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The fine structure of the dorsal lingual epithelium of the scincine lizard *Chalcides ocellatus* Forscal (Scincidae, Sauria, Reptilia)
I. Histogenesis of the lingual epithelium

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ABSTRACT

The purpose of the present study is to illustrate the histogenesis of the lingual epithelium of scincine lizard *Chalcides ocellatus* from the beginning of the appearance of the tongue at the floor of the buccal cavity to the truly functional tongue. Light microscopic examination revealed that the tongue of the embryo at the first chosen stage, was composed of a lamina epithelialis of stratified cuboidal cells, surrounding a core of mesenchymal cells. A peridermal layer surrounds the lamina epithelialis. A condensation of deeply staining mesenchymal cells was noticed underneath the lamina epithelialis. The interaction between these two layers was discussed in detail. The differentiation of the peridermal layer was studied in detail both by the light and scanning electron microscope. The dissociation of this layer at the chosen late stage before delivery was noticed by the aid of the SEM as ruptured cells cover the surface of the tongue. A few number of macrophage cells was observed also at different stage of phagocytosis. Therefore the light and the SEM findings seem to be complementary. In addition the differentiation of the lingual glands and lingual papillae was observed.

KEYWORDS: *Chalcides ocellatus,* reptiles, SEM, tongue, histogenesis, peridermis, macrophage.

INTRODUCTION

The tongue as a musculature organ in the buccal cavity shows great variability throughout the vertebrates. In most animals one or more types of lingual elevations, known as the papillae, cover the dorsal surface of the tongue. Theses papillae have different shapes, sizes, and functions (Wassif & El-Hawary 1998). All the papillae are covered by stratified squamous epithelium that differs only in the thickness of keratinization (Iwasaki & Miyata 1985). When the tongue of reptiles has been studied with scanning electron microscopy, marked variations in the architecture of the dorsal surface have been found (Iwasaki & Miyata 1985; Winokur 1988; Wassif & El-Hawary 1998; Lemell *et al.* 2000). Winokur (1988) postulated that, the presence or formation of lingual papillae is correlated with the adaptation of species to their habitat and feeding behavior. The fine structure of the dorsal lingual epithelium of the reptilian tongues has been studied by many authors (Nishida *et al.* 2000).

Many functions of the tongue have been proposed. Underwood (1971) suggested that feeding and collection of particles for transport to Jacobson's organ are the most important functions of the tongue within lizards, while McDowell (1972) mentioned that the tongue function is specialized for chemosensory behaviors. Schwenk (1986) found that *Sphenodon* also used its tongue for the prehension of small prey items during feeding. These views reflect the common possibility of a functional link between these two uses, which implies mechanical constrain (Schwenk 1986). While there are many publications on the histogenesis of the epidermal scale in different groups of reptiles (Mohammed 1987; Alibardi 1998; Alibardi & Thompson 1999a,b; and Alibardi 2001), little is known regarding the morphological, histological and histochemical changes occurring during the development of the reptilian tongue, although Mohammed (1992) described the structure of the tongue during late embryonic stages and at birth in *Mabuya aurata.*

The present study reports the histogenesis of the tongue of the scincine lizard

Chalcides ocellatus Forskal (Scincidae, Sauria, Reptilia) during embryonic stages and at birth using light and scanning electron microscopy. Scanning electron microscopy was used in order to visualize the relationships between the three-dimensional features and the histological structures observed in the sections. Furthermore, this paper describes the histochemical properties of the tongue surface of the embryos.

MATERIALS AND METHODS

Twenty gravid female lizards were collected from Abu-Rawash (thirty kilometers north western to Cairo). The specimens were dissected at interval times of pregnancy. The embryos of *Chalcides ocellatus* were removed at three stages before birth, ranging from snout to vent 1.1 cm (paddle stage in the for-limb), 2.5 cm (separation of the 5 digits from each others, scales unpigmented) and 3.2 cm (more elongation of the digits and nails, with almost complete scale pigmentation).

