y. Bouvat for technical assistance. The work and manuscript preparation was supported by grants from National Institute of Health NS-13924, and from the PSC-BHE program 14056. Ms. Judith Steinberg typed the manuscript.

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