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Structure of the Internal Gills in Tadpoles of the Crab-Eating Frog, *Rana cancrivora*

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ABSTRACT—The morphology of internal gills was studied by light and electron microscopy in crab-eating frog tadpoles (T-K stages XIII-XVII) raised in tap-water. The internal gills consist of four pairs of gill arches, on which gill tufts are arranged linearly. Each gill tuft is composed of a stem and numerous ramifications. Histologically, a few PAS-positive cells are evident in the stem of the gill tuft. In fine structure, bi- or multilayered epithelia of internal gills are composed of pavement cells, mitochondria-rich (MR) cells and basal cells. The cuboidal pavement cells are abundant in the ventral epithelium of the gill arches and the epithelium of the stem of the gill tufts. On the other hand, the simple epithelium in the apical portion of the gill tufts consists of squamous pavement cells. The cuboidal and squamous pavement cells are identical in ultrastructure and contain many apical vacuoles and short microvilli covered with a fuzzy coat at the free surface. The MR cell is pleomorphic and characterized by numerous metochondria in the supranuclear area. This type of cell has a round, ovoid or pear-shaped profile in sections perpendicular to the surface. The matrix of the cytoplasm of the MR cells is electron-dense. Simple basal squamous cells located under the pavement or the MR cells are interconnected with the covering cells by desmosomes. Thus, four types of cell, pavement, MR, basal squamous and mucous, are distinguishable in the internal gill of the tadpole of the crab-eating frog.

INTRODUCTION

In anuran larvae, the internal gills are composed of well branched short tufts, which bulge from the ventral walls of the gill arches and function until metamorphosis [1]. In fish, the gills serve not only as an organ for respiration, but also as the main site of ion exchange and nitrogenous waste excretion [see 2]. In contrast, only one report has been published on the ultrastructure of the internal gills in anuran tadpoles [3]. Based on electron microscopy, Hourdry [3] suggested that the internal gills of tadpoles may play an osmoregulatory as well as a respiratory function. In fact, physiological studies have shown that the gill chamber is the major site of ion exchange [4], although no morphological evidence was presented.

The tadpole of the crab-eating frog, *Rana cancrivora,* is the only amphibian larva able to inhabit a brackish-water environment [5, 6]. Gordon and Tucker [6] suggested that the tadpole of the crab-eating frog is a good osmoregulator and that the gills may be important for ion exchange in euryhaline environments, although they did not show any cytological or physiological evidence. The present study was therefore undertaken to clarify the histological and cytological structure of the internal gills of the tadpole of the crab-eating frog. One purpose of this study was to provide basic cytological knowledge on the role of the internal gills in osmotic regulation of this euryhaline tadpole.

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MATERIALS AND METHODS

Adult males and females of the crab-eating frog,

Rana cancrivora, were captured in mangrove swamps (salinity 31‰) at Ang-Sila near Bangkok, Thailand, in April 1987. Tadpoles for the present experiments were obtained from these adults and raised in the laboratory at Niigata. Details of fertilization and development of tadpoles were described previously by Uchiyama *et al.* [7]. The embryonic stages were judged according to the developmental stages for *R. pipiens* by Taylor and Kollros [8]. Tadpoles (T-K stages XIII-XVII) were reared in tap-water at 24.5–26°C.