For light microscopic studies the specimens were dissected and the heads of the embryos and the lower jaw with the tongue of the lizards after birth removed. After fixation in Bouin's solutions and formalin, specimens were dehydrated in an ascending series of ethanol alcohol, embedded in paraffin wax, z`1haematoxylin and eosin (H&E) and Milligan's trichrome stains to study the general morphology (Humason 1979). Alcian blue (at pH 2.6) and periodic acid Schiff's reaction (PAS) was used to reveal mucus-secreting cells (Mowry 1956).

For scanning electron microscopy, the lower jaws with the tongues were removed and fixed in 5% glutaraldehyde solution for 2 hr, washed in (0.1 M) cacodylate buffer for one hour and postfixed in 1% osmium tetroxide solution (Millonig 1962) for 2 hours, followed by dehydration, critical point drying and gold-ion sputtering. These specimens were then examined in a JEOL scanning electron microscope (JSM-5455LV).

RESULTS

Macroscopic examination of the tongue of *Chalcides ocellatus* embryos (1.1cm in length from snout to vent) revealed that the tongue appeared as an elongated structure whose end is rounded and very slightly bifurcated. The tongue attached to the buccal floor along its entire length. At this stage of development, light microscopic examination of serially vertical sections of the tongue, from the anterior to the posterior direction, revealed that the tongue is composed of two layers. The lamina epithelialis of stratified cuboidal epithelium lies dorsal and is pseudo-stratified laterally, surrounding a core of mesenchymal cells. Some nuclei were seen in various stages of mitosis. Externally, a peridermal layer surrounds the lamina epithelialis of the tongue; this layer has been found to enclose all the structures found in the buccal cavity (figs.1, 2,). The peridermal cells often appear broken and have rounded nuclei. A narrow dense subepithelial zone of deeply staining cells was distinguished in the mesenchymal layer. There is an amorphous substance, which separates the lamina epithelialis from the subsequent mesenchymal layer (figs.1, 2). Proliferations and subsequent down growth of the epithelia at certain places were noticed (primordia of the lingual glands), and there was an aggregation of the mesenchymal cells towards the lamina epithelialis (primordia of the papillae) (figs.1, 2).

As development progressed, the primordia of lingual glands continue their downward proliferation from the dorsal and ventro-lateral surfaces of the tongue into the subjacent mesenchymal layer. The summits of the prospective lingual papillae become membranous and consist of one layer of flattened cells with oval or rounded nuclei below the peridermis (Figs.3, 4). The undifferentiated mesenchymal muscular tissue began to invade the core of such papillae (fig.4). At this stage of development the peridermal layer appears as one layer of cells with bulged rounded nuclei.

