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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'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,

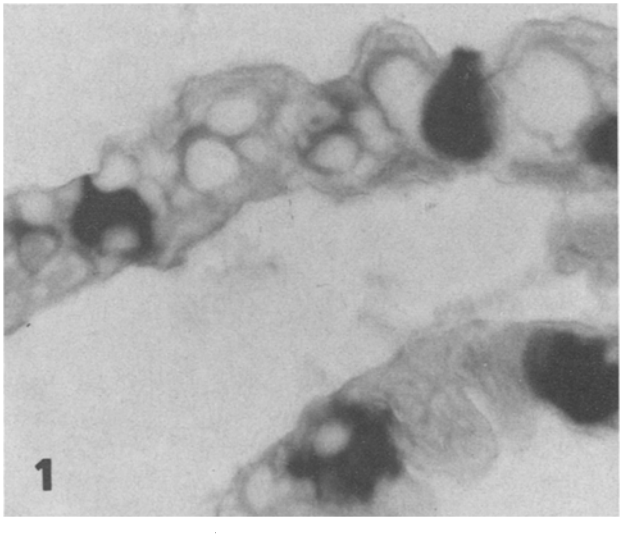

Figure 1. 10-µm-thick frozen section of a gill filament of a 2-day-old salamander larva. Several pear-shaped cells, CAH positive, can be seen. All of them open to the surface through a narrow neck. The pavement cells are negative. Control pieces of tissue incubated in the presence of acetazolamide[5]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

are found in deeper layers of the gill epithelium. Irrespective of the MRC localization they are densely filled with large, sometimes elongated mitochondria. An extensive membranous network of tubules fills almost the entire cytoplasmic space (figs. 3, 4). Connections between the lumen of the tubules and the extraepithelial apical space could sometimes be discerned. The MRC cytoplasm is rather electron dense, is rich in free ribosomes and extends into the microvilli (fig. 4).

Our previous observations that the epidermal MRC of salamander larvae are highly positive for CAH led us to suggest their possible involvement in CO_2 elimination[5]. Cutaneous respiration in Amphibia is a well established process[7]. Gills are responsible for a large proportion of CO_2 elimination in at least 1 aquatic urodele (*Necturus*[8]). It seems to us that the gills of *Salamandra* larvae could likewise be involved in CO_2 elimination. In these larvae gills are prominent during the cold season. There is evidence showing that CAH activity in gills is inversely related to temperature (in *Salmo*[9]). When water temperature rises in spring, gills are resorbed towards metamorphosis and the lungs assume their role in gas exchange in concert with the epidermis.

In the amphibian gill, a few cell types rich in mitochondria have been described in the past. None of them resemble the fish 'chloride-cell'[10,11], except for one MRC type found in the neotenous axolotl[12]. The 'chloride-cell'-like MRC of the salamander gill which we described in this study resembles ultrastructurally the larval epidermal MRC type II[5]. The main reasons for this are the presence of the extensive network of tubules and the enormous numbers of the mitochondria. These 2 traits are also characteristics of the fish 'chloride-cell'. But whereas in fish gill (and operculum) the ratio of tubules to mitochondria generally favors the first, in the salamander gill MRC it is in favor of the latter[2]. Moreover, whereas the fish 'chloride-cell' is characterized by opening to the surface in a typical crypt (pronounced in seawater-adapted fish), here the MRC protrude above the surface[13], being enclosed by a depression formed by the neighboring cells (figs. 2 and 3). Another difference between fish 'chloride-cell' and salamander larva MRC is that in fish there is a narrow band of apical cytoplasm which is devoid of the tubular system[14]. In contrast, in the salamander gill MRC the tubules reach out to the apical plasma membrane (figs. 3 and 4), and probably also open to the extracellular environment.

Fish 'chloride-cells' are largely involved in transport of ions[15], but appear also to have a role in acid-base regulation[16]. In

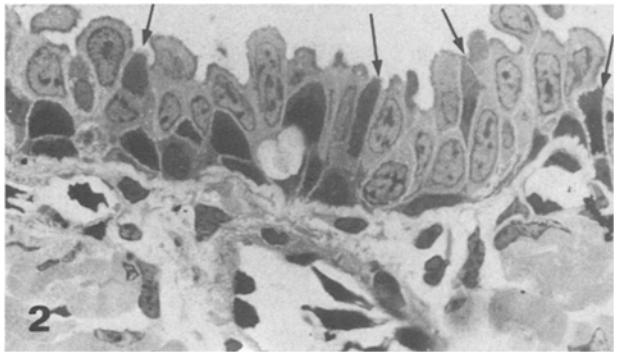

Figure 2. 1-µm-thick section of a gill filament from a 1-week-old salamander larva. The section demonstrates four MRC (arrows) in an interlamellar region of the gill filament. The MRC have a flask-form and a corona of microvilli. The MRC stain more intensely than their neighboring cells. (× 1050).

