Zoomorphology

On the fine structure of the skin of larval, juvenile and adult *Ichthyophis* (Amphibia, Caecilia)

Harold Fox

Department of Zoology and Cell Biology, University College, Gower Street, London WC1E 6BT, United Kingdom

Summary. The skin of a number of larvae at different developmental stages (in part determined by the level of differentiation of a variety of skin components), of a juvenile 9–10 cm specimen and of adults of *Ichthyophis* was examined by electron microscopy. More important results include descriptions of (a) the larval Leydig cells and their differentiation; (b) the fully developed Merkel cells and their synaptic association in older larvae and the postmetamorphic forms; (c) the structure and arrangement of the postmetamorphic flask cells and their associated nerves in the epidermis; (d) the relatively early development and differentiation of the mucous and granular glands in the dermis of the larva, comparable with those of adults; (e) the presence of iridophores in the dermis of the juvenile specimen and their relationship with the laminophore type cells. Some comparisons are made with previous descriptions of young larval and adult skin of *Ichthyophis*. The level of cellular differentiation of Leydig cells, Merkel cells and the glands would imply that they were fully functional in older premetamorphic larvae of *Ichthyophis*: indeed these components are recognizable, albeit in immature form, in young larvae 4.5 cm long.

A. Introduction

For more than a century the skin of adult caecilians has been extensively studied by light microscopy, but only within the last decade or so has information been published on its ultrastructure, including that of the scales (see references in Fox 1983, 1986 a).

Welsch and Storch (1973) first described the fine structure of adult skin of *Ichthyophis kohtaoensis* Taylor, 1960, and also that of a 3 cm larva. Subsequently, Fox and Whitear (1978) described Merkel cells in adult epidermis of *Ichthyophis*, and Welsch (1981) provided some information on the larval gills of *I. paucisulsus* Taylor, 1960. More recently Warnschaffe et al. (1985) investigated the fine structure of the lateral-line organs of the head of larval *I. kohtaoensis* 6.8–14.2 cm long. Fox (1983, 1985 a, 1986 b) reported on the skin of the adult body and tentacles of *I. kohtaoensis* and *I. orthoplicatus* Taylor, 1965, and also features of the epidermis of a 2 cm *Chthonerpeton indistinctum* Reinhardt and Lütken, 1861, and a 4.5 cm *I. kohtaoensis*. It is clear that so far, most of our knowledge of the fine structure of caecilian skin is limited to very young larvae and adults.

The present work attempts to fill the gap in our knowledge of the development of caecilian skin during larval life, towards metamorphosis. A rough developmental pattern of a variety of skin components is established during this period. A number of features are described, hitherto not reported ultrastructurally, in skin of several older larvae and a 9–10 cm juvenile of *I. kohtaoensis* and of adult *I. orthoplicatus*. Of particular interest, iridophores are reported for the first time in caecilian skin and their relationship together with cellular tissue containing 'crystals' (Jørgensen 1981), subsequently termed laminophores (Fox 1983, 1985a) is described. The precocious development of the dermal glands in larvae of *I. kohtaoensis* distinguishes this form from members of other groups of living amphibians, a feature which may possibly be an adaptation to the mode of larval life.

B. Materials and methods

All the larvae and the juvenile 9–10 cm specimen of *Ichthyophis kohtaoensis* (adult length about 28 cm; Taylor 1968) used in the present work, were sent to me by Prof. Ulrich Welsch of the University of Munich. Living adults of *I. kohtaoensis* and *I. orthoplicatus* were received from Dr. G. Vevers of the Zoological Society of London and Prof. Carl Gans of the University of Michigan.

Fixation methods for the skin of the 4.5 cm *Ichthyophis* larva, from N. Thailand, and the adults have been described (see Fox 1983, 1985a, 1986 b).

Skin from older larvae (stages A–C) and the postmetamorphic 9–10 cm juvenile from Sumatra was fixed similarly in glutaraldehyde, post-osmicated and then dehydrated and embedded in Araldite as previously described.

Skin of the 4.5 cm larva (Fox 1986 b) was taken from the branchial region (including the external gills) and the mid- and hind-body regions. In the present work, anterior body skin (stages A and B), mid-body skin (stage C and the 9–10 cm juvenile) and belly skin (adult *I. orthoplicatus*) was investigated. Details of the lengths of larval stages A–C were unavailable and so the stages were arbitrarily categorized in terms of their skin structure. The progressive sequence of development was referred to the degree of development of the figures of Eberth in the basal epidermal cells (Fox and Whitear 1986), the number and thickness of the plies of collagen of the basement lamella, the level of differentiation of the Leydig cells and Merkel cells and the complexity of the cellular structure of the dermal glands. The

9–10 cm juvenile is clearly postmetamorphic, for it has an epidermis of 5–7 layers of epithelial cells and a keratinized skin surface. The Leydig cells and figures of Eberth have disappeared, and flask cells are present in the epidermis and scales in the dermis.

Silver-grey Araldite-embedded sections were viewed under a JEM JEOL CX 11 electronmicroscope. Thick Araldite sections (approx. 1 μm thick) stained with toluidine blue were examined for general gross structure and photographed with a Zeiss photomicroscope.

C. Results

1. Epidermis

The epidermis of larval stages A–C typically has two to four layers, but cilia, present at the surface in earlier stages, have disappeared; it is now lined by microvillous cells. Many of them contain an extensive area of very fine lucent substance, sometimes seen surrounded by mitochondria, and situated proximal to the lobulated nucleus which has a prominent nucleolus. A fine granular area is not seen in surface cells of stage C, the putative oldest larval stage, nor in postmetamorphic forms. The basal cells include large well developed figures of Eberth and bobbins (larval hemidesmosomes). The extensive and massive skeins of tonofilaments extend from the bobbins to lead throughout the cell proximal and distal to the nucleus. The epidermis also includes melanocytes and an occasional mitochondria-rich cell and macrophage.

Leydig cells, with largest dimensions of about 18 μm by 15 μm, are common, albeit only found in the branchial epidermis of the 4.5 cm larva, though granular cells are still found in this region. In different regions of the body of older stages A–C larvae granular cells are not present.