The internal gills were dissected out quickly and

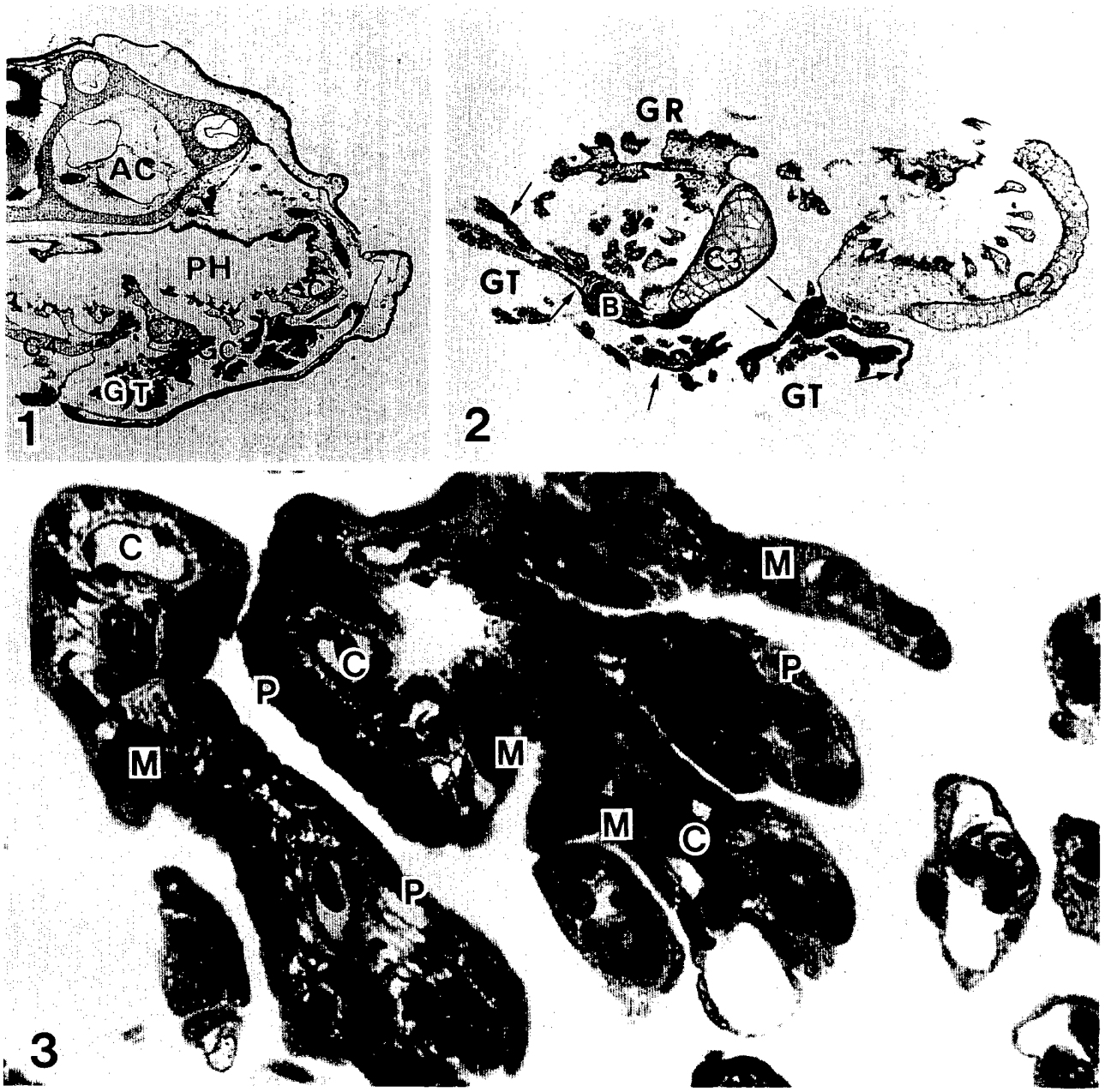


FIG. 1. Light micrograph of a cross-section at the ear level in a tadpole (stage XIII). Gill rakers and gills tufts are seen in the dorsal and ventral sides of ceratobranchialia, respectively. AC, auditory cavity; C1-C4, ceratobranchialia 1 to 4; GC, gill chamber; GT, gill tuft; PH, pharynx. Hematoxylin and eosin. ×40.

FIG. 2. Light micrograph of gill tufts of the 2nd and 3rd gill arches (stage XVI). B, branchial muscle; C2 and C3, ceratobranchialia 2 and 3; GR, gill raker; GT, gill tufts. Arrows indicate the location of eosinophilic cells. Hematoxylin and eosin. ×160.

FIG. 3. Light micrograph of semi-thin section of a gill tuft (stage XV). C, capillary; M, mitochondria-rich cell; P, pavement cell. Toluidine blue. ×630.