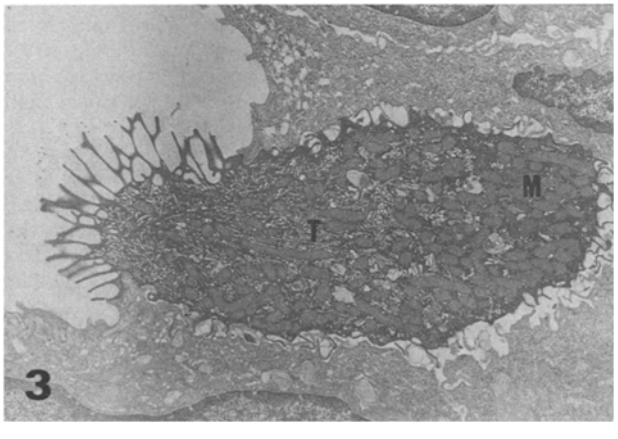

Figure 3. Transmission electron-microscope micrograph demonstrating a MRC from a gill filament of a 1-week-old salamander larva. The cell is fully packed with mitochondria (M). An elaborate tubular system (T) fills almost all the space in between the mitochondria. The cell is endowed with branching elongated microvilli. Its cytoplasm is much darker than that of its neighboring cells. (× 3000).

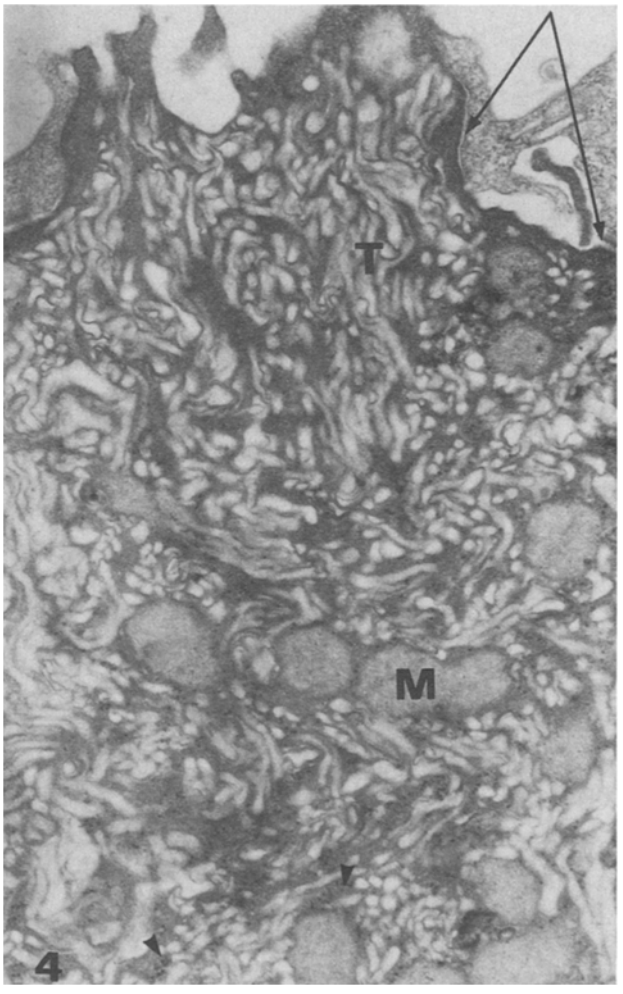

Figure 4. Higher magnification of the apical part of another MRC. The tubular system (T) reaches to the apical plasma membrane. One opening of its lumen to the extracellular space (short arrow) can be seen. The electron dense cytoplasm fills the little remaining space between the mitochondria (M) and the tubular system and extends into the microvilli. Free ribosomes (arrowheads) are scattered in the cytoplasm. A long tight-junction and a desmosome (long double arrows) connect the MRC with its neighbor pavement cells. (× 25,500).