They have differentiated into Leydig cells which are generally larger than those of the 4.5 cm specimen (Figs. 1–3). The Leydig cells are either round, oval or elongate, sometimes pear-shaped in profile, up to 25 μm long and variable in width. The nucleus is lobulated, in contrast to the smooth, rounded-shaped nucleus frequently seen in the Leydig cells of the 4.5 cm larva. In numerous profiles of a variety of Leydig cells of older larvae, the nucleus is seen to be surrounded by an RER (Figs. 2–4). Other areas may be filled, to a greater or lesser extent, with lucent vesicles which frequently merge with those adjacent and are derived from the dense granules of the granular cells. Dense granules and lucent vesicles usually coexist but the most highly developed Leydig cells mainly contain lucent vesicles (Figs. 2, 3). Occasionally, electron-dense vestiges of the granules are found within the vesicles, reflecting their developmental history (Fig. 5). Near the periphery of the cell there may be mitochondria, a Golgi complex, ribosomes, small vesicles, microtubules and aggregates of microfilaments, the major components of the Langerhans network (ln) (Hay 1961), though one or other of these components may be missing in a particular cell profile (Fig. 4). Indeed in some cases the periphery is composed of a dense band of ribosomes (Fig. 3).

Merkel cells originate (or at least are first recognizable) in the epidermis of Ichthyophis around the 4.5 cm stage. They are well-developed and have synaptic association with adjacent neurites in older larvae (Fig. 6) and postmetamorphic juveniles and adults (Fig. 8). This is in contrast to the Leydig cells, which have disappeared in post-larval forms (Fox 1983).

In a stage A larva, for example, an oval-shaped Merkel cell about 11 μm long (nucleus 9 μm × 9 μm), with several well-developed microvillous processes, contained more than 100 membrane-bound granules (mbg) in a single profile.

Fig. 1. Granular cell from the epidermis of the middle-body region of a 4.5 cm larva of Ichthyophis kohtaoensis. Note the heavy content of disc-like dense granules (g) in the region distal to the basal nucleus (n), which may be smooth-surfaced or lobulated

Fig. 2. Stage B older larva of I. kohtaoensis. Leydig cell in the epidermis showing distally located granules (g) of varied electron density, and lucent vesicles (v) derived from them. The basally-located, slightly lobulated nucleus (n) is surrounded by a granular endoplasmic reticulum (see Fig. 4)

Fig. 3. Stage C larva of I. kohtaoensis. Leydig cell with an extensive area of lucent vesicles (v) derived from the dense granules. The irregularly-shaped nucleus (n) is situated basally in the cell and the periphery is composed mainly of densely packed ribosomes and granular endoplasmic reticulum (rer)

Fig. 4. Stage B larva of I. kohtaoensis. Peripheral region of a Leydig cell showing granular endoplasmic reticulum (rer), mitochondria (m), a Golgi complex (gc), a large lucent vesicle and numerous small round vesicles, ribosomes and an aggregate of dense microfilaments near the periphery (arrowed) of the Langerhans network (ln)

Fig. 5. Stage C larva of I. kohtaoensis. Lucent vesicle (v) of a highly developed Leydig cell. A vestigial granule (g) is still present, in the process of dissolving into lucent substance of the vesicle

Fig. 6. Stage B larva of I. kohtaoensis. Highly developed Merkel cell in the epidermis. Microvillous processes originate from the cell (mp), and there are more than 300 membrane-bound granules (mbg) within it, a numerical level comparable to that frequently found in Merkel cells of adults but never seen in these cells in the younger 4.5 cm larva of Ichthyophis. The cell is closely enveloped by neurite branches (ne)

Fig. 7. Stage A larva of I. kohtaoensis. Merkel cell region showing membrane-bound granules (mbg), mitochondria (m), ribosomes, smooth and granular endoplasmic reticulum, tonofilaments (tf) and two small cytokeratin aggregates (ca), formed from masses of tonofilaments

