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Effect of ambient and dietary cadmium on pavement cells, chloride cells, and Na^+/K^+-ATPase activity in the gills of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water

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The effects of cadmium on the gills of the African freshwater cichlid *Oreochromis mossambicus* in water with normal and relatively high calcium concentrations were studied for periods up to 35 days. The exposure was either through the ambient water or via the diet. Changes in the ultrastructure of the gill epithelium upon exposure to cadmium in the ambient water indicated degeneration of pavement cells and chloride cells, and acceleration in the turnover of the chloride cells. Studies of the Na^+/K^+-ATPase activity of the gills indicated that a transient increase in the total number of chloride cells was not associated with an increase, but rather a decrease of the total ion-exchange capacity of the chloride cells. Macrophages, lymphocytes, rodlet cells and neutrophilic granulocytes infiltrated the filament epithelium. Recovery of the gills was observed after 35 days. Dietary cadmium caused similar, although delayed, effects. High water calcium concentration reduced the impact of water-borne cadmium, but had no ameliorating effect on dietary cadmium. The data indicate that the disturbance of ion regulation in fish caused by sublethal levels of dietary cadmium is the result of effects of the metals on the gills rather than on the kidney.

Key words: Calcium; Water-borne cadmium; Dietary cadmium; Gills; Na^+/K^+-ATPase activity; *Oreochromis mossambicus*

INTRODUCTION

Gill damage and structural changes caused by water-borne cadmium have been reported for several fish species (Bilinski and Jones, 1973; Eisler and Gardner, 1973; Gardner and Yevich, 1970; Voyer et al., 1975; Karlsson-Norrgren et al., 1985). Similar alterations of the gills have been observed in fish exposed to other toxic metals

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such as mercury (Lock et al., 1981; Naidu et al., 1983), copper (Gupta and Rajbanshi, 1981), zinc (Crespo et al., 1981), chromium (Van de Putte et al., 1982), tributyltin (Pinkey et al., 1989) and, in acidic waters, aluminium (Youson and Neville, 1987). In freshwater fish the damage results in electrolyte losses and, in more serious cases, impaired oxygen uptake (Evans, 1987; Pinkey et al., 1989; Wendelaar Bonga and Lock, 1993).

In fresh water, contaminated food forms a more important source of toxic metals than the water itself (Dallinger et al., 1987). This has been demonstrated in freshwater teleosts exposed to mercury (Phillips and Buhler, 1978), lead (Vighi, 1981), copper, and zinc (Dallinger and Kautzky, 1985), and is also applicable to cadmium. Although cadmium is widely distributed in natural waters as a result of industrial discharge and other human activities, it is mainly bound to ligands and this limits its toxicity to fish (Dallinger et al., 1987). Nevertheless, most studies on cadmium in fish have focussed on water-borne cadmium, and information on the effects of dietary cadmium is scarce. We have found that dietary cadmium uptake causes ionic disturbances of the blood plasma comparable to those caused by water-borne cadmium (Pratap et al., 1989). The effects of dietary cadmium on ion metabolism might be explained by the well-known effects of cadmium on the kidney. However, because of the similarity of the effects of water-borne and dietary cadmium on ion regulation, and because the gills are the main ion regulatory organ of fish, cadmium absorbed via the gut might also cause structural and functional alterations in the gills.

In the present study, our objective was to compare the effects of water-borne and dietary cadmium on the ultrastructure of the gills of the African cichlid *Oreochromis mossambicus*. We paid particular attention to the chloride cells (ionocytes) of the gills, the cells engaged in the active exchange of ions between the fish and the ambient water (Mayer-Gostan et al., 1987). Proliferation of chloride cells has been observed in teleosts exposed to, e.g., zinc (Matthiesen and Brafield, 1973; Crespo et al., 1981), chromium (Temmink et al., 1983), copper (Baker, 1969; Crespo et al., 1981) and cadmium (Oronsaye and Brafield, 1984). This has been interpreted by most of the authors as a reaction of these cells to compensate for ion losses, or, by Oronsaye and Brafield (1984) and Pascoe et al. (1986), as an attempt to increase the excretion of these toxicants. In a recent study on fish exposed to acid water we concluded that the rapid increase in chloride cell numbers under this condition in fact reflected a higher turnover rate of these cells rather than an increased ion transport capacity, because most of the cells were immature or degenerating (Wendelaar Bonga et al., 1990). We have now investigated whether the cadmium-induced proliferation of chloride cells might represent a similar process.

It is known that divalent cations, in particular Ca^{2+}, may reduce the impact of water-borne cadmium on fish (Coombs, 1979; Pascoe et al., 1986; Wicklund and Runn, 1988). We therefore compared the effects of water-borne and dietary cadmium in fish acclimated to water with normal (0.2 mM Ca^{2+}) and relatively high (0.8 mM Ca^{2+}) calcium in the water.

MATERIALS AND METHODS

Freshwater laboratory stock of male *Oreochromis mossambicus* of about 20 g body weight were used in the present study. The fish were kept in tap water (0.8 mM Ca^{2+}) and maintained in 100-l aquaria with continuous aeration and circulation of filtered water (pH 7.4), at 28°C on a daily 12-h photoperiod.

Experimental design

Fish were divided into six groups of ten fish each. Three groups were kept in normal calcium water (0.2 mM Ca^{2+}) and three in high-calcium water (0.8 mM Ca^{2+}). Three weeks before the experiment, fish were transferred to artificial freshwater prepared according to Flik et al. (1985). Acclimation of fish to water with 0.2 mM Ca^{2+} was performed by gradual reduction of the water calcium concentration from 0.8 mM Ca^{2+} to 0.2 mM Ca^{2+} during 1 week, followed by a period of 2 weeks in water of 0.2 mM Ca^{2+}. The fish were subsequently exposed to either 10 μg Cd/l of ambient water or were fed cadmium-containing food averaging 10 μg Cd/fish/day. Fish maintenance, preparation of cadmium food and administration of ambient or dietary cadmium were reported earlier (Pratap et al., 1989). The experimental set-up is shown in Table 1.