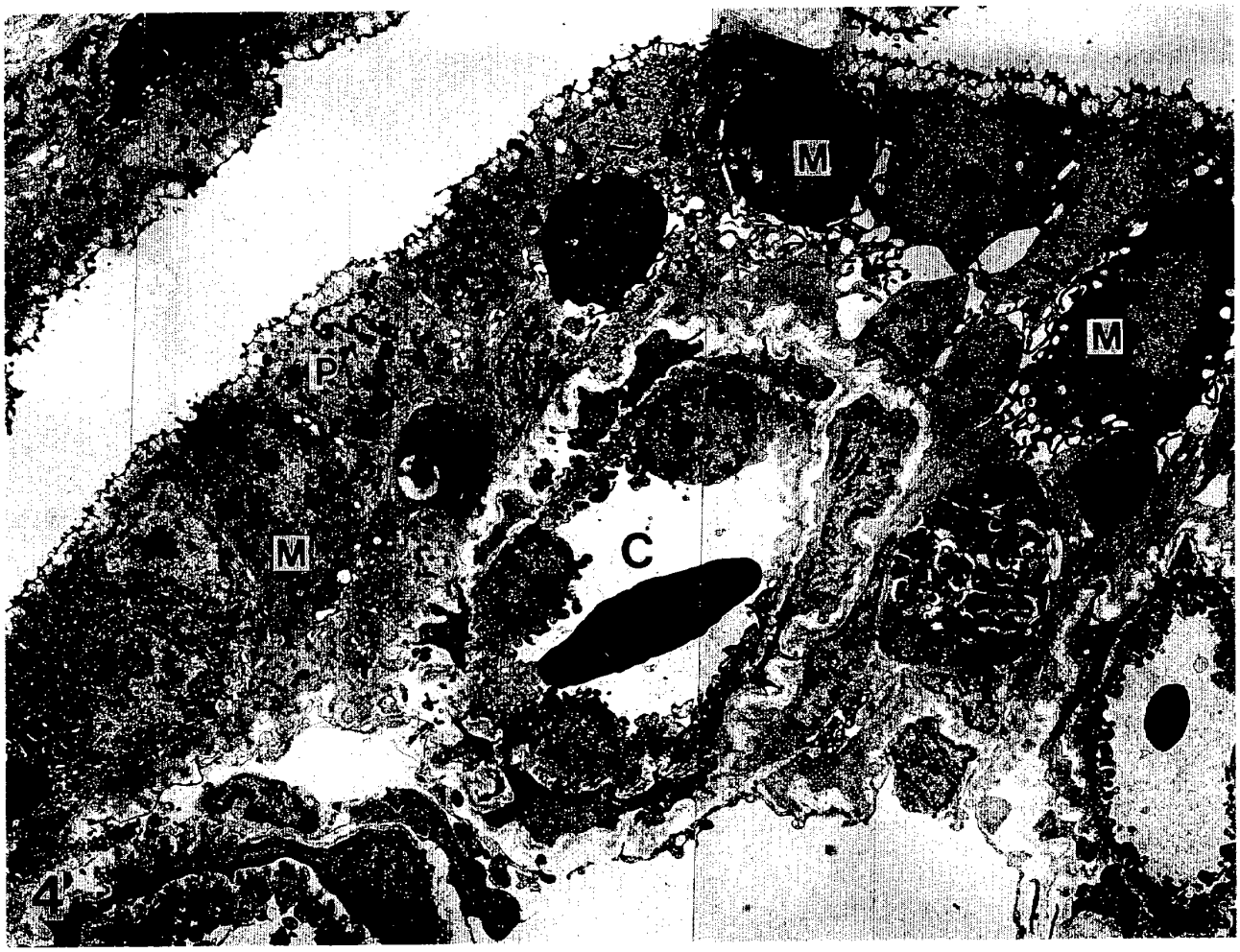
treated as follows. For light microscopy, they were fixed with Bouin's solution, dehydrated and embedded in paraffin. They were then sectioned serially at 6 or 8 μm by the routine paraffin method. The stains used were: 1) Mayer's hematoxylin and eosin, 2) Heidenhain's azan, or 3) periodic acid-Schiff reagent (PAS) and hematoxylin. Some semi-thin (1 μm) sections were stained with toluidine blue.

For electron microscopy, the internal gills were excised rapidly and immediately bathed with a solution of 2.5% glutaraldehyde—2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), then cut into small pieces and placed into fresh fixative for 6 hr. Tissue pieces were washed twice with 0.1 M sodium cacodylate buffer, then post-fixed with 1% osmium tetroxide for 1 hr. They were washed in distilled water, dehydrated in an ethanol series and embedded in epoxy resin. Thin sections were stained with methanolic uranyl acetate and alkaline lead citrate, and examined with a JEOL JEM-100B electron microscope.