Fig. 8. A Merkel cell in the epidermis of adult belly skin of Ichthyophis orthoplicatus, with highly developed reciprocal pre- and post-synaptic junctions (arrowed) against its surface. Numerous membrane-bound granules (mbg) are in the region of the synapse (sy), and the cytoplasm is heavily fibrous
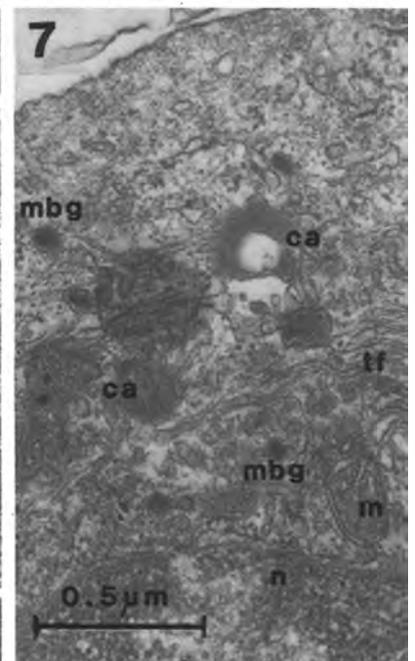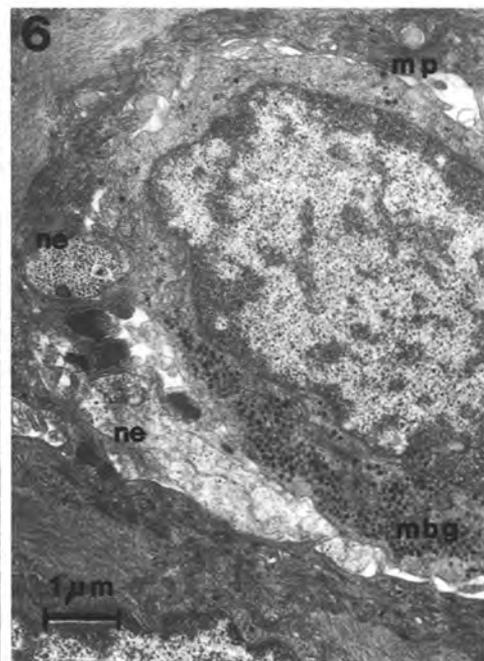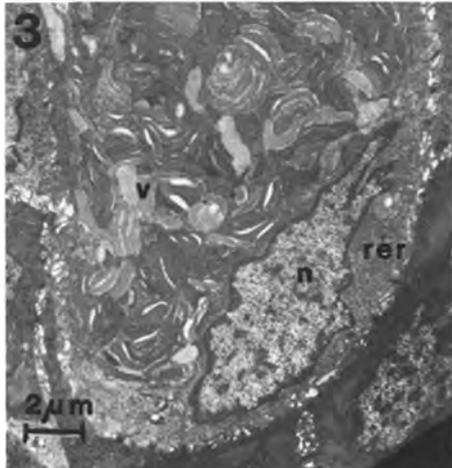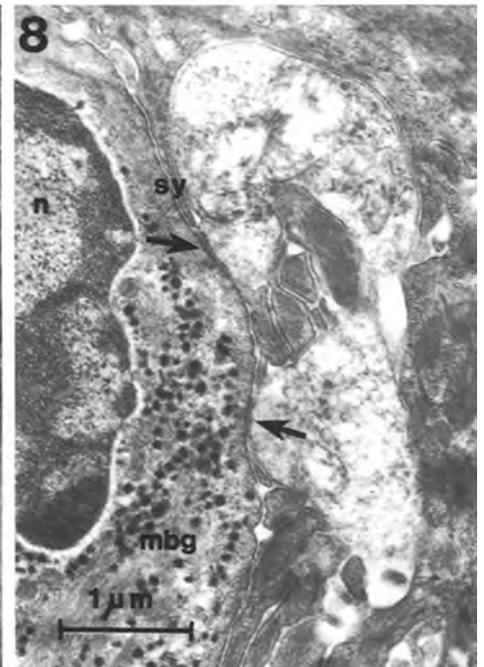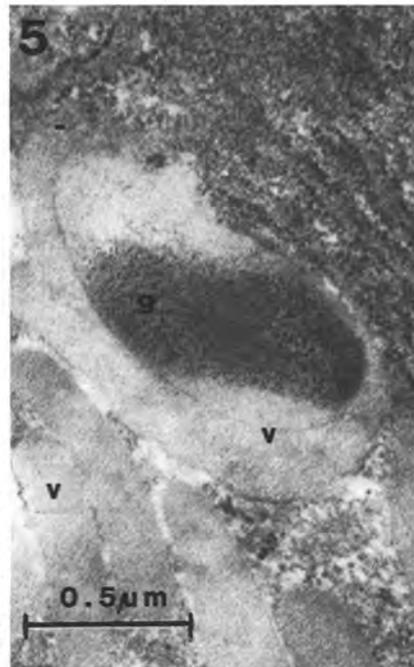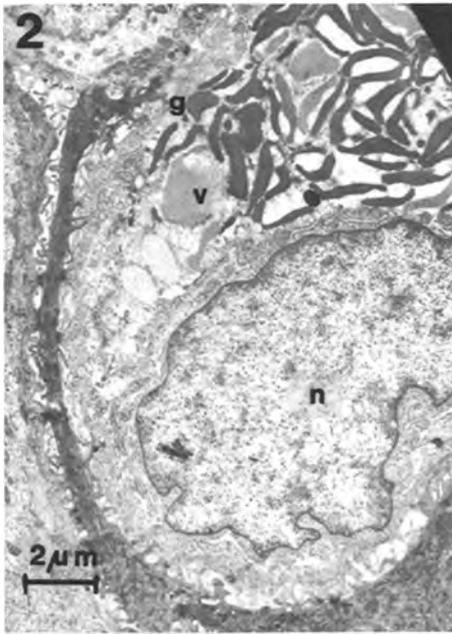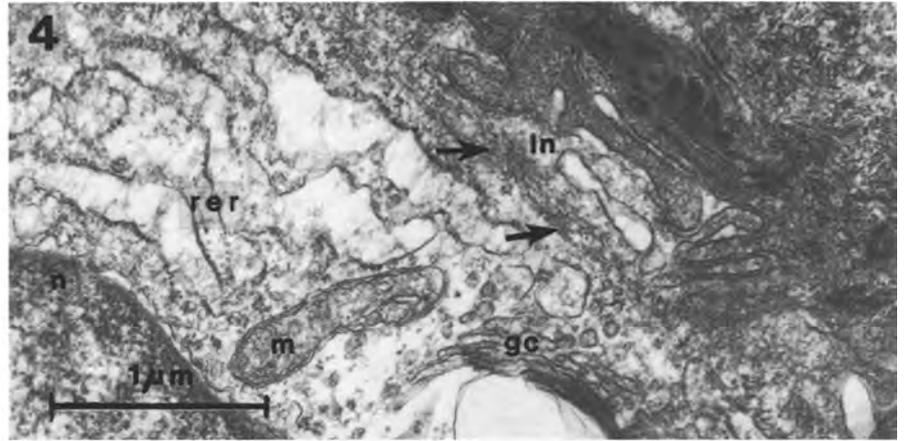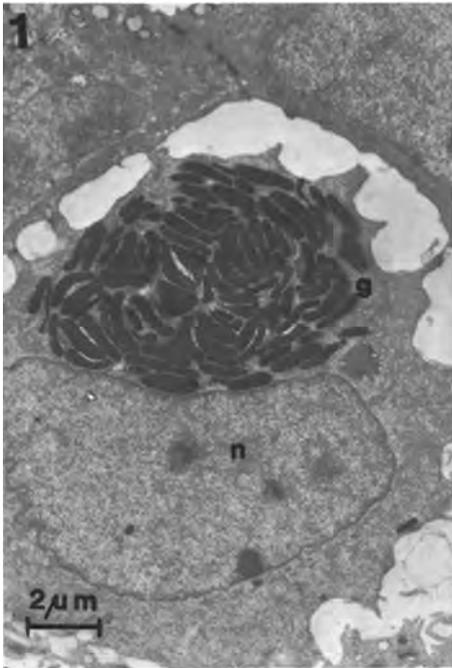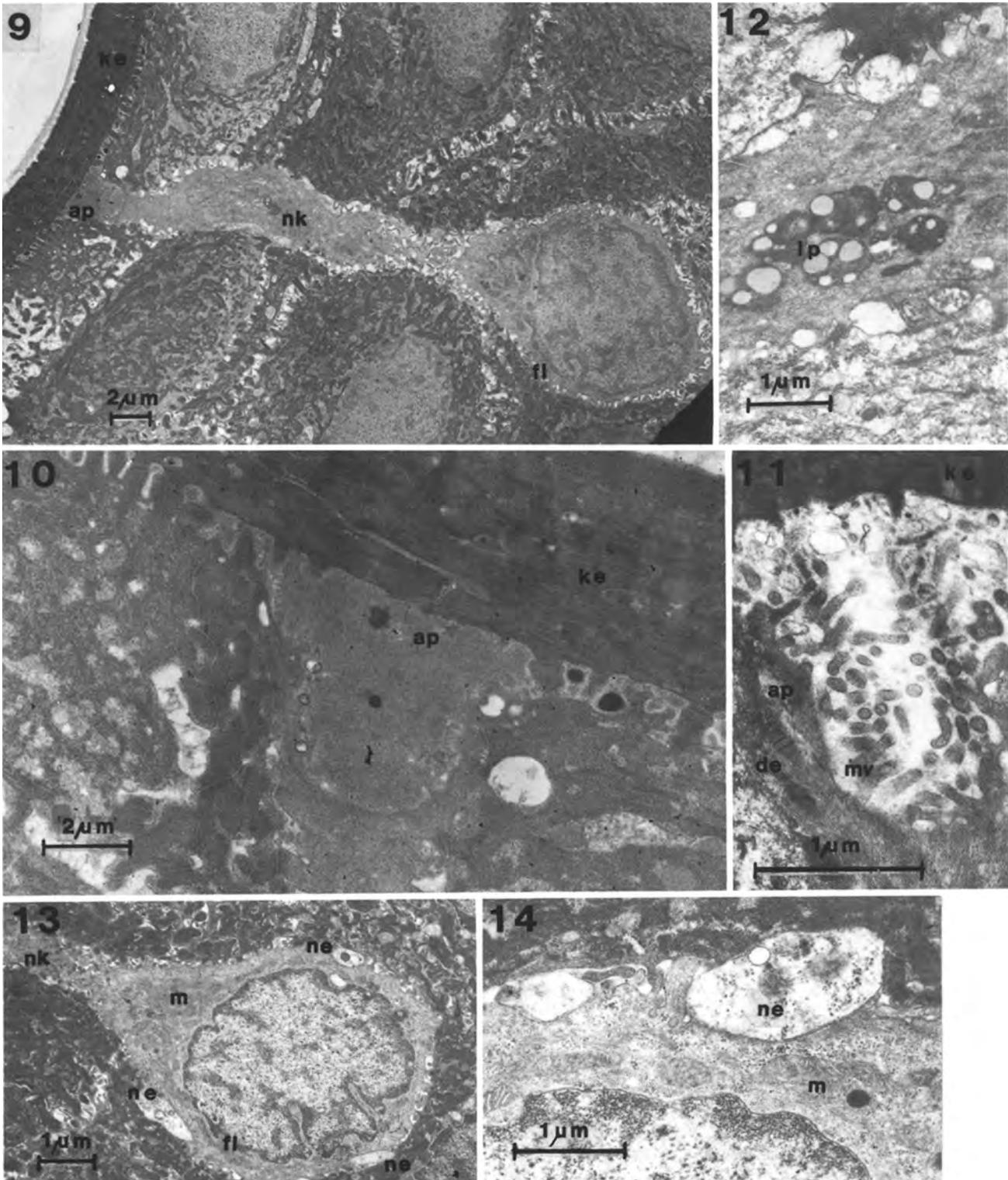
Fig. 9. Flask cell (approx. 33 μm long) in the epidermis of an adult *Ichthyophis orthoplicatus*, with an extended neck region (*nk*) leading from the flask (*fl*), at right angles to the skin surface and terminating at the apical region (*ap*), below the keratinized outer cell surface of the epidermis