Sampling and fixation procedure

Fish from each tank were sampled after 4, 14, and 35 days. The fish were lightly anesthetized in 0.2% 2-phenoxyethanol, and decapitated. The gill filaments were excised and prepared for electron microscopy as described by Wendelaar Bonga and Van der Meij (1989).

Estimation of chloride cell density

Numerical chloride cell densities (n/mm^2) in the gill area were determined in the inner opercular epithelium. Freshly dissected opercula were incubated for 1 h in a well

TABLE 1
Experimental design

Group	Ca in water (mM/l)	Cd in water (μg/l)	Cd in feed (μg/day/fish)
1	0.2	10	
2	0.8	10	
3	0.2		10
4	0.8		10
5	0.2		
6	0.8		

aerated solution in tap water (1 mg/ml) of dimethyl amino styryl pyridylethyl iodide (DASPEI; ICN Biochemicals, Inc., Plainview, NY) as described by Foskett et al. (1981). After rinsing in tapwater, the opercula were examined in a fluorescence microscope at a magnification of ×250. The number of cells per surface area (n/mm^2) was determined by counting the cells in 20 different squares of the epithelium with a total surface area of 5 mm^2 per fish.

The relative frequencies of accessory, immature, mature, apoptotic and necrotic chloride cells were determined on the basis of the classification of 100 chloride cells per animal, in groups of six fish per time point.

Determination of the Na^+/K^+-ATPase activity

For determination of the Na^+/K^+-ATPase activity of the gills, the fish were quickly anesthetized in a Tris-buffered (pH 7.4) MS-222 solution. The bulbus arteriosus was cannulated and the branchial apparatus was perfused with ice-cold isotonic saline containing heparin (20 U ml^{-1}) to remove the blood cells from the gills. Additionally, 0.2 mM phenylmethylsulphonyl fluoride, a protease inhibitor, was added to the perfusion fluid to increase enzyme recovery. The epithelium of the gills of 8 fish per group was scraped off onto an ice-cold glass plate with a glass microscope slide. The subsequent preparative procedure and the (Na^+/K^+)ATPase assay were performed as described by Flik et al. (1983).

Statistical analysis

The data were statistically evaluated with analysis of variance and Student's *t*-test, where appropriate. Statistical significance was accepted for $P < 0.05$.

RESULTS

Controls from water with normal or high calcium levels

The ultrastructure of the branchial epithelium of tilapia has been described earlier (Wendelaar Bonga and Van der Meij, 1989). Briefly, the lamellae are covered by one or two layers of pavement cells. The filament epithelium is multilayered and consists of pavement cells, chloride cells, mucous cells, and undifferentiated basal cells (Figs. 1 and 2). Most chloride cells are mature cells characterised by apical crypts in contact with the water, an extensive tubular reticulum, some apical vesicles and some granular endoplasmic reticulum, free ribosomes and numerous mitochondria evenly distributed throughout the cytoplasm (Fig. 1). They are occasionally associated with small and slender accessory (replacement) cells (Fig. 1) and immature chloride cells. The latter are smaller than the mature cells, with a less extensive tubular system and more strands of granular endoplasmic reticulum and Golgi areas than mature chloride cells. Apoptotic and necrotic cells (Wendelaar Bonga and Van der Meij, 1989) are very scarce. Macrophages, neutrophilic granulocytes, lymphocytes and rodlet cells are occasionally observed in the intercellular spaces. Macrophages have electron