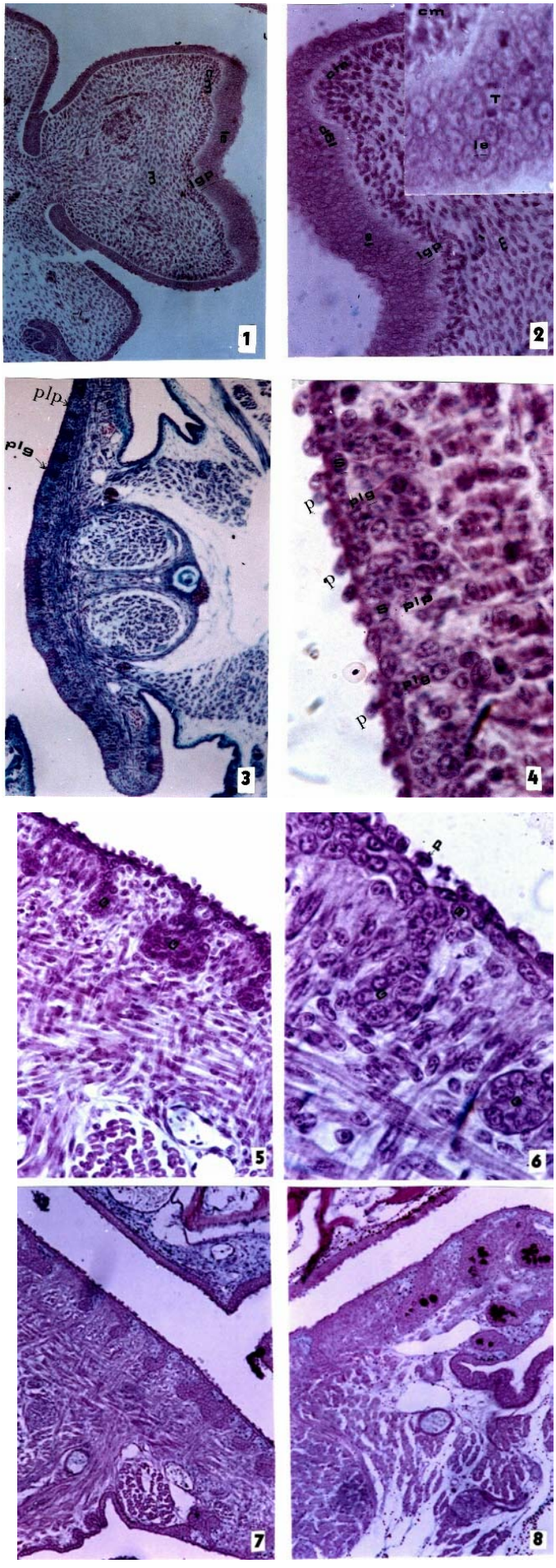

Fig. 1. Vertical section of the tongue of *Chalcides ocellatus* embryo at length 1.1 cm showing, the lamina epithelials (le), the mesenchymal condensation (cm) and the peridermis (arrow heads) surrounds all the structures found in the buccal cavity. Also showing the lingual gland primordia (lgp) . (H&E X 200).

Fig. 2 Higher magnification of figure (1) showing, lingual dorsum surface of stratified cuboidal epithelium and of pseudostratifed ones laterally. Also showing mitosis at any layer of the lamina epithelials arrows. (T). The demarcation between the lamina epithelials and mesenchymal layer (m) is clear. Insert, detail structure of the lamina epithelialis. (H&E. X 400 and insert X1000).

Fig. 3. Vertical section of the tongue of *Chalcides ocellatus* embryo at length 2.5 cm showing general structure of the tongue. (Milligan stain. X 100).

Fig. 4. Higher magnification of figure (3) showing the prospective lingual glands (plg) the prospective lingual papillae (plp), bulged peridermal cells (P) and stratum germinativum (S). (H&E X1000).

Fig. 5. Vertical section of the tongue of *Chalcides ocellatus* embryo at length 3.2 cm showing developing acinar glands (G) and invaded core of the papillae by developing muscle. (H&E X 400).

Fig. 6. Higher magnification of figure (5) showing the bulged peridermal cells (P), the stratum germinativum (S) and the developing acinar glands (G). (H&E X 1000).

Fig. 7. Vertical section of the mid-tongue of *Chalcides ocellatus* embryo at length 3.5 cm, showing multiple acinar lingual glands that stained negatively by Alcian blue-PAS stain. (X100).

Fig. 8. Vertical section of the hind-tongue of *Chalcides ocellatus* embryo at length 3.5 cm, showing intense polysaccharides staining of the acinar lingual glands. (Alcian blue-PAS stain. X100).

At the late embryonic stage, the multiple lingual glands continue their downward growth into the subjacent mesenchymal layer. These glands attain an acinar shape (figs.5, 6). The glands that lie in the anterior portion of the tongue are negatively stained with the alcian blue-PAS stain, while those found in the posterior portion of the tongue stain positively with the same stain (figs. 7&8). Thus such lingual glands secrete neutral mucopolysaccharides. The mucocytes are columnar and have basally located rounded nuclei. The epithelium overlying the dome of the papillae (stratum germinativum) is composed of simple, more or less cuboidal cells with rounded nuclei covered by the peridermal cells. These cells are flat and their nuclei appeared rounded at some places and flattened at others (fig.6).