Fig. 10. A higher magnification of the margin of the apical region (*ap*) of the flask cell of **Fig. 9**. This area is closely apposed to the lower surface of the keratinized surface cell of the epidermis

Fig. 11. Central region of the cup-shaped apex (*ap*) of an adult flask cell. Note the microvilli (*mv*) extending into the sub-surface cavity – the 'lake' – indicative of early moulting. There are no desmosomes in this microvillous layer. The desmosome (*de*) shown in the illustration joins the flask cell to an adjacent epithelial cell in the lateral region

This numerical feature, together with other examples in different Merkel cell profiles, provides an indication of the increase in number of these organelles compared with the relatively sparse number found in the Merkel cells of the younger 4.5 cm larva. In addition, there are numerous tonofilaments in the cytoplasm and occasionally a small cytokeratin aggregate (ca) was found (Fig. 7; see Fox and Whitear 1986). The adjacent synaptic neurone contained mitochondria, small lucent vesicles and glycogen granules, as with synapses in older specimens. In the 9–10 cm juvenile, among several processes seen in a profile of a Merkel cell 11 μm long and 6 μm wide, one almost 3 μm long deeply indented a neighbouring epithelial cell. In skin of previously investigated adult Ichthyophis, Merkel cells were not found with synaptic association (Fox 1983; Fox and Whitear 1978). However, in belly skin of adult I. orthoplicatus in the present work, one of a number of Merkel cells possessed an extensive innervation with paired synapses of pre- and post-synaptic densities on the opposing cell and neurite membranes (Fig. 8; see Fox and Whitear 1978; Hartschuh et al. 1986). Another Merkel cell had two large associated neurites, each with prominent synapses against the surface.

Flask cells, when oriented perpendicularly to the epidermal surface, are recognizable in the 9–10 μm juveniles and adults (Fig. 9) by their shape, desmosomal connexions with adjacent epithelial cells, high content of mitochondria and microvillar apical membrane, which extends into a sub-surface region beneath the keratinized surface cell. A prominent Golgi complex is frequently seen over the nucleus in the base of the neck. Flask cells previously described in the epidermis of adult Ichthyophis (Fox 1983), were up to 20 μm long, 7 μm wide at the flask and 3 μm at the neck. In adult belly skin of I. orthoplicatus in the present work, some flask cells reached up to 35 μm, the neck was 20 μm long and 4 μm wide and the flask 10 μm wide. The apical termination of the cell is typically cup-shaped, its periphery spread out and flattened somewhat (Fig. 10); the microvilli (mv) of the central region of the apex extend into the sub-surface cavity (Fig. 11), akin to the 'lakes' found during amphibian moulting (Fox 1986 a).