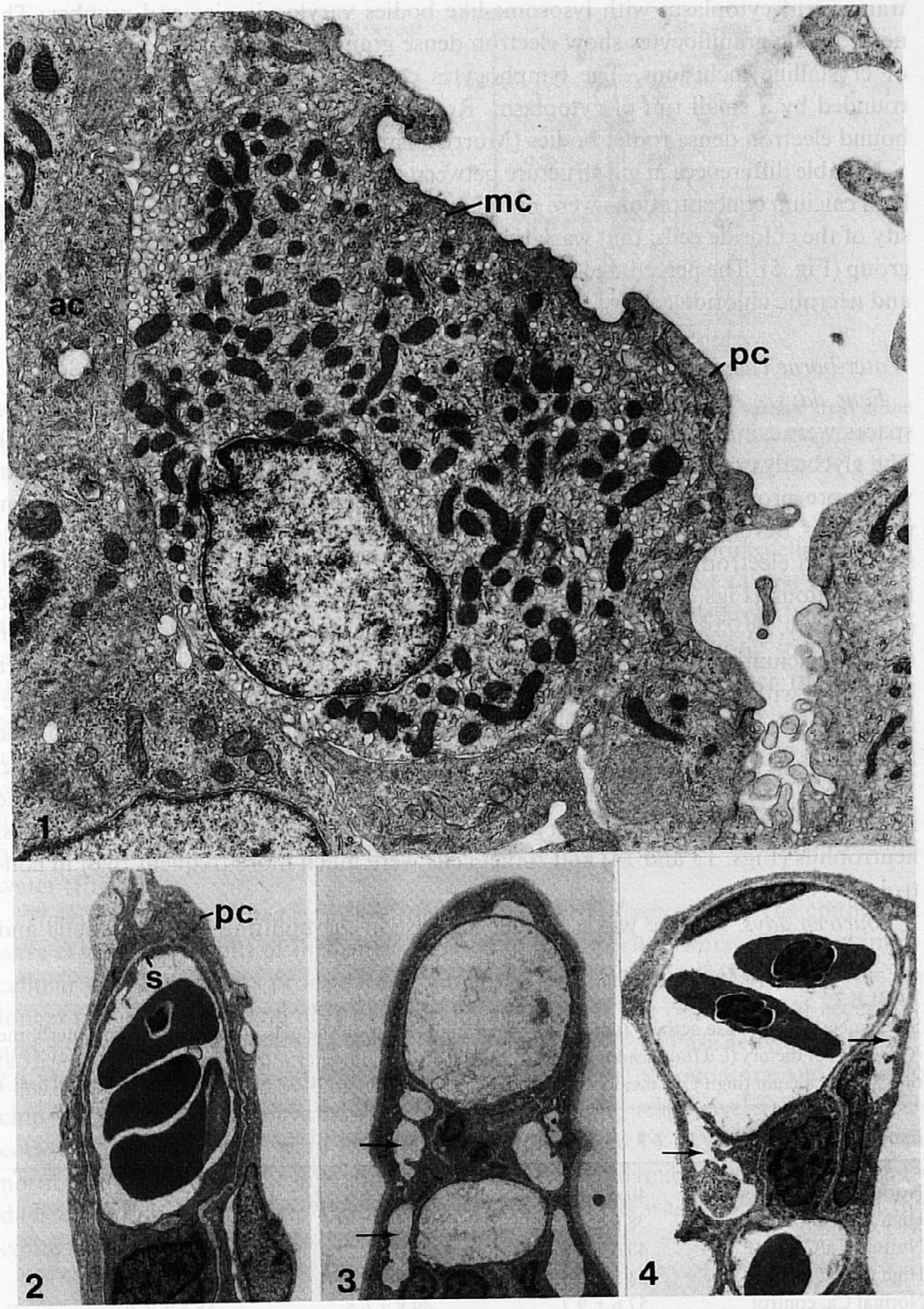


Fig. 1. Control. Mature chloride cell (mc) surrounded by pavement cells (pc) and accessory cells (ac); 8500×.
Figs. 2–4. Tips of lamellae with blood sinuses (s) and pavement cells (pc); arrows: enlarged intercellular spaces; 0.8 mM Ca^{2+} (2600×). 2, control; 3, water-borne cadmium, 4 days; 4, dietary cadmium, 14 days.

transparent cytoplasm with lysosome-like bodies varying in size and number. The neutrophilic granulocytes show electron dense granules, occasionally with band-like or crystalline inclusions. The lymphocytes contain a relatively large nucleus surrounded by a small rim of cytoplasm. Rodlet cells are characterised by membrane bound electron dense rodlet bodies (Morrison and Odense, 1978; Cenini, 1984).

Notable differences in gill structure between control fish from water with normal or high calcium concentrations were not observed, with exception of the numerical density of the chloride cells, that was about 50% ($P < 0.05$) higher in the normal-calcium group (Fig. 5). The percentage distribution of accessory, immature, mature, apoptotic and necrotic chloride cells of control fish is shown in Fig. 6.

Water-borne cadmium

Four days. After 4 days uplifting of epithelia and enlargement of intercellular spaces were common in the epithelium covering the filaments and lamellae (Fig. 3). The glycocalyx on the apical membrane of the upper layer of the filament epithelium was more pronounced. The microridges were irregularly formed and absent from many cells. Several of the tight junctions between the pavement cells and chloride cells showed an electron transparent gap between the membranes, indicating opening of the junctions (Figs. 18 and 19). The total number of chloride cells was increased significantly, by 73% in the fish from high-calcium water, and by 38% in the fish from normal-calcium water (Fig. 5). All stages of the chloride cells were observed more frequently with exception of the mature cells. In particular the number of immature, necrotic and apoptotic chloride cells increased. Dense bodies characteristic for the final stages of apoptosis were frequently found, particularly inside macrophages (Fig. 15). The Na^+/K^+-ATPase activity of the gills was significantly reduced in both the normal-calcium and high-calcium groups (Table 2). The macrophages, lymphocytes, neutrophils (Figs. 13 and 14) and rodlet cells were much more frequent than in controls.

Fourteen days. There was a further increase in the uplifting of the epithelia and

TABLE 2

Specific Na^+/K^+-ATPase activity (μmol Pi · h^{-1} per mg protein) in the gills of fish exposed to waterborne (Cd water) or dietary (Cd food) cadmium for 4, 14, and 35 days, in water with 0.2 mM calcium (normal Ca) or 0.8 mM calcium (high Ca); means ± SD of eight fish per group; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Exposure time (days)	4	14	35
Normal Ca/Cd water	40.6 ± 8.5*	29.4 ± 8.5***	52.6 ± 8.0
High Ca/Cd water	38.8 ± 7.2**	39.8 ± 7.9**	51.6 ± 8.4
Normal Ca/Cd food	48.6 ± 9.6	34.0 ± 7.7*	38.4 ± 3.2**
High Ca/Cd food	53.5 ± 6.3	40.8 ± 5.8*	45.2 ± 4.6*
Normal Ca, control	57.6 ± 9.7	49.8 ± 6.5	55.4 ± 7.3
High Ca, control	55.4 ± 4.6	53.0 ± 10.6	54.0 ± 8.8

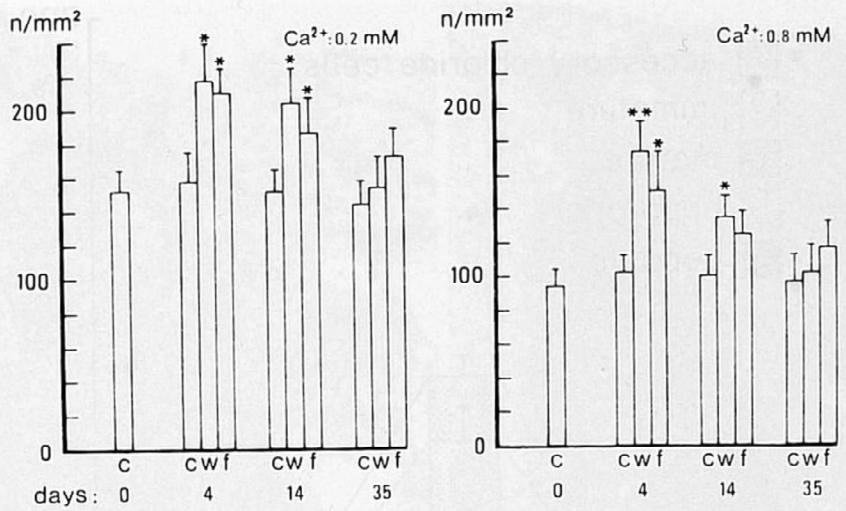


Fig. 5. Numerical density of the chloride cells (number of cells per mm² of epithelial surface area) in the opercular epithelium, as determined after DASPEI staining; c, controls; w, water-borne cadmium; f, dietary cadmium; means ± SD of eight fish per group from normal or high calcium water; $*P < 0.05$; $**P < 0.01$.