At birth, the tongue exhibits more organization and complication of lingual structure (fig.9). The papillosed part of the dorsal lingual surface is covered by scale-like, short, often-flattened papillae. A few taste buds are fond at the summit of the papillae at the anterior portion of the tongue (fig. 10). Numerous lingual, simple and branched, acinar glands having short ducts were noticed between the lateral sides of the papillae (fig. 11). Mucocytes are columnar with flattened, basally located nuclei. These cells produce an acidic mucus substance that stains positively with PAS and alcian blue (at a PH 2.6)(fig. 12). Neither the tongue tips nor the most anterior portion of the tongue of *C. ocellatus* have glands (fig. 9). The summits of the papillae are covered by a stratified squamous epithelium, keratinized anteriorly but not posteriorly. The papillae have longitudinally arranged striated muscle fibres that extend well into their connective tissue cores (figs.9&12). A thin sheet of collagenous fibres lies beneath the lingual epithelium as seen by Milligan stain (fig.11). The presence of pigment cells was not observed in the anterior portion of the tongue, neither before nor immediately after birth.

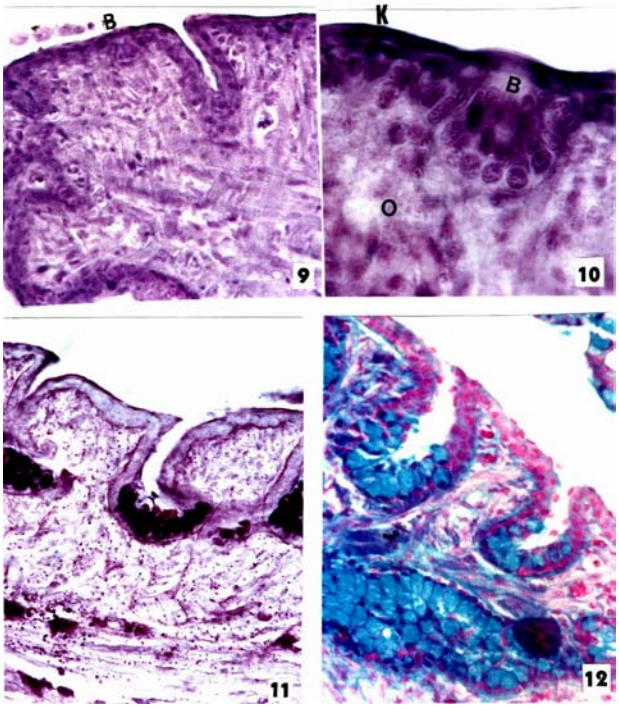

Fig. 9. Vertical section of the fore-tongue of *Chalcides ocellatus* after birth showing lingual papillae covered by keratinized stratified squamous epithelium and taste bud (B) at the summit of the papillae. (H&E. X 400).

Fig.10. Higher magnification of figure (9) showing the detail structure of the taste bud (B) and the keratin layer (K). (H&E. X 1000).

Fig. 11. Vertical section of the hind-tongue of *Chalcides ocellatus* after birth showing the intense polysaccharide staining of the acinar lingual glands. (Alcian blue-PAS stain. X 400).

Fig. 12. Vertical section of the hind-tongue of *Chalcides ocellatus* after birth showing dorsal and ventral lingual glands. The summit and the lateral sides of the papillae are covered by non-keratinized stratified squamous epithelium. Also showing collagenous sheath underneath the epithelial layer. (Milligan stain. X 400).