Located in the neck of a flask cell, over a mass of mitochondria and amidst an extensive array of tonofilaments, a group of lipid-like organelles (lp) within a myelin-like configuration is occasionally seen. Similar, though isolated, lipid-like organelles may be found in the flask region of other cells (Fig. 12). These structures may be metabolic features of cellular differentiation, or perhaps lysosomal bodies concerned with degeneration, though necrotic areas were not seen in the rest of the cell.

Flask cells may be closely associated with neurites and up to three separate nerves, or their branches, were seen closely apposed to the surface of the flask or against the base of the neck or higher up (Fig. 13). Examination of serial sections of numerous flask cells, however, never revealed any convincing examples of synaptic junctions between neurites and the flask cell surface, notwithstanding their intimate association (Fig. 14).

2. Dermis

The dermis of the older larvae of stages A–C of Ichthyophis, in the present work, is substantial and varies in thickness between 0.6 mm and 1 mm (Fig. 15). The dermis is more highly developed than in the 4.5 cm stage, though even in the same specimen some, at least, of the anterior body skin components may show a greater degree of development than similar, more posterior components (Fox 1986 b; Fox and Whitear 1986). In the 4.5 cm larva, a moderately developed basement lamella is about 7 μm thick, made up of 9–10 loosely associated orthogonally arranged bundles of collagen. In the older larvae the basement lamella (bl) is up to 10 μm thick and contains up to 15–16 piles of collagen. The stratum laxum includes glands, fibroblasts, melanophores, laminophores (see Fox 1983, 1986 a), capillaries and myelinated and non-myelinated nerves with Schwann cells. This region is the site of future scale formation (Zylberberg et al. 1980). Below the glands, a stratum compactum (sc) is about 20 μm thick, made up of about 20 orthogonally arranged bundles of collagen, limited by elastic fibres and a thin bounding inner layer of endothelial cells.

The glands are well developed in older larvae. Ducted mucous and less common granular glands (Figs. 16, 17, 19) are composed of secretory cells and peripherally situated myoepithelial and mitochondria-rich cells. Myoepithelial cells (mec) may have discrete membrane densities at their external surface, reminiscent of early developed hemidesmosomes. There is a comparable 'adepidermal membrane' enclosing an 'adepidermal space', similar to these features found beneath the basal epidermal cells (Fig. 18).

The dermis of the 9–10 μm juvenile Ichthyophis has an extensive content of iridophores (ir) with stacks of reflecting platelets (rp), originally occupied by purine crystallates destroyed during preparation (Fig. 19). The adenine, guanine, hypoxanthine and uric acid in life are involved with other chromatophores in imparting structural colouration (Bagnara et al. 1979).

Frequently iridophores are found closely associated with melanophores and laminophore tissue. Although these tissues are usually clearly separate when seen side by side, in other cases iridophore and laminophore tissue appear fused, so that laminated bodies (lb) and reflecting platelets (rp) apparently coexisted within the same cell (Fig. 20). Though this feature may well be an artefact, a genuine conjoint arrangement of these organelles in a common cell type should perhaps be seriously considered (see below). Iridophores are not found in larvae of Ichthyophis. Nor have they been recognized, so far, in different regions of

Fig. 12. Lipid-like bodies (lp) associated with myelin-like configurations in the neck of a flask cell in the epidermis of an adult I. orthoplicatus

Fig. 13. The extended region of a flask cell of an adult I. orthoplicatus, showing part of the neck (nk), the flask (fl), the high content of mitochondria (m) and three neurite branches (ne) against the flask surface

Fig. 14. A higher magnification of a neurite (ne) in intimate contact with a flask cell in the epidermis of an adult I. orthoplicatus, but no synapse was recognized. Nor were they found in any other examples of a similar kind. Mitochondria (m) may be present all around the interior of the flask
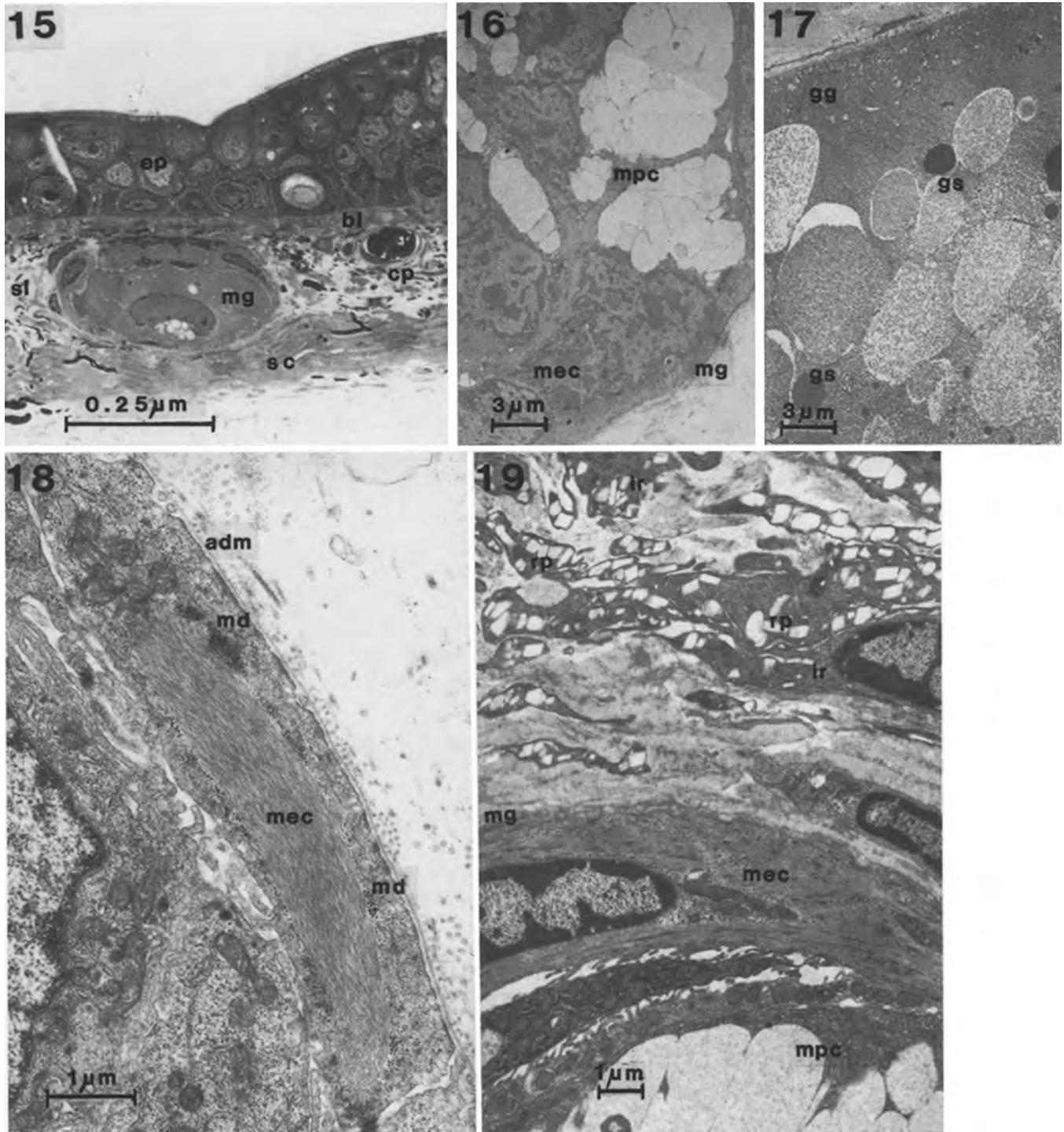
Fig. 15. Thick Araldite section (approx. 1 μm thick) of skin of a stage C larva of *Ichthyophis kohtaoensis*, stained with toluidine blue. The disposition of the outer basement lamella (*bl*) and the inner stratum compactum (*sc*), above and below the gland respectively, is clearly apparent. *cp*, capillary; *ep*, epidermis; *sl*, stratum laxum