RESULTS

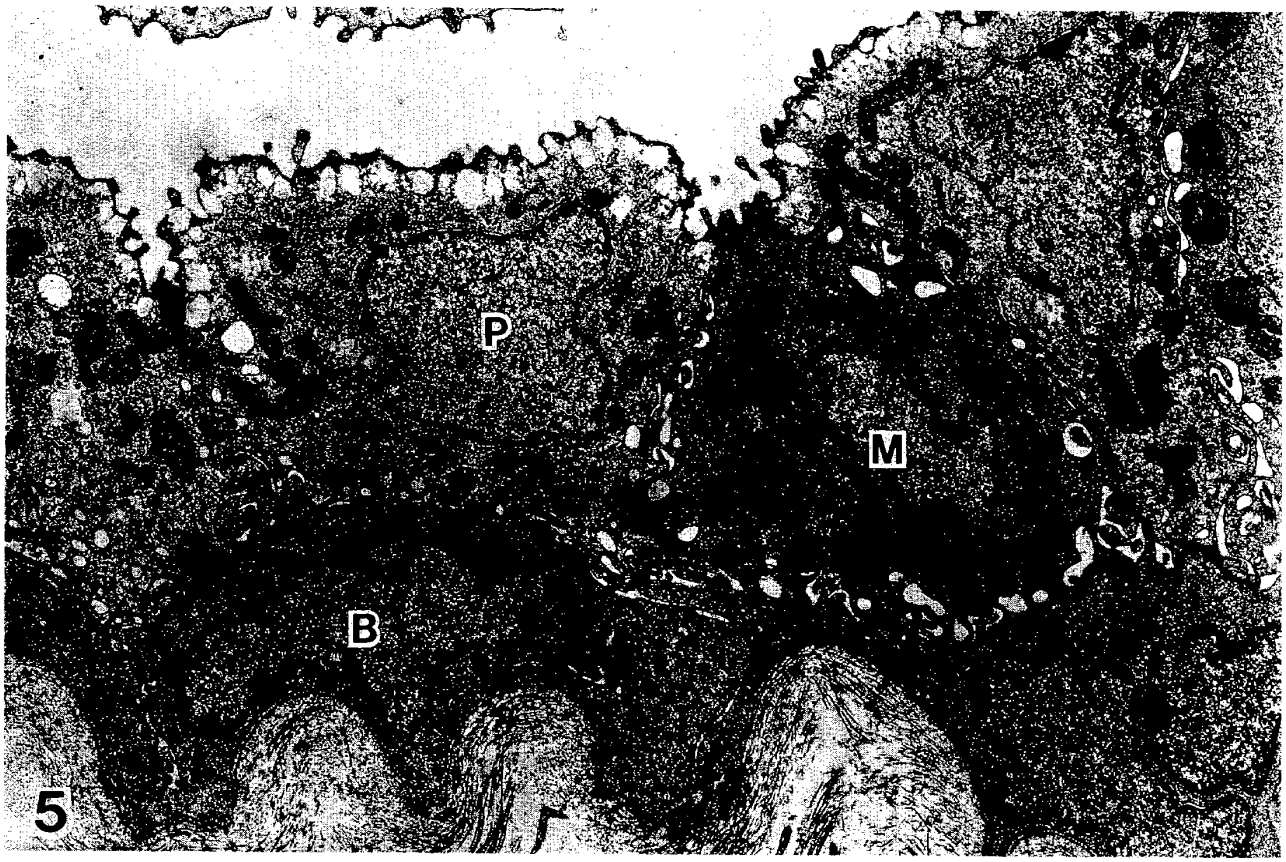
Light microscopy

There are four pairs of gill arches, which are supported by cartilaginous skeletons, the ceratobranchialia 1–4. Ventral and dorsal terminals of the ceratobranchialia are joined with each other, thus forming a gill basket. On the inside of this structure, the dorsal parts of the gill arches are covered with the gill rakers, which are composed of a bilayer of cells which are columnar and squamous.



FIGS. 4–9. Electron micrographs of tadpole gill tufts (stage XV and XVII).

FIG. 4. Basal parts of ramifications of a gill tuft (stage XVII). C, capillary; M, mitochondria-rich cell; P, pavement cell. ×2,700.



