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Effects of Water-Borne Cadmium on the Skin of the Common Carp (*Cyprinus carpio*)

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Abstract. The skin of carp, *Cyprinus carpio*, was studied at the ultrastructural level after exposure of the fish to low and high concentrations of cadmium in the water (22 and 560 μg/L, respectively) for different periods. The effects of the low concentration of cadmium were similar to those of the high concentration, although they appeared later. The basal lamina and the skin surface became highly undulating. Chloride cells appeared between the pavement cells. Necrotic pavement cells were seen from the first day on, while apoptotic pavement cells appeared after several days. Filament cells contained many electron-transparent and electron-dense secretory vesicles. Mitotic cells were commonly seen, mainly in cells adjacent to club cells or close to the epidermal surface. Mucous cells differentiated close to the skin surface. They became elongated and synthesized highly electron-dense mucosomes. The epidermis became infiltrated by many leucocytes. As the experiment progressed, many leucocytes degenerated, and their remnants were found within macrophages and club cells. Fibroblasts displayed intense synthesis and, in fish from the low cadmium concentration, deposited a dense network of collagen fibers in the dermis. Melanosomes were located in the extensions of melanocytes. In these cells aggregation of melanosomes and apoptotic processes were common. Several of these changes were observed earlier under the impact of stressors other than cadmium. Some changes, such as the appearance of tumorlike bodies at the skin surface, the appearance of Merkel cells throughout the epidermis, and the coupling of leucocytes, may be specific for cadmium.

Heavy metals, including cadmium, exert a wide range of pathological effects on fish and other aquatic organisms (Dethlefsen and Tiews 1985; Mallatt 1985). Short-term exposure to cadmium causes changes in several blood parameters, such as a decrease of haematocrit and leucocrit values (Tort and Torres 1988), reduction of plasma electrolytes (Pratap *et al.* 1989), and elevation of plasma cortisol and glucose levels (Pratap and Wendelaar Bonga 1990; Fu *et al.* 1990). Long-term exposure may induce vertebral lesions (Bengtsson *et al.* 1988) or the formation of tumorlike bodies in the skin (Iger 1992).

The gills of fish belong to the most important target tissues of water-borne as well as dietary cadmium (Pratap and Wendelaar Bonga 1993). The reported changes in plasma electrolytes by cadmium exposure are probably caused by malfunctioning of the chloride cells, the cells responsible for active ion exchange across the gills, and by increased permeability to ions of the branchial epithelium (Verbost *et al.* 1989; Wendelaar Bonga and Lock 1992). Many authors have reported histopathological changes in the branchial epithelium after exposure to cadmium (Oronsaye and Brafield 1984; Karlsson-Norrgren *et al.* 1985; Mallatt 1985; Pratap and Wendelaar Bonga 1993).

In contrast to the gills, the skin outside the branchial area has received little attention, although this tissue is also in intimate contact with external pollutants. Like the gills, the skin may contain chloride cells, albeit in small numbers (Whitear 1986). It is covered by a mucus layer that may reduce the penetration of cadmium into the body (Pärt and Lock 1983), and possibly may serve as a vehicle for cadmium excretion (Bryan 1979). However, there is no information available on the effects induced, and the responses evoked, in the skin of cadmium-exposed fish.

This paper describes ultrastructural changes of the epidermis and the dermis of carp exposed to low or to high cadmium concentrations in the ambient water.

Materials and Methods

Fifty-four carp (*Cyprinus carpio*), both males and females of 18–25 g body weight, were kept in three groups for an acclimation period of 3 weeks. The fish were maintained in artificial freshwater (demineralized water with the following additives in mmol/L: 3.8 NaCl; 0.8 $CaCl_2$; 0.335 $NaHCO_3$; 0.06 KCl; 0.2 $MgSO_4$, pH 7.5) at 22°C, under continuous aeration and filtration, and with a daily water replacement of about 25%. One group served as control. For two groups appropriate

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amounts of Cd [from a stock solution of $Cd(NO_3)_2$] were added gradually to create (in 3 h) concentrations of 0.2 and 5 μmol/L Cd (22.4 μg Cd/L and 560 μg Cd/L, respectively). The actual cadmium concentrations were measured at least once per day, using atomic absorption spectrophotometry (PU 9200X, Philips), and were adjusted when the deviation was more than 10% from the nominal concentration.