Using SEM at low magnification, the tongue of the first stage embryo (the paddle stage of the forelimb) was seen to be as an elongated structure with very slightly bifurcated rounded tips. There is a depression at the midline of the tongue, which extends from the anterior to the posterior portion of the tongue (fig.13). Closely packed bulges are densely distributed all over the surface of the tongue. These bulges seem to be similar to the peridermal cells observed by the light microscope. Using higher magnifications revealed that each bulge seems to be coincident with a single cell. The limits between the cells are not clear. The diameter of the bulge is about 5-7 μm, and the surface of these cells is wrinkled and

irregular. Some cells are concave, while others seem to be convex (figs.14&15).
As development proceeds, the tongue of the embryo was seen to be deeply bifurcated, flattened and triangular in shape (fig.16). Dome-shaped bulges are densely distributed all over the surface of the tongue (fig.17). Using higher magnification revealed that each bulge seems to be coincident with a single cell. These bulges resemble the nuclei of the peridermal cells as seen by the light microscope. The limits between cells are not clear. The diameter of the dome-shaped bulge is about 4-5 μm. Some cells seem to have microvilli. The number of the cells bearing microvilli increases posteriorly (figs.18&19).

At the late embryonic stage of development, serrated scale-like and ridge-like papillae appear at the base of the tongue. The peridermal cells also cover these papillae. These cells have flattened, rounded lens-shaped nuclei with long axis-oriented parallel to the lingual surface. The limits between cells are clear (fig.21&22), and their surface appears more or less smooth. Some cells are shown floating on the surface of the papillae (fig.22). Anteriorly, the dome-shaped bulges cover all the surface of the tongue. These bulges are similar to those described in the preceding stages, These bulges seem to be similar to the peridermal cells observed by the light microscope. Many ruptured peridermal cells were seen also (fig. 23). At this stage of development, a mucous substance is noticed between the papillae at the posterior portion of the tongue. In addition a number of macrophage cells were observed, only by the aid of the SEM, at different stages of phagocytosis (figs. 24). Therefore, the SEM and light microscopic findings seem to be complementary.