the occurrence of intercellular spaces (Fig. 7). The numbers of chloride cells were still elevated (Fig. 5), and the Na^+/K^+-ATPase activity was reduced (Table 2). Many mature chloride cells formed deep apical crypts, sometimes with electron dense granular material. Similar to day 4, the immature, necrotic and apoptotic stages of the chloride cells dominated and their numbers were significantly higher than in the controls ($P < 0.01$; Figs. 6, 8 and 9). Dense bodies characteristic of the final stages of apoptosis were frequently found. Leucocytes, in particular macrophages and rodlet cells, were also common. No specific structural differences were observed between the fish from water with normal or high calcium levels. The numbers of chloride cells in fish from normal-calcium water remained higher than those of fish from high-calcium water (Fig. 5).

Thirty-five days. After exposure for 35 days, signs of recovery of the epithelium were evident in the gills of the fish acclimated to high-calcium water. Enlarged intercellular spaces were less pronounced than at day 14, and epithelial uplifting was no longer found. The total number of chloride cells (Fig. 5), the percentage of accessory and apoptotic chloride cell stages (Fig. 6) and the Na^+/K^+-ATPase activity of the gills (Table 2), were back to control values. The percentage of immature and necrotic chloride cells was still elevated ($P < 0.05$ and $P < 0.01$, respectively; Fig. 6). Leucocytes and rodlet cells were less prominent than after 14 days. In the fish from normal-calcium water, the gills showed the same type of damage as observed after 14 days of cadmium exposure. The number of chloride cells was similar to that of the controls (Fig. 5).

Dietary cadmium

Four days. Administration of cadmium via food for 4, 14 and 35 days had similar

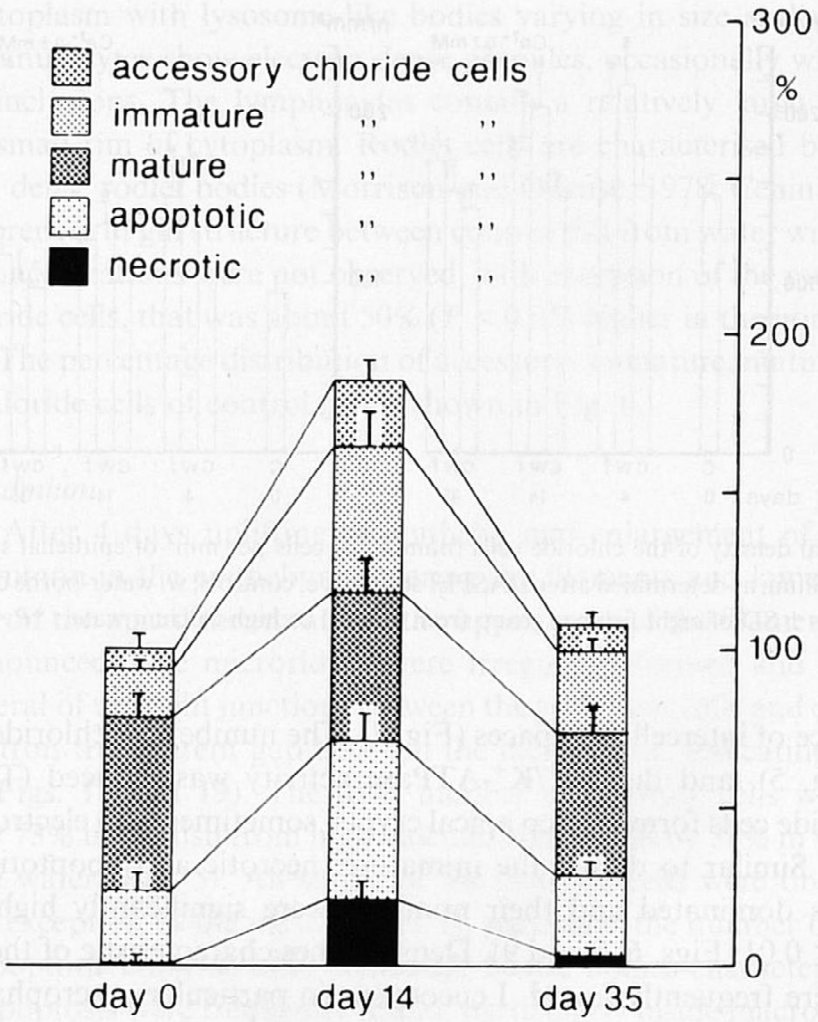
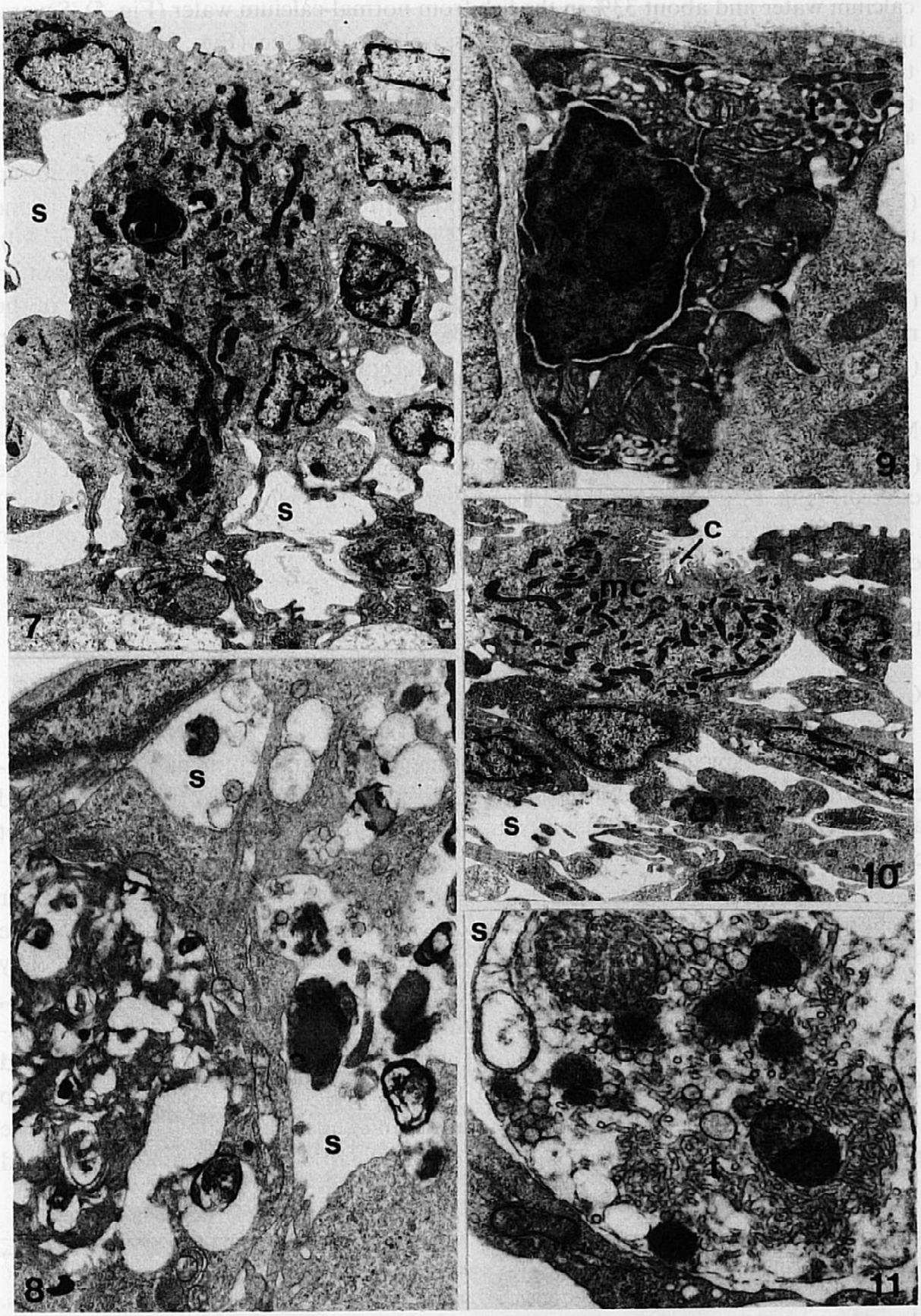


