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A New Type of Cell in the Larval Epidermis of the Green Toad, *Bufo viridis viridis*

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The flank skin of the premetamorphic stage of the green toad, *Bufo viridis viridis,* was studied by transmission electron microscopy. The skin was typically larval amphibian, made up of a highly fibrous and vascular dermis to the inside and a highly cellular epidermis to the outside with a distinct basal lamina separating the two layers. The epidermis is three-cells thick. The epidermal cells, mostly keratinocytes, are closely packed together and are attached to each other through desmosomes and interdigitations. The basal keratinocytes are anchored to the basal lamina through hemidesmosomes. In addition to the keratinocytes, some other types of cells known in the amphibian larval skin were found. These cells include mucus-secreting, mitochondria-rich cells, Merkle cells and flask cells. Anew type of cells, the dark cells, are described in this paper. The dark cell rests on the basal lamina. It is a ramified cell with a number of cytoplasmic processes intervening in between the keratinocytes. The cytoplasm is strikingly dark and rich with polysomes and granular endoplasmic reticulum.

Key words: Anurans, green toad, skin, epidermis, dark cell.

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The larval amphibian skin is a very interesting organ. It performs several functions including physical and mechanical protection (AMICHE *et al.* 2000), respiration (VAN DRIESSHe & HILLYARD 1985), ion (STIFFLER 1994; 1996; SORENSEN & LARSEN 1996) and water (JORGENSEN 1993, 1997) exchange. This versatile function is reflected in the structural details of the skin, particularly the epidermis and successive cell populations that appear during the larval and metamorphic stages (FOX 1986chyba za; WARBURG *et al.* 1994).

Several types of cells have been recorded in the larval and adult anuran epidermis (FOX 1986; QAR & Al-ADHAMI 2003). The epidermal cell types that appear during early amphibian larval life include the ciliary cells, ordinary epidermal cells (keratinocytes), mucus-secreting (club) cells and mitochondria-rich cells (FOX 1985). Other types of cells that appear at later larval stages include Merkel cells, Leydig cells and flask cells (WARBURG *et al.*1994).

In a previous work, QAR and Al-ADHAMI (2003) described the flank skin of the green toad, *Bufo viridis viridis,* in which a new type of cell was described. The present work extends the description of another type of rare cell in the epidermis of the premetamorphic skin of the same species.

Material and Methods

Premetamorphic larvae (corresponding to stage 44-45 of NIEUWKOOP and FABER 1956) of the toad, *B. viridis viridis,* were collected during the spring of 2003 from ponds in Alghor Alshamali, Jordan. In the laboratory, the animals were kept in aquaria containing dechlorinated water under laboratory conditions. The tadpoles were sacrificed by an overdose of tricane sulphonate (MS222).

Small pieces of the flank skin were fixed in 2.5% gluteraldehyde in 0.1% M sodium cacodylate buffer (pH 7.4) for two hours, washed and fragmented under a dissecting microscope. Postfixation was performed in 1% osmium tetroxide in the same buffer for three hours, dehydrated through a graded ethanol and embedded in Arladite. Sectioning was done using a Reichert-Jung Ultracut E ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate, and examined and photographed under a Zeiss EM 10 CR electron microscope at 60 KV.

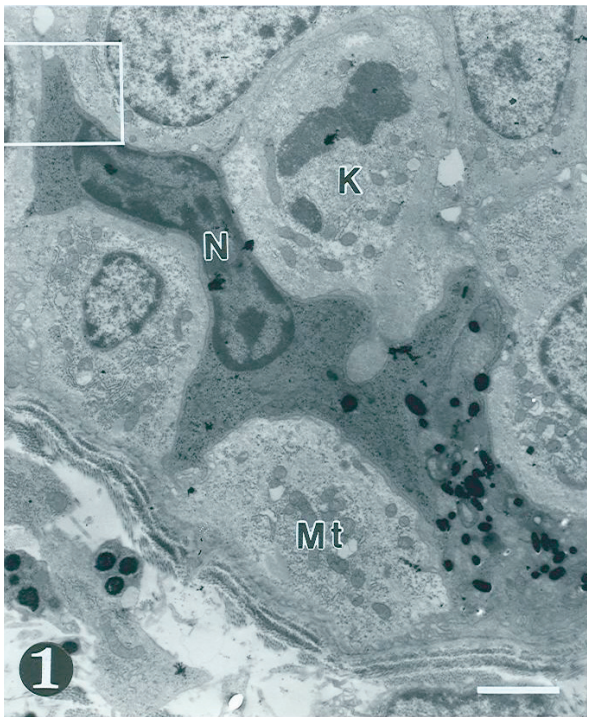

Fig. 1. Basal epidermis of the premetamorphic tadpole showing the dark, highly branched cells surrounded by a number of keratinocytes (K). N: nucleus of the dark cell, Mt: a dividing keratinocyte. Bar = 1 μm.