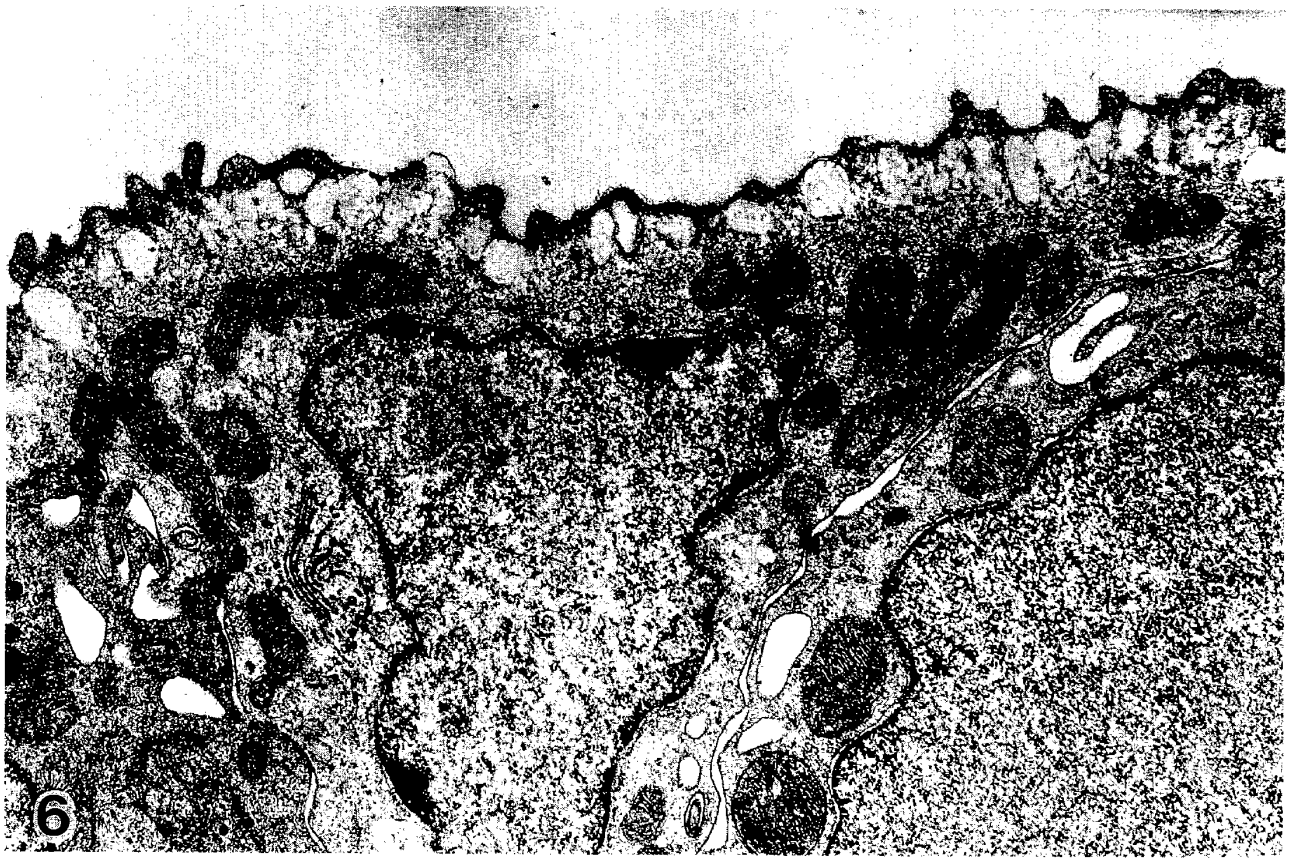


FIG. 5. Ventral epithelium of the gill arch (stage XVII) consist of a bilayer of cells, including mitochondria-rich (M), pavement (P) and basal squamous (B) cells. ×6,900.

FIG. 6. Apical site of pavement cell (stage XVII). Note many apical vacuoles and short microvilli covered with a fuzzy coat at the free surface. ×13,200.