For electron microscopy, small pieces (3 × 3 mm) of skin of three fish from the different groups were excised 1 h, 24 h, and 3, 7, 14, and 21 days after reaching the actual Cd concentration. Samples were taken from dorsal areas of the heads of fish that had been anaesthetized lightly with hypno-calmer (Jungle, Texas). The tissues were fixed in 3% glutaraldehyde in sodium cacodylate buffer (0.09 M, pH 7.3), washed in buffer, and post-fixed in osmium tetroxide (1%) in the same buffer. After dehydration in ethanol the tissues were embedded in Spurr's resin. Thin sections were contrasted with uranyl acetate and lead citrate, and were examined in a Jeol 100 CX transmission electron microscope.

Results

Control

The epidermis of the control fish contained filament cells, oval mucous cells and club cells. Mucous cells were composed of electron-transparent mucosomes and were found from the second innermost cell layer of the epidermis (newly differentiated cells), up to the skin surface (mature cells). Club cells were located in the mid-epidermal layers. Merkel cells were seen exterior to the club cells. The outer layer of the epidermis was composed of pavement cells with few electron-transparent vesicles. Chloride cells were not found. At the basal pole of the filament cells adjacent to the basal lamina, numerous endocytotic vesicles were found. In the dermis, the outer zone contained loosely arranged collagen fibres, among fibroblasts and pigment cells, in particular melanocytes. Melanosomes were mostly located in the cell bodies of the melanocytes, rather than in the cytoplasmic processes of these cells. Capillaries were found in the inner areas of the dermis. Leucocytes, mainly lymphocytes, were occasionally observed in both epidermis and dermis. This skin structure of the control fish was similar to that of other carp under normal conditions, as previously described (Whitear 1986; Iger 1992).

Cadmium-Containing Water

At 560 μg Cd/L, mortality occurred already after 3 days and no fish survived for more than 8 days. During the first 24 h, fish of this group appeared darker than the controls. Later on, they were paler than the controls.

At 22 μg Cd/L, all fish survived the experimental period. No color changes were noticed when compared to controls.

Time (and intensity) of the appearance of morphological changes in the skin of fish of the two groups exposed to cadmium are presented in Table 1.

Epidermis

The epidermal surface and the basal lamina of fish exposed to cadmium had a highly wavy appearance throughout the experiment.

Filament Cells and Pavement Cells: Necrotic pavement cells (swollen cells showing disruption of membranes, increased electron transparency of the cytoplasm and fragmentation of nuclear heterochromatin) were common (Figure 1). Apoptotic cells (showing cellular shrinkage, condensation of cellular components, and loss of junctions) were occasionally found. Such cells were rarely found in controls. Necrotic pavement cells were absent in controls.

The non-degenerative pavement cells showed signs of increased secretory activity: many Golgi systems, well-developed rER, and many vesicles. However, while, at first, electron lucent vesicles prevailed, later on the majority of the vesicles were composed of a homogeneous matrix of high electron density. Such vesicles were found also in filament cells of the three to four outermost layers of the epidermis (Figures 1 and 2) and were particularly numerous in cells adjacent to Merkel cells. Occasionally, filament cells were found with osmiophilic vesicles of 0.6–0.8 μm diameter (Figure 3). Up to four of such vesicles per cell were found. Other filament cells contained crystal-like granules, with a core of high electron density or high electron transparency (Figure 5). Filament cells also contained lysosome-like bodies as well as phagosomes and apoptotic bodies (Figure 4). Several filament cells contained rounded bodies with a diameter of 1.8–3 μm (Figure 2). These bodies were mostly membrane-bounded, and composed of amorphous material of high electron transparency. They were regarded as autophagosomes.

From day 3 onwards, many pavement cells were cylindrical rather than flattened. The terminal web of pavement cells, their ridges, and the glycocalyx around the ridges, were pronounced during the whole experiment. Tight junctions remained intact, although occasionally the intercellular spaces were enlarged. In inner filament cells most of the cellular components (Golgi systems, rER, secretory vesicles) were located apically from the nucleus and resembled the general structure of pavement cells.

Mitotic filament cells, absent in control fish, were detected. Such cells were mostly adjacent to club cells, but were also seen close to the skin surface. Apoptotic filament cells were also found neighbouring club cells. At the skin surface abnormal cell aggregates protruding into the water were found. These structures (Figure 6) had a width of 20–35 μm and a height up to 55 μm, and were solely composed of filament cells. The latter appeared very active as was reflected by the presence of well-developed rER and many Golgi systems as well as many transparent and high electron-dense vesicles. The nucleus of such cells was enlarged, irregular in shape, and composed of euchromatin.