Amphibia, however, the gills do not seem to be essential for ion regulation as neither ligation nor amputation of gills had much effect on transport[17,18].

In spite of the morphological differences mentioned, CAH activity is a common denominator of fish 'chloride-cell'[19,20] and salamander gill MRC. Based on these observations we feel that the amphibian gill MRC could be involved to a large extent in gas exchange and acid-base regulation, thereby providing a possible link between the fish gill 'chloride-cell', the amphibian larval epidermal MRC, and hence the adult amphibian epidermal flask-cell[5,6].

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Scanning and transmission electron microscopic evidence of epithelial phagocytosis of spermatozoa in the terminal region of the vas deferens of the cat

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Summary. Scanning and transmission electron microscopic observations have been made in the terminal region of the vas deferens of the cat, with emphasis on the occurrence of spermiophagy. The present study has revealed that epithelial cells as well as luminal macrophages are extensively and actively involved in phagocytosis of spermatozoa. The mechanism of the spermiophagy is discussed, in relation to a possible role of the epithelial cells, as one function of the vas deferens.
Key words. Cat, vas deferens; vas deferens, cat; spermatozoa; phagocytosis, epithelial; spermiophagy.

Phagocytosis of spermatozoa by the epithelial cells was first demonstrated extensively in the rat vas deferens, and especially in its terminal portion, by Cooper and Hamilton[1]. Phagocytotic activity of the epithelial cells in the vas deferens has since been confirmed in its ampullary region in monkeys and humans[2–5]. Sporadic occurrence of spermiophagy by the epithelial cells in male reproductive tracts other than the vas deferens, such as seminiferous tubule, rete testis and efferent ductule has recently been documented in some mammals[6–8]. However, at present, it is not known whether spermiophagy by the epithelial cells in the vas deferens is a common event in mammalian species or is a phenomenon peculiar to certain species. In this report, the terminal region of the cat vas deferens was studied by SEM and TEM with special attention to the spermiophagic ability of its epithelial cells.

Materials and methods. Four adult male domestic cats were used in this study, and the experiments were carried out in March and April. The animals were anesthetized with Nembutal injected intramuscularly and perfused vascularly through the ascending aorta first with physiological saline solution and next with 2.5% paraformaldehyde and 2% glutaraldehyde in cacodylate buffer (pH 7.2). After perfusion, the whole reproductive tract was removed rapidly. The vas deferens was dissected out and subdivided grossly into proximal, distal and terminal regions. The proximal region was located within the scrotum and the latter two were in the pelvis. A short segment of each region was cut into small tissue blocks. They were immersed in the same paraformaldehyde-glutaraldehyde fixative for another 1–2 and postfixed in 2% phosphate buffered OsO_4 (pH 7.2) for 1 h. Following dehydration by ascending acetone, the tissue was embedded in Epon 812. Thin sections were cut with diamond knives, stained with lead citrate and either uranyl acetate or tannic acid, and viewed in a H-500 or JEM-100S transmission electron microscope (TEM). Thick sections of 1 μ were cut with glass knives and stained with 1% toluidine blue in phosphate buffer. Tissues for scanning electron microscopy (SEM) were prepared and processed as for TEM examination but after dehydration they were dried in a critical point dryer using liquid CO_2, coated with gold-palladium in an Eiko sputter coater, and examined with an HFS-2 field emission scanning electron microscope.

Results and discussion. The terminal vas deferens of the adult cat was less than 10 mm in the region just before it entered the prostate to form the ejaculatory duct (prostatic urethra) (fig. 1). The lumen of the terminal portion was characterized by a rather distended and circular profile. The epithelium lining the lumen was uniform in height and consisted of 1 or 2 layers of low cuboidal cells (fig. 2).

In thin sections, the epithelial cells were somewhat smooth in outline and possessed slender microvilli and an occasional single cilium projecting into the lumen. In the cytoplasm of the cells, there were a few organelles, many lysosomal dense bodies and abundant fine filaments oriented randomly (fig. 3).

When viewed by SEM, the luminal surface of the epithelial cells of the terminal vas was flattened, with a hexagonal cell boundary, and was covered by stubby microvilli which varied slightly in length from cell to cell. A large number of spermatozoa and a few macrophages were located on the epithelial sur-