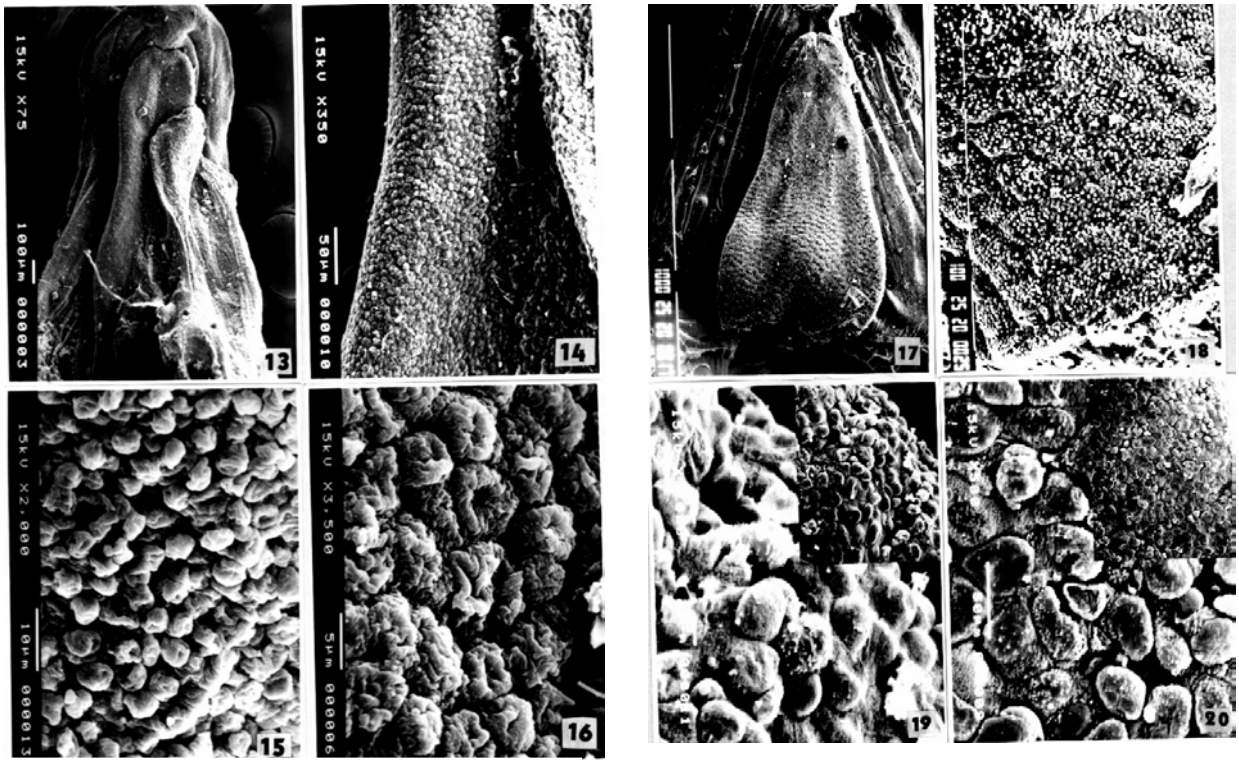

Fig. 13. Scanning electron micrograph of the dorsal surface of the tongue of *Chalcides ocellatus* embryo at 1.2 cm. showing the general structure of the tongue at this stage. (X75).
Fig. 14. Higher magnification of figure (13) showing closely compact bulges covering the dorsal surface of the tongue. (X355).
Fig.15. Higher magnification of figure (14) showing the nut –shaped bulges and the limit between cells are not clear.(X2000).
Fig. 16. Higher magnification of figure (15) showing the wrinkled and irregular surface of the epithelial membrane of the cells.(X3500).

Fig.17. Scanning electron micrograph of the dorsal surface of the tongue of *Chalcides ocellatus* embryo at 2.3 cm. showing the general structure of the tongue at this stage. (X75).
Fig. 18. Higher magnification of figure (17) at the posterior portion of the tongue showing the papillae covered by bulges. (X 350).
Fig. 19. In site, higher magnification of figure 16 at the anterior portion of the tongue showing the loosely backed bulges cover all the surface of the tongue at this portion. (X500). Higher magnification of the in site showing each bulge coincident with one cell; the bulge resemble the place of the nucleus. Some of these bulges bearing fine granules. (X 3500).
Fig. 20. In site, higher magnification of figure 16 at the middle portion of the tongue showing the closely backed bulges cover all the surface of the tongue at this portion. (X500). Higher magnification of the in site showing each bulge coincident with one cell; the bulge resemble the place of the nucleus. The number of bulges bearing micovilli increased. (X 3500).

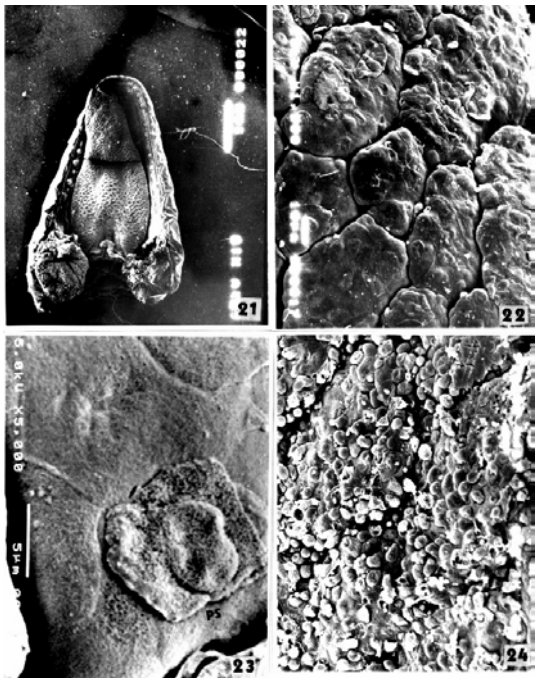

Fig. 21. Scanning electron micrograph of the dorsal surface of the tongue of *Chalcides ocellatus* embryo at 3.5 cm. showing the papillosed surface of the tongue excluding the tongue tip. (X75).

Fig.22. Higher magnification of figure (21) showing the serrated scale- like papillae at the posterior portion of the tongue. Covered by flattened epithelial cells. The limit between cells are clear. (X500).