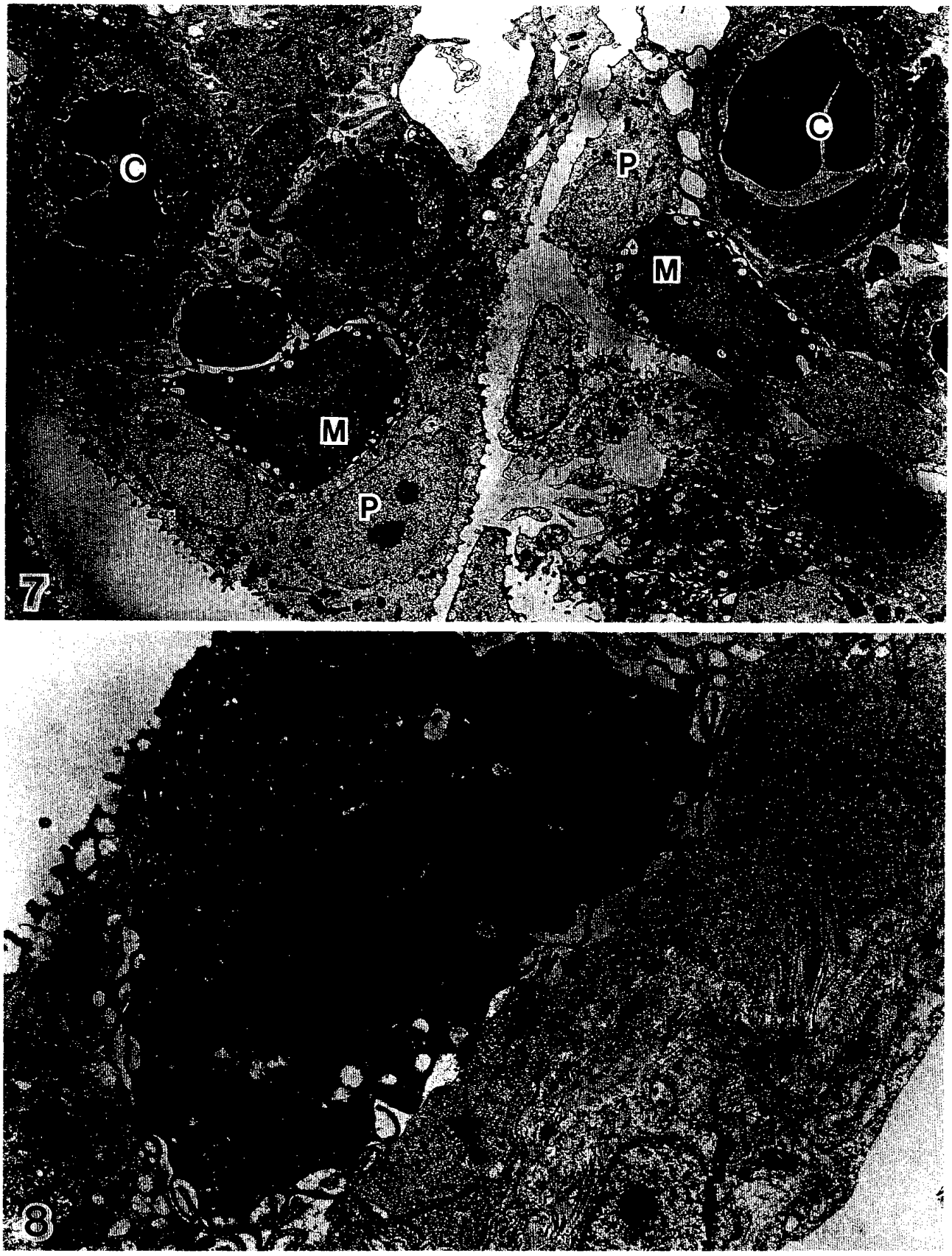


FIG. 7. Cross-section of the apical portion of a gill tuft (stage XV). Note cytoplasmic extensions of pavement cells covering the apical surfaces of neighboring cells. C, capillary; M, mitochondria-rich cell; P, pavement cell. ×3,300.

FIG. 8. Progressive stage of degeneration of a mitochondria-rich cell, showing condensation of the cytoplasm, and condensation and indention of the nucleus (stage XVII). ×9,300.

The internal gills consist of soft gill tufts situated linearly on the ventral and lateral parts of the gill arches. The gill tuft is composed of a stem and numerous ramifications and is well vascularized. The epithelium of the stem of the gill tuft consists of bi- or multilayers of cells which are similar to those of the ventral epithelium of the gill arches. In the 2nd and 3rd gill arches the gill tufts are more numerous than in the other gill arches. Figure 1 shows the gross morphology.

Large round or ovoid cells characterized by eosinophilic cytoplasm are scattered in the ventral and lateral epithelium of the gill arches and the stem of the gill tufts, but not in the dorsal epithelium of the gill arches (Fig. 2). The cytoplasm of this cell type is stained densely by toluidine blue in semi-thin sections (Fig. 3). A few PAS-positive cells are evident in the gill tufts. The columnar cells of the gill rakers are also PAS-positive, showing the presence of mucopolysaccharides.

Electron microscopy

Bi- or multilayered epithelia composed of cuboidal and squamous cells are evident in the ventral parts of the gill arches and the stems of the gill tufts. These cells are connected with each other by tight junctions at their apical portion and bear a few irregular, lateral and basal infoldings that interdigitate with each other. Broad intercellular spaces are sometimes observed at the lateral and basal portion (Fig. 4).

At the ventral and lateral epithelia of the gill arches and the basal parts of the gill tufts, simple squamous cells are situated under the cuboidal cells (Fig. 5). In these squamous cells the cytoplasmic organelles are sparse and the nucleus-cytoplasm volume ratio is high. The basal squamous cells are interconnected with the covering cells by desmosomes (Fig. 5).