Fig. 6. Relative frequency of the different stages of the chloride cells in control fish (day 0) and fish exposed to water containing 10 μg cadmium and 0.8 μM calcium for 14 and 35 days, as estimated in the electron microscope. The length of the bars reflects the numerical density of the chloride cells as determined in the inner opercular epithelium after DASPEI staining (density of chloride cells in control fish at day 0: 100%). Means ± SD of six fish per group.

effects on the gills of tilapia from both normal-calcium water and high-calcium water. The microridges on the apical pavement cells were not affected. After 4 days, structural alterations such as epithelial uplifting or enlarged intercellular spaces were not seen. The total number of chloride cells increased about 47% in the fish from high-

Figs. 7–11. Filament epithelium of cadmium-exposed fish from water with 0.2 mM calcium. The intercellular spaces (s) are enlarged. (7) Water-borne cadmium, 14 days. Mature chloride cell with a large lysosomal body (l); 4000×. (8) Water-borne cadmium, 4 days. Necrotic cells; 9600×. (9) Water-borne cadmium, 14 days. Apoptotic chloride cells, showing electron-dense nucleus and cytoplasm, and swollen tubular system (t); 14,000×. (10) Dietary cadmium, 14 days. Mature chloride cell (mc) with large apical crypt (c); 1800×. (11) Dietary cadmium, 4 days. Necrotic cell showing cytoplasmic disorganization; t, tubular system; 15,000×.


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calcium water and about 33% in the fish from normal-calcium water (Fig. 5). Several immature chloride cells were present as well as necrotic (Fig. 11) and apoptotic cells. Most other chloride cells appeared rather electron dense, with signs of apoptosis. The branchial Na^+/K^+-ATPase activity was unchanged (Table 2). Leucocytes and rodlet cells were increased in number.

Fourteen days. In the mature chloride cells enlarged apical crypts were found (Fig. 10), similar to 4-day exposure. The only difference was the enlargement of the intercellular spaces in both the lamellar (Fig. 4) and filament epithelium (Fig. 10). Newly developing necrotic and apoptotic pavement and chloride cells were common (Figs. 16 and 17). The total number of chloride cells was significantly higher in the normal-calcium group, but not different from the controls in the high-calcium group (Fig. 5). The Na^+/K^+-ATPase activity was reduced in both groups (Table 2).

Thirty-five days. The effects of dietary cadmium were the same as after 14 days. Many newly developing, necrotic, and apoptotic pavement cells and chloride cells (Fig. 15) were still present. The total number of chloride cells was similar to that of the controls (Fig. 5). The Na^+/K^+-ATPase activity was still below control level (Table 2).

No differences were observed between the fish from water with normal or high calcium levels.