Fig.23. Higher magnification of figure (22) showing the surface of the flattened cells are more or less smooth. The limit between cells are clear, also showing the floated cell at the surface of the papillae (macrophage cells). Showing also the pseudopodia (PS). (X5000).

Fig. 24. Higher magnification of figure (21) showing the scale- like papillae at the middle portion of the tongue covered by flattened epithelial cells, and bulged cells. Also many ruptured cells are seen.(X500).

DISCUSSION

In the present investigation, lingual papillae morphogenesis of *Chalcides ocellatus* does not involve lingual placodes, as in the developing tongue of *Mabuya aurata* (Mohammed 1992). At early stages of development, the lamina epithelialis of the tongue consists of stratified cuboidal epithelium dorsally and pseudo-stratified type laterally covered by peridermis. Such a period of development was not described by Mohammed (1992) in *Mabuya aurata;* he reported that the lingual epithelial cells are hardly distinguished from the underlying mesenchymal flat cells. In the present investigation, by using different types of staining techniques, these two layers are well distinguished from each other at all stages. Mohammed (1992) stated that the formation of the lingual keratinized papillae appears to be favored with respect to the formation of epidermal scales. The sub-epithelial condensation of mesenchymal deeply staining cells was noticed at the paddle stage of forelimb development. This is contradicted by the dermis of epidermal scale morphogenesis which shows no sign of mesenchymal condensation in *Lacerta vivipara* (Maderson 1965), *Lacerta muralis* (Dhoually & Maderson 1984), *Chalcides ocellatus* (Mohammed 1987) and *Lampropholis guichenoti* (Alibardi & Thompson 1999b). Mohammed (1992) stated that the sublingual tissue plays a pattern-determining role in lingual appendages formation. Ferguson (1988) stated that in all vertebrates, regional, temporal and species-specific epithelial differentiation is specified by the underlying mesenchyme. He mentioned that signaling of this interaction is complex but involves both extracellular matrix and soluble factors. These soluble growth factors have a biphasic effect: directly on the epithelia and on the mesenchyme where they stimulate or inhibit cell division and synthesis of specific extracellular matrix molecules. The extracellular matrix molecules (and bound growth factors) synthesized by the mesenchymal cells may then directly affect the epithelium. These signals cause differential gene expretion *via* an intracellular second messenger systems e.g. cyclic AMP, like intracellular calcium, pH and phosphatidylinositol lipids. The genes for such molecules are probably expressed in response to mesenchymal signals.

Alibardi (1998) noticed that in scale morphogenesis of *Anolis lineatopus and Podarcis muralis* fibroblasts under the inner side of the scale made few contacts with the basement membrane, and their cytoplasmic elongations were mostly oriented parallel to the dense lamina. Instead fibroblasts under the basal layer of the outer scale surface made numerous

contacts with the basal layer of the outer scale surface, suggesting that more dermal epidermal interactions take place on this side of the scale.

Alibardi & Thompson (1999a;b) stated that the epidermis in the developing scales of *Emydura macquarii* is initially composed of an external flat peridermis and a basal layer of cuboidal cells. The present findings in the developing tongue support the work of Alibardi & Thompson (1999a,b). Because such cells are found only during embryogenesis, Alibardi & Thompson (1999a,b) has referred to these layers as embryonic epidermis.

Using TEM, Alibardi & Thompson (1999b) described the peridermal cells in the developing scale of *Lampropholis guichenoti* as a single, variably electron-dense outer periderm with microvilli covering a basal layer. Most of this outer peridermis is flat and narrow, except those adjacent to the nucleus, which are bulged. This finding is in agreement with the present work as revealed by the light and scanning electron microscopy. Alibardi (1998) mentioned that the basal layer produces the various supra-basal epithelial layers, and mentioned that during scale differentiation, the peridermis darkens, flakes off and is partially lost before hatching. This would seem to be in agreement with the present investigation as revealed by the scanning electron microscope only, since dissociations of the peridermal cells were noticed. Furthermore, the present findings have revealed the presence of flattened peridermal cells floating over the surface of the peridermal layer, and in addition many ruptured cells and a number of macrophage cells were seen at different stages of phagocytosis. At birth this layer is not found. As here, Alibardi (1998) mentioned that the narrow outer peridermis is torn and partially lost during scale morphogenesis. The present investigator attributed the disappearance of the peridermal layer partly to the phagocytosis of these cells by macrophage cells, which were seen only by the aid of the SEM at late stage of development.