The endocytotic activity at the inner side of the basally located filament cells was at first almost completely suppressed. It was resumed later, but remained only 30–40% of that in the controls. Many of the basal filament cells had extensions of about 1.5–2 μm, projecting into the dermis.

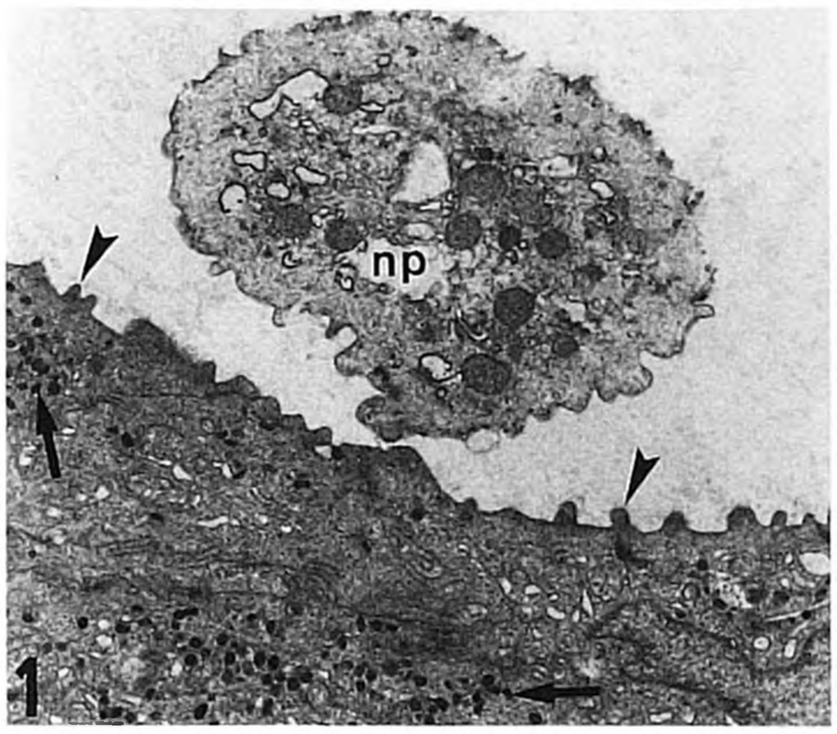
Mucous Cells: Enhanced mucus secretion was found after the start of the experiment. This was concluded from the location of most mucous cells in the external layers of the epidermis, the appearance of many secreting cells at the skin surface, and (in skin of fish exposed to 560 μg Cd/L) also by the dramatic reduction in the number of mucous cells in the epithelium. Under the impact of both concentrations of cadmium, most mucous cells were elongated rather than oval-shaped. These cells contained numerous Golgi systems, rER, and small vesicles at their basal pole, while apparently extruding mucus from their apical pole, at the epidermal surface. Many mucous cells contained highly electron dense mucosomes (Figure 7). There were also mucosomes with a rounded or elongated core of high

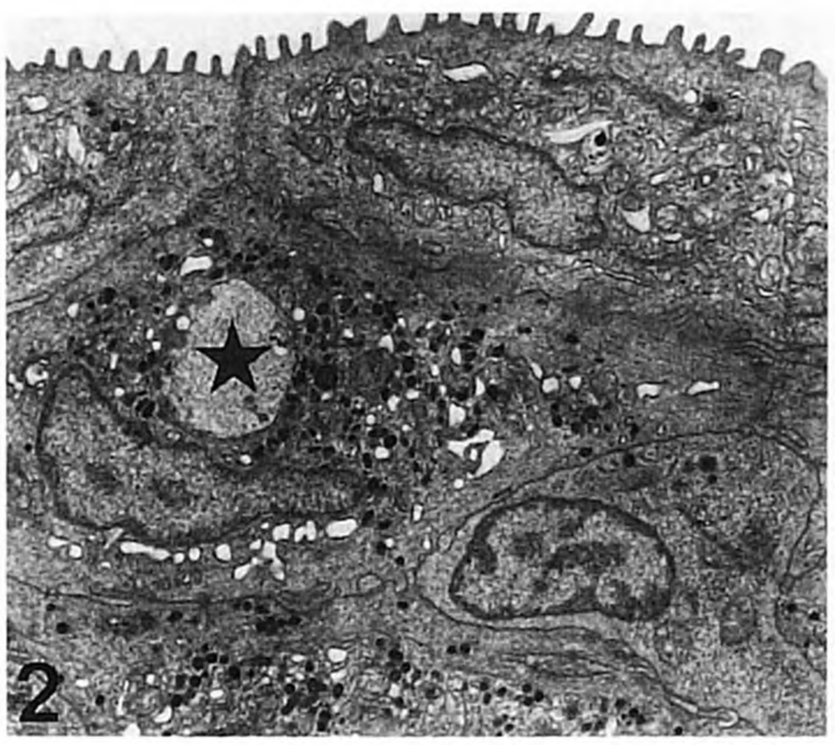
Table 1. Summary of the effects of high (560 μg/L; H) and low (22 μg/L; L) concentrations of cadmium on the skin of carp; = similar to controls; + increase; ++ marked increase; − reduction; −− marked reduction (compared with the controls); at the high concentration no fish survived longer than 8 days