Fig. 16. Outer area of a mucous gland (*mg*) in the dermis of a larva of a stage A *I. kohtaoensis*. There are myoepithelial cells (*mec*) at the surface, and secretory cells (*mpc*) with a swollen mucus content contain an extensive granular endoplasmic reticulum

Fig. 17. Part of the surface of a granular gland (*gg*) of a stage B larva of *I. kohtaoensis*, with a heavy content of granules (*gs*) of varied electron density, amid a fine, granular, lucent matrix

Fig. 18. A margin of a relatively undifferentiated dermal gland of a stage B larva of *I. kohtaoensis*. A myoepithelial cell (*mec*) contains a large skein of tonofilaments and has surface membrane densities (*md*), like early developing hemidesmosomes of basal epidermal cells. There is also an 'adepidermal membrane' (*adm*) enclosing an 'adepidermal space'

Fig. 19. Iridophore cellular tissue alongside a mucous gland (*mg*) in the dermis of a 9–10 cm juvenile of *I. kohtaoensis*. Reflecting platelets (*rp*) are clearly seen. Iridophores (*ir*) are widely distributed throughout this region of the dermis. *mec*, myoepithelial cell; *mpc*, secretory cell of mucous gland

Fig. 20. Cellular tissue with conjoint platelets (*rp*) and laminated bodies (*lb*) in the dermis of a 9–10 cm juvenile of *Ichthyophis kohtaoensis*. Such tissue is frequently seen in addition to separate iridophore and laminophore tissue in this region of the dermis

the body as a feature of adult skin, though laminophore tissue is well represented.

D. Discussion

1. Epidermis

The skin of 3 cm and 4.5 cm larvae of *Ichthyophis kohtaoensis* has been described (see Welsch and Storch 1973; Fox 1986 b). To summarise briefly, the epidermis of the 3 cm larva is composed of 2–3 layers of epithelial cells, some ciliated at the surface, including those of the external gills. In the basal region, it contains characteristic cells with large, disc-shaped, electron-dense granules. The epidermis of the 4.5 cm larva generally has a similar ultrastructure but the basal epithelial cells have newly-developed figures of Eberth. The figures develop from increased numbers of tonofilaments, which join to form bundles arranged in loops and are associated with the hemidesmosomes of the basal plasma membrane (Fox and Whitear 1986). There are granular cells in all regions of the body and Leydig cells also in the branchial epidermis, clearly derived from them.

Young Merkel cells occur basally in all regions along the body, though they are not common. At this stage they possess neither cell processes nor synaptic association with neurites, but a small number of membrane-bound granules within the 80–120 nm range is present in the cytoplasm (see Fox 1986 b). Ciliated cells are restricted to the branchial surface and the external gills, like those of the larva of *Ichthyophis paucisulsus* (Welsch 1981).

a) Leydig cells. A consideration of the series of larvae in the present work adds further support to the view that the epidermal granular cells of Welsch and Storch (1973), seen in younger 3 cm and 4.5 cm larvae of *Ichthyophis*, are the progenitors of the Leydig cells (Fox 1986 b). In older larvae, the Leydig cells have an extensive content of lucent vesicles, which originate from material of the dense, disc-like granules of the granular cells. Indeed, many of these granules are still retained in the Leydig cells of older larvae. Variation in the appearance of different Leydig cells, that is in the number of granules and the degree of lucency of the vesicles, presumably reflects their degree of differentiation. It could also relate, perhaps, to the level of their functional activity. Leydig cells occur along the entire length of the body of older larvae (Breckenridge 1983). Likewise, they were found at different levels of the body in older larvae in the present work. The exact time of their disappearance in *Ichthyophis* presumably occurs during metamorphosis, as in *Taricha torosa* Rathke, 1833 (Kelly 1966) and *Salamandra salamandra* Linnaeus 1758 (Rosenberg et al. 1982), though Leydig cells are retained in neotonous forms like *Necturus* (Lindinger 1984). They were recently described to disappear at the onset of the juvenile form in *I. glutinosus* (Breckenridge et al. 1987).

b) Merkel cells. Merkel cells are well developed and apparently fully differentiated in older larvae of *Ichthyophis*, and they have junctional synapses as in the 9–10 cm juvenile and adults. The arrangement is generally similar to that described in other amphibians (Fox and Whitear 1978). Merkel cells appear more frequently in belly epidermis than in skin from other regions of the body. However, their disposition throughout the body has yet to be documented.

c) Flask cells. Among amphibians, flask cells occur in the epidermis at the end of metamorphosis and afterwards (Warburg and Lewinson 1977; Fox 1985 b, 1986 a). They are the '*birnförmige Zellen*' (Welsch and Storch 1973) in the adult *Ichthyophis kohtaoensis*, and are common and fully developed both in the 9–10 cm juvenile and the adults of *I. orthoplicatus*.