DISCUSSION

General gill structure

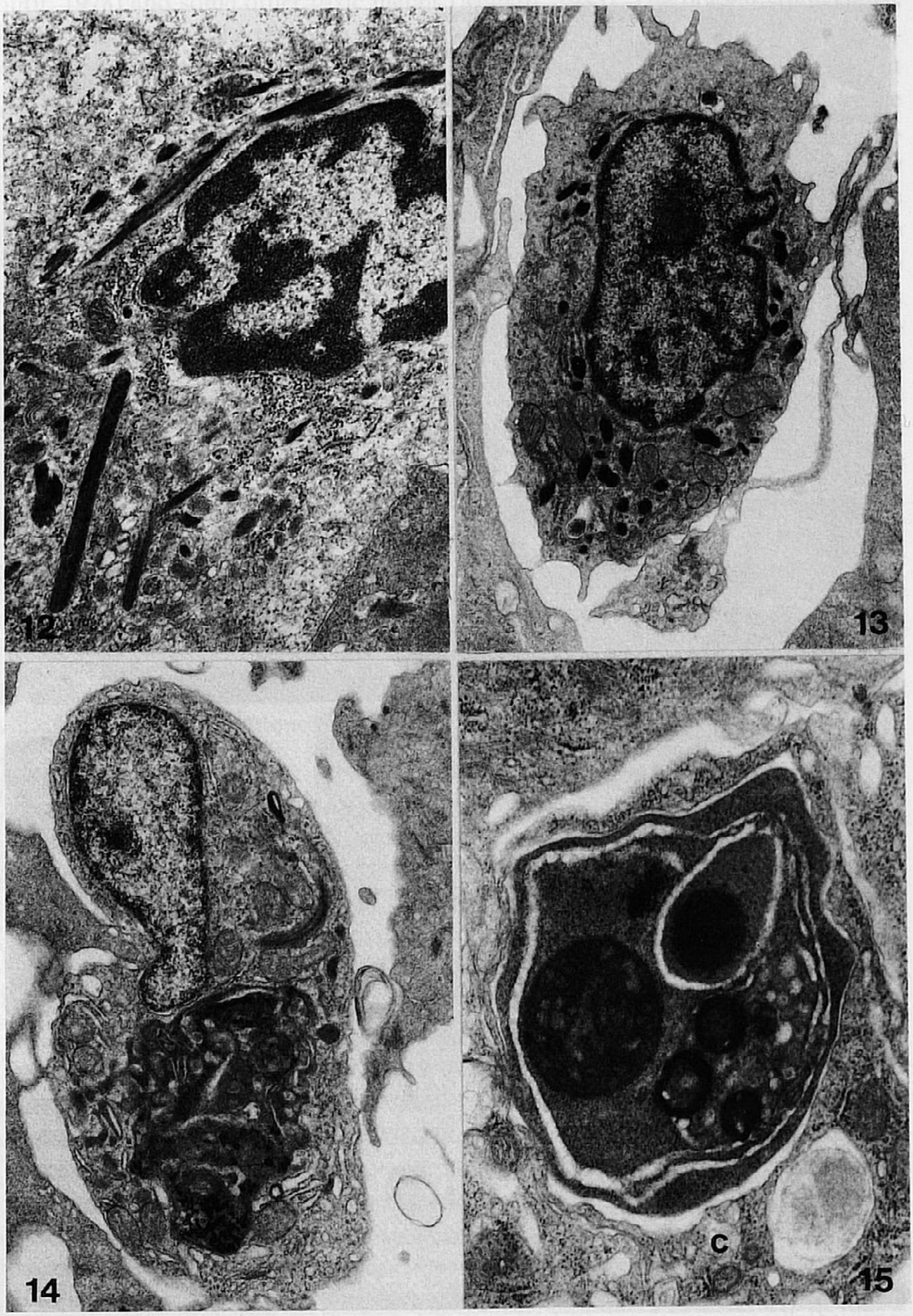
The most conspicuous effects observed in the branchial epithelium of cadmium-exposed fish were epithelial lifting, increased intercellular spaces, proliferation and apoptosis of chloride cells, and infiltration of the epithelium by leucocytes and rodlet cells.

