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0014-4754/84/090953-03$1.50 + 0.20/0

The choroid in the eye of the eel *(Anguilla anguilla)*

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Summary. By means of light and scanning electron microscopy evidence of the existence of a normally organized choroid in the eye of the eel, *Anguilla anguilla,* is presented.
Key words. Anguilla anguilla; eel, eye; eye, eel; choroid.

The eye of the eel, *Anguilla anguilla,* is of particular interest, since its retina is directly fed by the membrana vasculosa retinae[1,2], whose vessels are derived from the hyaloid artery, spread on the inner surface of the optic cup and penetrate within the retina. If one considers that only angiotic mammals and the snake *Tarbophis* show intraretinal vessels, the unusual organization of the eye of the eel is clear. Furthermore, according to several authors[3-6], the fish is unique in having no demonstrable choroid[7]. Therefore, the present work was undertaken to provide a description of the organization of the ocular layers of the eel, with particular regard to existence of the choroid.

Male eels *(Anguilla anguilla)* were collected in their natural habitat and killed by decapitation. The eyes were rapidly enucleated, cut into 2 halves and fixed in 4% glutaraldehyde in phosphate buffer (pH 7.4; 0.2 M). One half was critical point dried, gold-sputtered and examined in an ETEC Autoscan scanning electron microscope, the other was trimmed into small pieces (1–2 mm^3), post-fixed in 1% OsO_4 in phosphate-sucrose buffer (pH 7.4; 0.2 M) and embedded in Durcupan. Semithin sections were cut in a LKB Ultrotome V ultramicrotome, stained with toluidine blue-pironine[8], and examined using an Olympus BH-2 microscope.

The existence of the choroid could be demonstrated in both kinds of micrograph. In fact, when observed with the scanning electron microscope (fig. 1), the eye of the eel shows blood vessels within the neuroretina and, under the pigment epithelium layer, a regular row of wider vessels filled with erythrocytes. A semithin section of the same region (fig. 2) shows a vascular

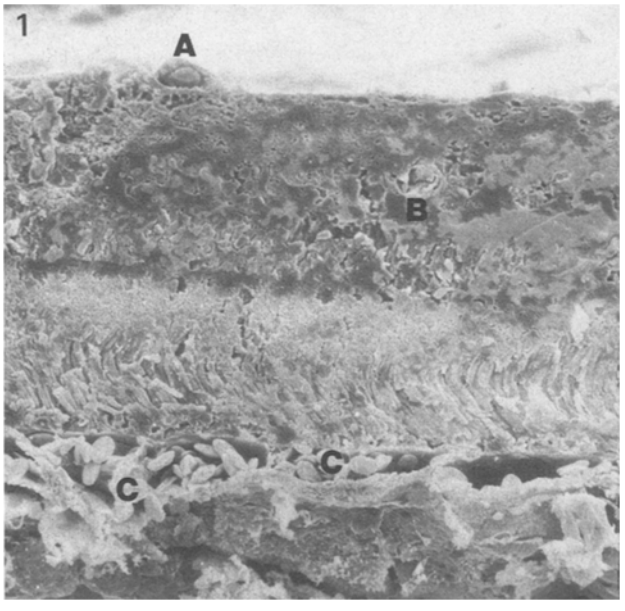

Figure 1. Longitudinal section, obtained with a razor blade, of the optic cup of the eel. A, membrana vasculosa retinae; B, intraretinal vessels; C, choroidal vessels. × 560.

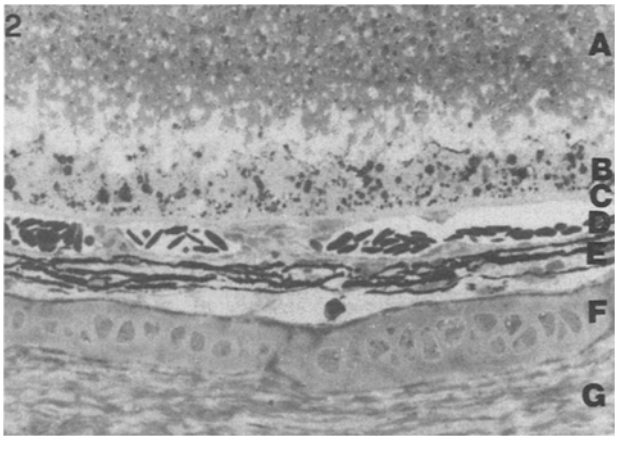

Figure 2. Semithin section of the eye of the eel. A, photoreceptors; B, pigment epithelium; C, Bruch's membrane; D, choroidal vessels; E, argentea; F, scleral cartilage; G, sclera. × 520.

layer formed either by large vessels or by small capillaries between the pigment epithelium and the choroidal lamina argentea.
The conclusion can be drawn that all vertebrates, with no exception, possess a choroidal circulation. The retina of the eel, in fact, is fed not only, directly, by the branches of the «membrana vasculosa retinae», but also, indirectly, by the choroidal vessels, via the pigment epithelium.