In the ventral and lateral epithelia of the gill arches and the gill tufts, two types of cuboidal cell are distinguishable. One, the clear cell, has electron-lucent cytoplasm and the other, the dark cell, has electron-dense cytoplasm. The clear cells, which are pavement cells, sometimes take a squamous profile and are abundant in the ventral

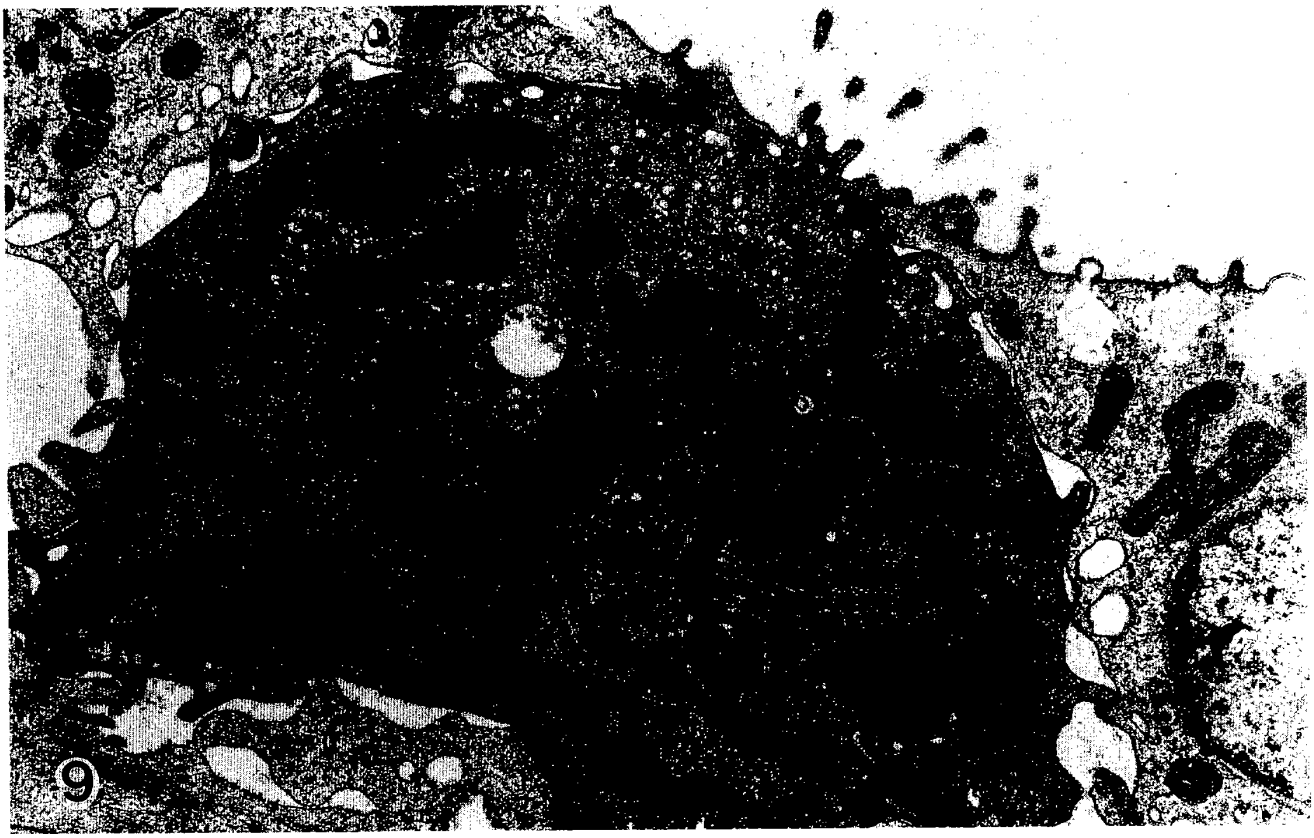


FIG. 9. Note many clear or dense vesicles and simple tubular structures in the supranuclear area of a mature mitochondria-rich cell (stage XV). ×13,000.

and lateral epithelia and the gill tufts. In the pavement cells, there are many apical vacuoles and short microvilli which are covered with a fuzzy coat at the free surface (Fig. 6). The pavement cells contain a Golgi apparatus with numerous associated vesicles, such as rough endoplasmic reticulum and free ribosomes. The apical part of the gill tufts consists of a monolayer of the squamous pavement cells, which may function in respiration (Fig. 7). Electron-lucent cytoplasm resulting from poor development of cellular organelles and fewer microvilli are observed in some squamous pavement cells in the apical parts of the gill tuft. Between the squamous pavement cells and the basal layers extracellular spaces are observed.