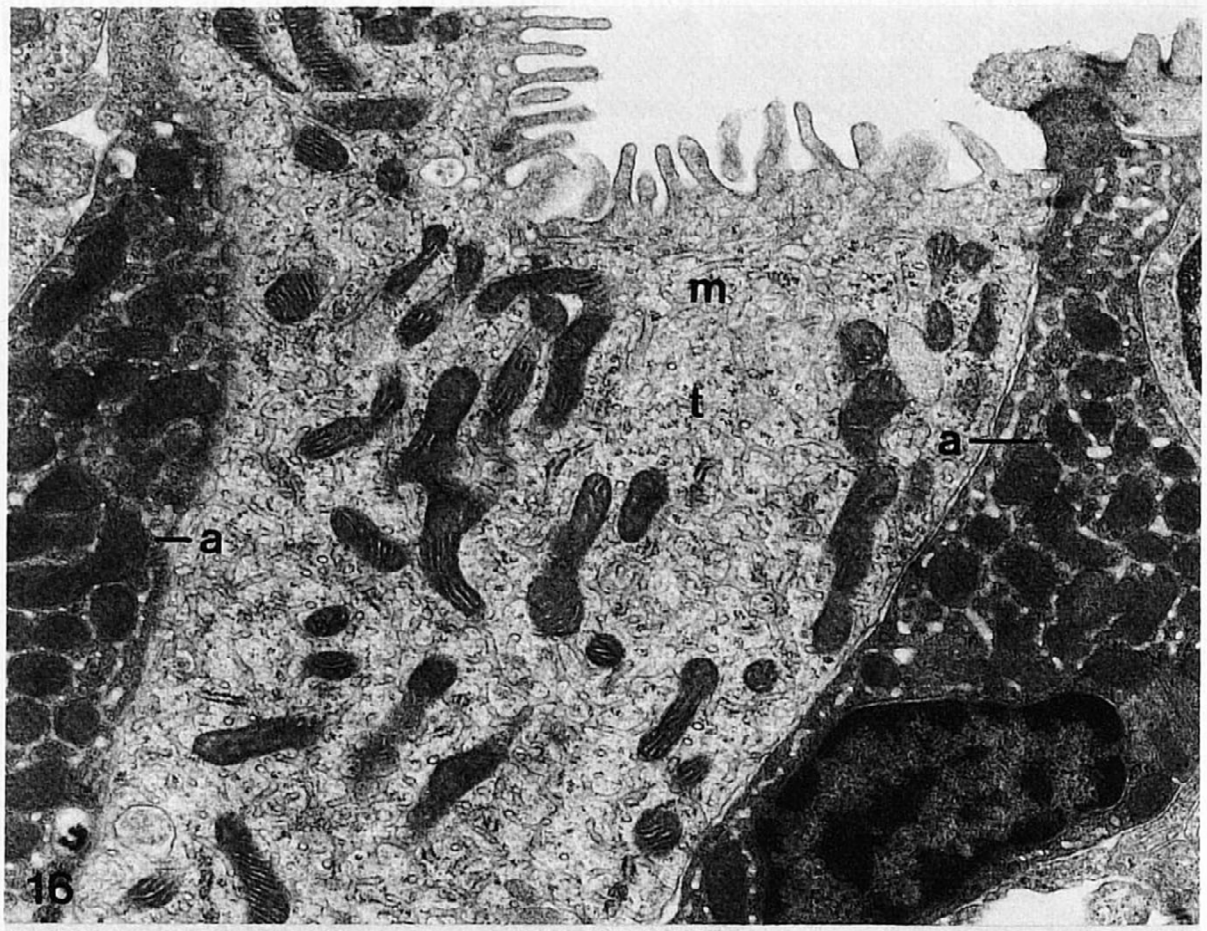
With exception of chloride cell apoptosis and the infiltration of rodlet cells, similar phenomena have been reported in light microscopic studies of fish gills after exposure of fish to a variety of noxious agents in the water, such as pesticides, phenols, heavy metals including cadmium, tributyltin, and aluminium in water of low pH (Mallatt, 1985). Epithelial uplifting and dilation of the intercellular spaces may impair respiration (Pinkey et al., 1989). Our data show that in tilapia these phenomena are caused by cadmium in the water as well as in the diet. Apparently, cadmium taken up through the gut can enter the body circulation and cause substantial damage to the gills. Although infrequently, this has been reported earlier for cadmium (Patrick and

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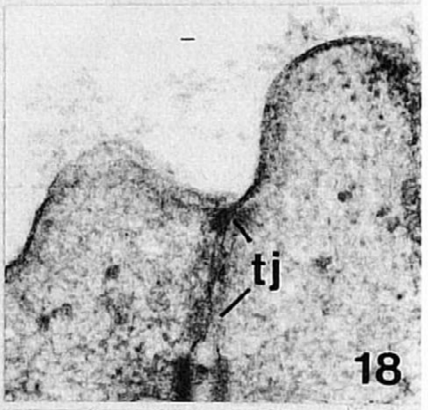
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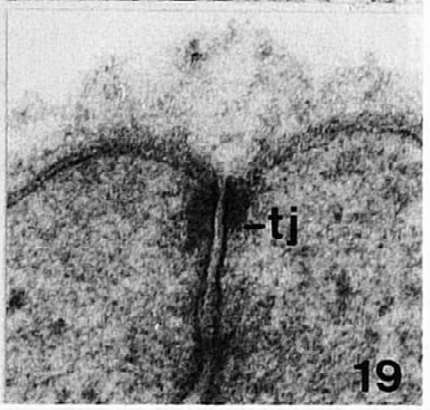
Figs. 12–15. Filament epithelium of fish from water with 0.8 mM calcium. (12) Control. Rodlet cell; 4000×. (13) Water-borne cadmium, 4 days. Neutrophilic granulocyte; 9600×. (14) Water-borne cadmium, 4 days. Macrophage containing an apoptotic body; 11,000×. (15) Dietary cadmium, 35 days. Apoptotic body, probably remnant of a chloride cell, surrounded by cytoplasm of a macrophage (c); 19,000×.


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Loutit, 1978) and some other toxic metals (Mallatt, 1985). The present study shows that the damage and the response of the gill epithelium are very similar at the ultrastructural level for both types of exposure. The structural changes were transient at the concentrations used. Within 5 weeks partial recovery became noticeable. In the group receiving dietary cadmium the recovery was delayed. A similar delay was observed with respect to the appearance of the gill damage. This delay may be caused by the circumstance that dietary cadmium follows a more complicated route to the gills than cadmium present in the ambient water. However, the differences between the effects of dietary and ambient cadmium should be interpreted with caution, because the dose of cadmium received by both treatments may not be comparable. Elevation of the calcium content in the ambient water enhanced the recovery. In a comparable experiment we have shown earlier that high water calcium levels reduce the plasma electrolyte losses in tilapia exposed to cadmium (Pratap et al., 1989). The protective action of high water calcium levels and water hardness against toxic metals has been reported by several authors (e.g., Pascoe et al., 1986; Wicklund and Runn, 1988). The present results show that protection of both pavement and chloride cells is involved.

Lamellar fusion, excessive proliferation of pavement cells and disruption of pillar cells (Mallatt, 1985) were not found. Together with the absence of mortality and the clear signs of recovery of the gill damage this indicates that the conditions used were not exceptionally stressful even though rather high levels of cadmium exposure were used. In this respect tilapia is more resistant to cadmium than zebrafish and brown trout are (Karlsson-Norrgren et al., 1985). The recovery of tilapia was likely caused by induction of metallothionein-like proteins in the gill tissue (see Fu et al., 1990).