Flask cells, in the three subtaxa of living amphibians, are frequently found closely associated with nerves. Thus, Whitear (1974) showed a preferential association of nerves and flask cells in the skin of adult *Rana temporaria*, though some of them did not have nerves against them, a feature similarly noted with flask cells of *Ichthyophis kohtaoensis* and *I. orthoplicatus*. Likewise nerves surround flask cells of *Triturus cristatus* Laurenti, 1768 (Bani 1968). In *Rana*, Whitear (1974) described up to four adjacent nerves running up the sides of the flask cell and travelling round the neck at the level of the second and third tiers of epithelial cells. No synapses were found, for they are not sensory cells. Nor are they found for any nerves alongside the flask cells of *Ichthyophis*. Whitear (1974) considered that nerves lie adjacent to the flask cells as a consequence of the pattern of moulting in the epidermis. It is probable, therefore, that the close spatial association of flask cells and nerves in the skin of amphibians is fortuitous, imposed by the overall growth and moulting phenomena of the epidermis.

Flask cells of amphibians are believed to contain a cellular pathway for chloride (Katz and Larsen 1984; Katz 1986). Some of their enzyme cytochemical activities have recently been described (Zaccone et al. 1986).

d) Iridophores. The absence of iridophores in the dermis of all the premetamorphic larvae studied suggests that they arise during metamorphosis, as in other amphibians (Fox 1985 b). Iridophores were only found in the dermis of the 9–10 cm juvenile *Ichthyophis*. The apparent coexistence of laminated bodies and reflecting platelets in cell tissue profiles may not be artefactual. There is evidence that chromatophores, or chromatophore-type cells, of amphibians are frequently variable in fine structure. In *Rana clamitans* Latreille, 1802, Berns and Narayan (1970) described a cell type which was either an immature xanthophore or a new form of cell. In *Centrolenella fleischmanni* Boettger, 1893, there are melanophores, xanthophores, iridophores and chromatophore C cells with pigment organelles, granules of various shapes similar to pterinosomes and also carotenoid vesicles. Schwalm and McNulty (1980) suggested that the pigment organelles originated from pterinosome lamellar tissue. Again, in clonal cultures of cells of *Rana catesbeiana* Shaw, 1802, tadpoles, iridophores can transform into melanophores and melanophores into xanthophores (Ide and Hama 1976; Ide 1978).

It is known that different organelles can occur in the same chromatophore-type cell. Bagnara et al. (1979) proposed that different pigment cells ere originally derived from a stem cell containing primordial organelles, which differentiate into any of the known chromatophoral organelles.

In the neon tetra (*Paracheirodon innesi* Myers, 1936), a freshwater teleost, Lythgoe and Shand (1982) illustrated an iridophore with reflecting platelets (spaces) where guanine crystals are missing, together with some guanine crystal bodies (600 nm long and 100 nm thick) retained within them. Though these dense structures are not shown to have laminations, their presence together with the reflecting platelets in the same cell, is similar to the conjoint arrangement in the iridophore-laminophore cell type of *Ichthyophis*, where reflecting platelets and laminated bodies coexist. It is possible, therefore, that iridophores in the 9–10 cm juvenile *Ichthyophis* are derived from laminophore tissue cells (see Fox 1983), which lose their laminated bodies, as seen in Figs. 19 and 20. In adults, where iridophores have yet to be found, either they disappear during later post-juvenile development or they are extremely rare in the dermis and only localised in specific regions of the body, possibly a more likely view. In some cases, at least in adults, laminated bodies of the laminophores are stabilised and not lost either normally or as a result of preparation. Thus only the so-called laminophores remain, not he mixed-type cells or iridophores with their reflecting platelets.

2. Dermis

In *Ichthyophis*, indeed in apodans generally, mucous glands are more numerous than the larger granular glands, which tend to be located dorsally in the skin (Physalix 1910).

In the 3 cm and 4.5 cm *Ichthyophis* larvae, the dermal

glands are relatively undifferentiated and without ducts (Welsch and Storch 1973; Fox 1986 b). The well developed mucous and granular glands of older *Ichthyophis* larvae are generally similar in appearance to those of the 9–10 cm juvenile and adults. The glands are precociously developed in these larvae, compared with those of some other amphibians; indeed they are present at hatching in *I. glutinosus* (Breckenridge et al. 1987). In anurans, they differentiate around metamorphosis (Fox 1986a), and in urodeles like *Salamandra terdigitata* Lacépède, 1788, only the first signs of glands are apparent in stage 5. This is the period when the branchial apparatus, Leydig cells and neuromasts are lost and when there is keratinization of the skin and a terrestrial existence (Delfino et al. 1982).

Newly-emerged larvae of *Ichthyophis* are usually quiescent, concealed under stones or moss. They swim by characteristic eel-like movements and burrowing on land is probably a gradual process, beginning at some early stage. Within a year they are firmly established in the soil (Breckenridge et al. 1987).

Amphibian dermal glandular secretion may serve a number of different functions (Whitear 1977). A precocious glandular development and hence secretion in *Ichthyophis* larvae could, among other things, be bound up with a particular life style. For example, in addition to its free-swimming activity, it may burrow more in the soil of its aquatic habitat and thus there is an early need for surface lubrication, compared with other amphibian larvae. The subject would seem to merit further investigation.

In conclusion, general examination of the fine structure of caecilian skin in larvae and adults reveals its strong similarity with comparable skin in other subtaxa of living amphibians. The presence of Leydig cells, ampullary organs and cell size of various cellular components of the epidermis shows that caecilians have features more in common with living urodeles. This could well have phylogenetic significance (Fox 1986 b). However, caecilian skin is unique among living amphibians as it has dermal scales (see Zylberberg et al. 1980; Fox 1986 a). The precocity of development of the dermal glands in *Ichthyophis* larvae may be a further characteristic distinguishing this group from anurans and urodeles, a developmental feature possibly related to the mode of life.