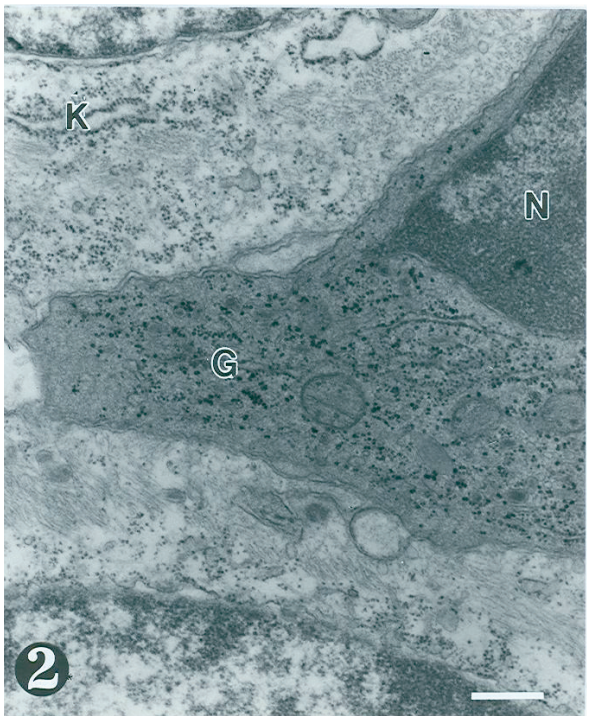

Fig. 2. Inset shown in figure 1 magnified to show the granular endoplasmic reticulum (G). (K): keratinocyte, N: nucleus of the dark cell. Bar = 0.5 μm.

Results and Discussion

The flank skin of the premetamorphic tadpole of the green toad, *B. viridis viridis,* is made up of an inner, highly fibrous dermis and an outer cellular epidermis with a discrete basal lamina in between (Fig. 1). The epidermis is, as reported in most other anurans previously studied (ROBINSON & HEINTZELMAN 1987; ROSENBERG & WARBURG 1995), three-cells thick. The vast majority of the constituting cells are keratinocytes (Fig. 1). These are roughly round, organelle-rich, tightly packed cells with specialized intercellular junctions linking neighboring cells. Such junctions include primarily desmosomes and interdigitations. Basal cells are anchored to the basal lamina by a large number of hemidesmos1omes and figures of Eberth (FOX & WHITEAR 1986). The basal lamina often shows perforations (Fig. 1). Other types of cells observed include surface mucuos cells and mitochondria-rich cells. This general histological description of the premetamorphic skin of the green toad is in general accordance with descriptions of the larval skin in other amphibians, particularly anurans, previously studied (BILLET & GOULD 1971; ROSENBERG & WARBURG 1992; 1995). It is also in conformity with a previous description of the larval skin of the same species extended by the present authors previously (QAR & AL-ADHAMI 2003).

A very rare type of cell was observed during the present study. These cells are slender and highly branched. They occupy the intercellular spaces between the basal and the overlying keratinocytes. Cells of this type partially rest on the basal lamina but do not attach to it through hemidesmosomes or inter-digitations (Fig. 1) as do ordinary basal keratinocytes omit (FOX 1986). It is worth mentioning that the non-epidermal cells reported previously (QAR & AL-ADHAMI 2003) did not contain hemidesmosomes, even though they rest on the basal lamina as do the dark cells of the present study.

The nucleus is, in turn, compressed with dense, peripheral and some central clumps of condensed chromatin. The basal cytoplasm contains a large number of melanosomes. The ground cytoplasm is strikingly darker than that of the surrounding keratinocytes. It contains a small number of spherical mitochondria and a large number of polysomes and is rich with granular endoplasmic reticulum (Fig. 2). A few vesicles with moderately electron-dense homogeneous content are also present. Cytoplasmic processes intervene between the surrounding keratinocytes and approach the basal lamina in one direction and the free surface on the other. However, no specialized junction of any type between the dark cells and the surrounding cells could be detected. A few large, lucent to moderately electron-lucent omit vesicles are present between the dark cells and the surrounding keratinocytes.

The morphology and ultrastructural description of the dark cells does not correspond to any of the previously described cells observed in the larval skin of anuran or urodelan amphibians (FOX 1986; WARBURG *et al*. 1994; ROSENBERG & WARBURG 1995). Their structure does not give a clear clue regarding their function. However, the ramified outline of the cell and its intimate relation to many epidermal cells and the basal lamina may suggest a supportive and/or a nutritional role. Further investigation is needed to test this hypothesis and to determine the origin of this type of cell.

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