Pavement cells

In tilapia exposed to cadmium in the water, but not dietary cadmium, the microridges present on the surface of the upper layer of epithelial cells became irregular in shape and partially disappeared. This has been reported earlier for fish exposed to, e.g., cadmium, iron, chromate, and mercury in the water (Hart and Oglesby, 1979; Temmink et al., 1983; Karlsson-Norrgren et al., 1985; Pereira, 1988). The opening of some of the tight junctions between the pavement cells of fish exposed to cadmium in the water is in line with findings of Prozialeck and Niewenhuis (1991), who demon-

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Figs. 16–19. Filament epithelium of fish from water with 0.8 mM calcium. (16) Dietary cadmium, 14 days. Mature chloride cell (m) with well-developed tubular system (t), surrounded by apoptotic chloride cells (a) with electron-dense nucleus and cytoplasm, and swollen tubular system; 14,000×. (17) Dietary cadmium, 14 days. Part of developing chloride cell showing extensive granular endoplasmic reticulum (er) and developing tubular system (t); 22,000×. (18) Control. Contact area between two pavement cells with a tight junction (tj); 60,000×. (19) Water-borne cadmium, 4 days. Contact area between two pavement cells showing an electron-transparent gap in the junctional area (tj). 81,000×.

strated that cadmium can disrupt tight junctions, and in this way can increase the permeability to ions of epithelia.

Chloride cells

The most prominent effect of the exposure to cadmium on the chloride cells was a rapid increase in cell number in the inner opercular epithelium. Changes in the frequency of the chloride cells in this epithelium reflect the changes in cell numbers in the epithelium covering the gill filaments and lamellae, areas that are less accessible for routine cell counts than the opercular epithelium (Wendelaar Bonga et al., 1990). The highest numbers of chloride cells were found after four days of exposure to both water-borne and dietary cadmium. We further showed that after four days of exposure the specific Na^+/K^+-ATPase activity, which is located in the chloride cells of the gills, was slightly reduced. This reduction in enzyme activity seemed at variance with the marked increase in chloride cell numbers. The discrepancy can be explained by the results of our analysis of the ultrastructure of the cells. In earlier studies (Wendelaar Bonga and Van der Meij, 1989; Wendelaar Bonga et al., 1990) we have identified different subtypes of chloride cells: accessory, immature, mature and apoptotic cells – that we have interpreted as successive stages in the chloride cell cycle – and necrotic cells. Because mature cells contain a well developed tubular system and are in contact with the water via the cell apex that frequently is differentiated into an apical pit, the ion-exchange activity of the chloride cells is most likely concentrated in this cell stage. The accessory chloride cells of tilapia are considered functionally inactive replacement cells. They are frequently not in contact with the water and are lacking the apical interdigitations with cytoplasmic processes of neighbouring cells that are typical for accessory cells in seawater fish (Wendelaar Bonga and Van der Meij, 1989). The immature cells may display functional activity in the course of their maturation. Apoptotic cells rapidly lose contact with the water and, similar to necrotic cells, are unlikely to be functionally active. The rapid increase in the total number of chloride cells following cadmium exposure was mainly due to increased numbers of accessory cells and immature cells. The number of mature cells was reduced, apparently because of apoptotic and, to a lesser extent, necrotic degeneration. Thus, as a result of cadmium exposure the total ion exchange capacity of the gills is probably reduced, even though the total number of chloride cells was increased significantly. This conclusion is supported by the observed reduction of the Na^+/K^+-ATPase activity of the gills, the driving force for Na^+-dependent ion exchange (Mayer-Gostan et al., 1987).

The marked increase in the number of apoptotic cells was the most conspicuous change following cadmium exposure. Apoptosis is the physiologically controlled form of cell death, resulting in cellular densification and eventually removal of the cells by macrophages (Wyllie et al., 1980). We have observed this phenomenon before in tilapia transferred to seawater and in tilapia exposed to acid water (Wendelaar Bonga and Van der Meij, 1989; Wendelaar Bonga et al., 1990). Enhanced occurrence

of apoptosis reflects reduction of the cell cycle, and this can also be deduced from our present data. From this point of view the chloride cells most likely are more sensitive to cadmium than the pavement cells. Studies in our laboratory have shown that the Ca^{2+}-ATPase activity of the chloride cells of teleosts is extremely sensitive to cadmium, with 50% inhibition at a concentration of 3 nM Cd^{2+} (Verbost et al., 1988). Because Ca^{2+} extrusion from the cytoplasm is dependent on this enzyme activity, it is well possible that cadmium induces a rapid rise in the intracellular Ca^{2+} concentration, which may result in chromatic fragmentation and, eventually, apoptotic degeneration. The percentage of necrotic chloride cells also increased in the first days after cadmium exposure. Necrosis was less prominent than apoptosis, indicating that acute lethal damage to the cells was less commonly induced by cadmium than accelerated aging. The chloride cell density was higher in fish from normal-calcium water than in fish from high-calcium water, under control conditions as well as during cadmium exposure. Increased chloride cell densities after reduction of the water calcium concentration have been reported earlier for trout. It is unclear whether this effect is related to the function of chloride cells in calcium uptake, or a compensatory response to the increased diffusional losses of monovalent ions in water with lowered calcium levels (Laurent and Perry, 1991).

Leucocytes

Leucocyte infiltration of the intercellular spaces has been reported frequently after exposure of fish to heavy metals including cadmium (Gardner and Yevich, 1978; Karlsson-Norrgren et al., 1985) and many other toxic substances present in the water. The cells were identified as macrophages, neutrophils, lymphocytes and rodlet cells lymphocytes on the basis of the relatively scarce literature on fish leucocytes (e.g., Ferguson, 1976; Cenini, 1984; Diepen et al., 1991). The high incidence of leucocytes points to mobilisation of the immune system during cadmium exposure of both water and dietary origin. Cadmium probably increases the liability to infections of the branchial epithelium.

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