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0014-4754/84/090955-02$1.50 + 0.20/0

'Chloride-cell' – like mitochondria-rich cells of salamander larva gill epithelium

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Summary. Two types of mitochondria-rich cells (MRC) are described ultrastructurally in the gill epithelium of salamander larva. They resemble MRC found in larval ventral epidermis. Histochemical localization of carbonic anhydrase indicated numerous positive reacting cells, most of them flask-shaped. Morphological and functional similarities to fish 'chloride cells' are discussed.
Key words. Mitochondria-rich cells; amphibian gill; carbonic anhydrase; chloride cell.

The 'chloride-cell' described in fish gills, operculum and skin is widely accepted as an osmoregulatory unit of this group[1]. These cells have been demonstrated both in fresh and in seawater fish[2]. An equivalent cell in Amphibia has not yet been described. Mitochondria-rich cells (MRC) of possible osmoregulatory function were observed in the epidermis of both larval and adult Amphibia[3-5]. These cells were characterized by the unique possession of carbonic anhydrase (CAH) activity[5,6]. A possible involvement of these CAH-positive epidermal MRC in CO_2 elimination was suggested by us. It was only natural to look for similar cells in the amphibian larval gill epithelium. Ultrastructural and histochemical studies on the gill epithelium of *Salamandra* larvae revealed the presence of abundant MRC which are endowed with an elaborate network of membranous tubules and which demonstrate CAH activity. This is the first report describing 'chloride-cell'-like MRC in Amphibia. Their possible role in gas-exchange is discussed.
Materials and methods. Gills were taken from *Salamandra salamandra* larvae at the age of 1, 4, 8 and 12 weeks. Tissue specimens were fixed in 3% glutaraldehyde buffered with 0.1 M cacodylate buffer (pH = 7.4) for 2 h at 4 °C, postfixed in buffered 1% OsO_4 for 1 h (4 °C), dehydrated in graded ethanols and embedded in Epon 812. 1-µm-thick sections stained with 1% toluidine-blue in 1% borax and silver to gold sections were cut on an LKB Ultratome III, mounted on uncoated copper grids and stained with 2% uranyl acetate and lead citrate. Grids were then examined in a Jeol 100B TEM operated at 80 kV.
For the demonstration of CAH activity, gills were removed from larvae at the corresponding ages and the histochemical reaction and its control were carried out using Hansson's method as previously described by us in detail[5] on 10–20 µm prefixed frozen sections.
Results and discussion. In the salamander larva gill epithelium of all ages studied, numerous CAH-positive cells, many of which were flask-shaped, could be observed (fig. 1). Toluidine-blue stained, 1-µm-thick sections of the gills revealed the distribution pattern of the flask-shaped cells, which were relatively intensely stained (fig. 2). These cells were found mostly in the interlamellar regions of the main gill filament. They are hardly ever found in the secondary lamellae. Although no quantitative analysis of MRC numbers was undertaken, because of the highly delicate nature of the gill tissue, we were much impressed by the density of MRC in this tissue compared to the epidermis.
Ultrastructural examination has shown the cells to be darker than their neighboring surface cells (fig. 3). Many of these cells are found at the surface where they are endowed with long and branching microvilli. Most of these cells assume the typical flask form, opening to the surface with a narrow neck, whereas others display a broad apical front. (A more detailed ultrastructural description is in preparation). Some cells, however,

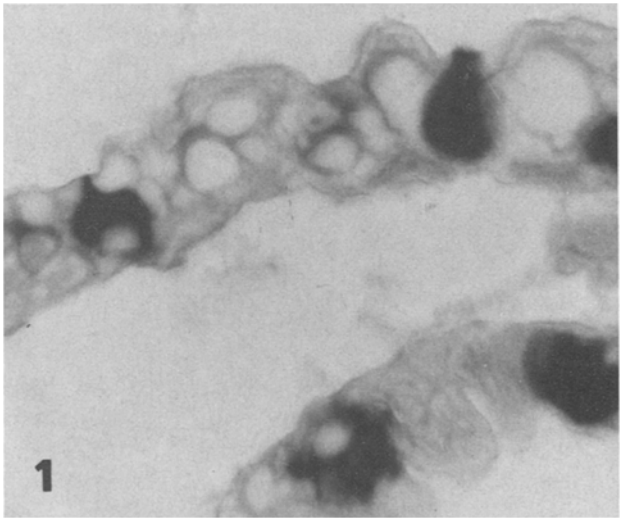

Figure 1. 10-µm-thick frozen section of a gill filament of a 2-day-old salamander larva. Several pear-shaped cells, CAH positive, can be seen. All of them open to the surface through a narrow neck. The pavement cells are negative. Control pieces of tissue incubated in the presence of acetazolamide[5] were totally negative. (× 1875).