References

Bagnara JT, Matsumoto J, Ferris W, Frost SK, Turner WA, Tchen TT, Taylor JD (1979) Common origin of pigment cells. Science 203:410–415

Bani G (1968) Cellule particolari, probabili reccettori, nell'epidermide di *Triturus cristatus carnifex*. Boll Soc Ital Biol Sper 44:244–246

Berns MW, Narayan KS (1970) An histochemical and ultrastructural analysis of the dermal chromatophores of the variant ranid blue frog. J Morphol 132:169–180

Breckenridge WR (1983) Integumentary histology of larval *Ichthyophis* (Amphibia: Gymnophiona). Proc Sri Lanka Ass Adv Sci 39:65

Breckenridge WR, Nathaneal S, Pereira L (1987) Some aspects of the biology of *Ichthyophis glutinosus* (Amphibia, Gymnophiona). I Zool Lond 211:437–449

Delfino G, Brizzi R, Calloni C (1982) Development of cutaneous glands in *Salamandra terdigitata* (Lacépède, 1788) (Amphibia: Urodela); findings by light and electron microscopy. Z Mikrosk Anat Forsch 96:948–971

Fox H (1983) The skin of *Ichthyophis* (Amphibia:Caecilia): an ultrastructural study. J Zool Lond 199:223–248

Fox H (1985a) The tentacles of *Ichthyophis* (Amphibia:Caecilia) with special reference to the skin. J Zool Lond 205:223–234

Fox H (1985b) Changes in amphibian skin during larval development and metamorphosis. In: Balls M, Bownes M (eds) Metamorphosis, Symp Vol Brit Soc Dev Biol No 8., Clarenden Press Oxford, pp 59–87

Fox H (1986 a) The skin of Amphibia. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) Biology of the integument. Vol 2. Vertebrates. Springer, Heidelberg, pp 78–135

Fox H (1986 b) Early development of caecilian skin with special reference to the epidermis. J Herpetol 20:154–167

Fox H, Whitear M (1978) Observations on Merkel cells in amphibians. Biol Cell 32:223–232

Fox H, Whitear M (1986) Genesis and regression of the figures of Eberth and occurrence of cytokeratin aggregates in the epidermis of anuran larvae. Anat Embryol (Berl) 174:73–82

Hartschuh W, Weihe E, Reinecke M (1986) The Merkel cell. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) Biology of the integument. Vol 2. Vertebrates. Springer, Heidelberg, pp 605–620

Hay ED (1961) Fine structure of an unusual intracellular supporting network in the Leydig cells of *Amblystoma* epidermis. J Biophys Biochem Cytol 10:457–463

Ide H (1978) Transformation of amphibian xanthophores into melanophores in dermal culture. J Exp Zool 203:287–294

Ide H, Hama T (1976) Transformation of amphibian iridophores into melanophores in clonal culture. Dev Biol 53:297–302

Jørgensen JM (1981) On a possible hair cell turnover in the inner ear of the caecilian *Ichthyophis glutinosus* (Amphibia:Gymnophiona). Acta Zool (Stockholm) 62:171–186

Katz U (1986) The role of amphibian epidermis in osmoregulation and its adaptive response to changing environment. In: Bereiter-Hahn J, Matoltsy AG, Richards KS (eds) Biology of the integument. Vol 2. Vertebrates. Springer, Heidelberg, pp 472–498

Katz U, Larsen EH (1984) Chloride transport in toad skin (*Bufo viridis*). The effect of salt adaptation. J Exp Biol 109:353–371

Kelly DE (1966) The Leydig cell in larval amphibian epidermis. Fine structure and function. Anat Rec 154:685–700

Lindinger MI (1984) Fine structure of the abdominal epidermis of the adult mudpuppy, *Necturus maculosus* (Rafinesque). Cell Tissue Res 238:395–406

Lythgoe JN, Shand J (1982) Changes in spectral reflexions from the iridophores of the neon tetra. J Physiol (Lond) 325:23–34

Physalix M (1910) Repartition et signification des glandes cutanées chez les batraciens. Ann Sci Nat Zool Biol Anim 12:183–201

Rosenberg M, Lewinson D, Warburg M (1982) Ultrastructural studies of the epidermal Leydig cell in larvae of *Salamandra salamandra* (Caudata, Salamandrida). J Morphol 174:275–281

Schwalm PA, McNulty JA (1980) The morphology of dermal chromatophores in the infrared-reflecting glass frog (*Centrolenella fleischmanni*. J Morphol 163:37–44

Taylor EH (1968) The caecilians of the world. A taxonomic review. Univ Kansas Press, Lawrence, Kansas, pp 1–848

Wahnschaffe U, Fritzsch B, Himstedt W (1985) The fine structure of the lateral-line organs of larval *Ichthyophis* (Amphibia:Gymnophiona). J Morphol 186:369–377

Warburg M, Lewinson D (1977) Ultrastructure of epidermis of *Salamandra salamandra* followed throughout ontogenesis. Cell Tissue Res 181:369–393

Welsch U (1981) Fine structural and enzyme histochemical observations on the respiratory epithelium of the caecilian lungs and

gills. A contribution to the understanding of evolution of the vertebrate respiratory epithelium. Arch Histol Jpn 44:117–133

Welsch U, Storch V (1973) Die Feinstruktur verhornter und nicht-verhornter ektodermaler Epithelien und der Hautdrüsen embryonaler und adulter Gymnophionen. Zool Jahrb Abt Ontog Tiere 90:323–342

Whitear M (1974) Flask cells and epidermal dynamics in frog skin. J Zool (Lond) 175:107–149

Whitear M (1977) A functional comparison between the epidermis of fish and amphibians. In: Spearman RIC (ed) Comparative biology of skin. Symp Vol Zool Soc No 39. Academic Press, London, pp 291–313

Zaccone G, Fasulo S, Cascio PL, Licata A (1986) Enzyme cytochemical and immunocytochemical studies of flask cells in the amphibian epidermis. Histochemistry 84:5–9

Zylberberg L, Castanet J, Ricqles A de (1980) Structure of the dermal scales in Gymnophionen (Amphibia). J Morphol 165:41–54