Alibardi & Thompson (1999b) stated that in scale formation in embryos of lizard *Lampropholis guichenoti,* the fast growth of the epidermis into pointed scales probably causes disruption of the peridermis, with some cells being sloughed into the amniotic fluid. This process allows lipid-like secretions to exist externally from the inner periderm layer and coat the whole scale surface. In *Lacerta muralis* (Dhouailly *&* Maderson 1984*)* and in *Anolis lineatopus* and *Podarcis muralis* (Alibardi 1997), the inner peridermal cells are capable of secreting mucosubstances*,* whereas in *Lampropholis guichenoti* the secretory product is lipid-like (Alibardi & Thompson 1999b). The lingual mucocytes exhibit a positive PAS reaction in the late period of the prenatal life, especially at the posterior dorsal and ventral surfaces. These findings agree with the work of El-Gammal (1997) in the developing rat tongue of 18-day fetuses and newborn young. This study describes taste bud differentiation soon after birth, Mohammed (1992) who pointed out to the taste buds in *Mabuya aurata* at birth. In cod, Harvey & Batty (1998) described taste bud differentiation soon after hatching. In rat, taste buds have been differentiated in the dome of the central part of the papillae at 18^{th} prenatal day (Ahpin *et al.* 1989 and El- Gammal 1997). This would seem to be contradicted with Torrey (1940) who reported that taste buds were observed at the 9^{th} day after birth in rat.

In conclusion, it may be suggested that the removal of the ruptured peridermal cells be partly due to the phagocytosis of these cells by macrophage cells, which were seen at late stage of development only by the aid of the SEM. The lingual mucocytes exhibit a positive PAS reaction in the late period of the prenatal life. Differentiation of the taste bud takes place immediately after birth. The differentiation of the papillae proceeds from the posterior to the anterior portion of the tongue.

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الملخص العربى

التركيب الدقيق للطلائية اللسانية الظهرية للدفان الكبير (زواحف: سفنفوريات)

أولا: التطور الجنيني للطلائية اللسانية

إقبال نادرس واصف
قسم علم الحيوان - كلية العلوم - جامعة أسيوط - مصر

استهدف هذا البحث دراسة التطور الجنيني للسان الدفان الكبير (زواحف: سفنفوريات) في ثلاث مراحل عمريـه وهـى مرحلـة تكوين أصابع الطرف الأمامي دون انفصالها - مرحلة انفصال الأصابع وظهور الأظافر في الطرف الأمامي وظهور الحراشيف عديمة اللون- مرحلة استطالة أصابع وأظافر الطرف الأمامي وكذلك تلون الحراشيف، وذلك باستخدام كل من المجهر الضـوئي والمجهر الإلكتروني الماسح. ولقد وجد في المرحلة الجنينية الأولي أن اللسان في القطاع الرأسي يتكون من ثلاث طبقات وهى من الداخل للخارج الطبقة الميزنشيمية والطلائية اللسانية والطبقة الخارجية والتي تعرف بغلاف البشرة0 ولقد وجدت تجمعـات من الخلايا الميزنشيمية أسفل طلائية اللسان , كما نوقش بالتفصيل تأثير هذه الخلايا على الطلائية التي تلامسـها. ودرس كـذلك التطور الجنيني للحلمات اللسانية والغدد المخاطية وأيضا درس بالتفصيل تطور طبقة غلاف البشرة بكل من المجهـر الضـوئي والإلكتروني الماسح. وبواسطة المجهر الإلكتروني الماسح وجد أنها تتفكك وتضمحل قبل الوضع. وأيضا وجدت بعـض الخلايـا الأكولة أثناء هذه المرحلة من النمو.