The dark cells are characterized by electron-dense cytoplasm and numerous mitochondria in the supranuclear area. These cells are termed mitochondria-rich cells (MR cells). The MR cells are pleomorphic and often show a round, ovoid or pear-shaped profile in sections perpendicular to the surface. In this type of cell, the apical surface is furnished with microvilli and the nucleus is usually present at the basal portion (Figs. 4, 8 and 9). The MR cells are fewer in the apical portion and numerous in the basal parts of the gill tufts and the ventral epithelium of the gill arches. The MR cells seem to be absent in the gill rakers. The apical surfaces of MR cells are often covered by cytoplasmic extensions of neighboring pavement cells. Degenerating MR cells are frequently observed, being characterized by cellular shrinkage, condensation of cytoplasm, condensation and indentation of the nucleus and the presence of many lysosomes (Fig. 8). In contrast, many clear or dense apical vesicles, tubular structures and Golgi apparatus are present in the mature MR cells (Fig. 9).

DISCUSSION

The present observations using light microscopy make it clear that the basic structure of the gill of the present tadpoles is similar to that of *R. catesbeiana* [9]. Hourdry [3] reported from ultrastructural studies that the respiratory squamous cell and two types of cuboidal cell can be recognized in the gill tufts of tadpoles of *Discoglossus pictus*. Greven [10] reported that the epithelium of the external gills of larval intrauterine *Salamandra salamandra* is composed of pavement cells, basal cells, pea-shaped cells and ciliated cells. In teleosts, the gill is composed of five types of cell: squamous pavement, chloride, mucous goblet, neuroepithelial, non-differentiated [see 2, 11]. The epithelia of gill arches and the gill tufts of the tadpoles of *R. cancrivora* consist of four types of cell: pavement, MR, basal squamous, mucous. The function of the basal squamous cell is unknown from the present study. Only a few mucous cells, which are PAS positive, are present in the gill tufts, although mucous cells are numerous in the gill rakers, which are considered to function as a filter [9].

Pavement cells

The pavement cells form a sheet covering the epithelia of the gill arches and the gill tufts. In the present study, two types of pavement cell, the cuboidal and the squamous, are observed at the stem and the apical parts of the gill tuft, respectively. Ultrastructural features of the cuboidal pavement cells and the squamous pavement cells are identical. The feature of these cells is also similar to that of the squamous pavement cells which have been reported in filament epithelium and secondary lamellae of teleosts [see 2, 12]. Columnar pavement cells have also been reported in the filament epithelium of teleost [see 2]. Although the exact function of vesicles concentrated beneath the apical membrane is unknown, they may be involved in regulation of the permeability of the gill surface as well as the fuzzy coat of the apical membrane.

Mitochondria-rich cells

Hourdry [3] first reported that the MR cell is present in the gill tufts of tadpoles of *D. pictus*. The presence of MR cells has also been observed in the integuments of anuran tadpoles and the integuments and gill epithelium in the larval urodele [10, 13]. This type of cell is considered to have a function in transport of ions [3, 13]. In the present study, eosinophilic cells which seem to be equivalent to MR cells were observed in the epithelia of gill arch and gill tufts of tap-water-

adapted tadpoles of *R. cancrivora*. In contrast, Gordon and Tucker [6] reported no signs of acidophilic cells similar to those found in the gills of teleosts in the gills of tap-water-, 60% seawater- and 80% seawater-adapted *R. cancrivora* tadpoles. The chloride cells are present in both euryhaline and stenohaline species of fresh- and seawater teleosts, and function specifically in relation to environmental salinity. It has been reported that the chloride cells of teleosts take up small quantities of ions in freshwater [14] and secrete large amounts of salts in seawater [15, 16]. Ultrastructural characteristics of the chloride cells have been elucidated in both fresh- and seawater teleosts [see 12]. It has also been reported that freshwater larval lampreys possess chloride cells which are rich in apical vesicles and mitochondria without a distinct tubular system or apical pits [17, 18]. This type of chloride cell is characteristic of freshwater teleosts and has been suggested to participate in the active uptake of ions [17, 18]. Hourdry [3] also indicated a similar type of MR cell in the gill tufts of *D. pictus* tadpoles.

In the present study the features of the MR cell (Fig. 9) are consistent with those of the MR cells reported previously in ammocoetes and tadpole gills [3, 17, 18]. It is suggested, therefore, that the gills of *R. cancrivora* tadpoles may play a role in the osmoregulation of their body fluid by means of mechanisms similar to those of teleosts in freshwater. On the other hand, the mechanisms of salt water adaptation remain to be elucidated in the tadpole of the present species.

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