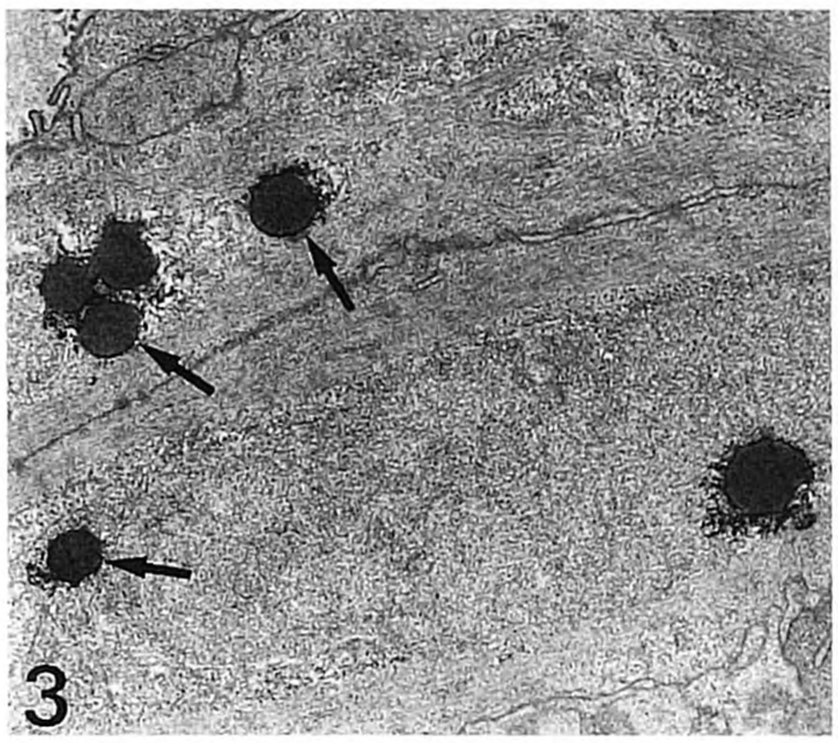
Exposure period	Group	1 h	24 h	3 d	7 d	14 d	21 d
Epidermis							
Undulation of surface and basal lamina	H:	++	++	++	++		
	L:	=	+	+	+	+	+
Pavement cell necrosis	H:	+	++	++	++		
	L:	=	++	=	=	+	+
Pavement cell apoptosis	H:	=	=	+	+		
	L:	=	=	+	+		
Secretion of electron dense vesicles	H:	=	=	++	++		
	L:	=	=	+	++	+	+
Mitosis of filament cells	H:	=	=	+	+		
	L:	=	=	+	+	+	+
Filament cell aggregates	H:	=	=	+	+		
	L:	=	=	=	=	=	+
Cytosis of basal filament cells	H:	−−	−	−	−		
	L:	−	−−	−	−	−	−
Number of mucous cells	H:	=	−	−−	−		
	L:	=	=	=	=	=	=
Elongation of mucous cells	H:	+	++	+	+		
	L:	=	=	+	++	++	+
Electron dense mucosomes	H:	=	+	+	+		
	L:	=	=	+	+	+	+
Number of club cells	H:	=	=	−−	−−		
	L:	=	=	=	=	=	=
Fusion of club cells	H:	=	+	+	++		
	L:	=	=	+	=	+	=
Club cell phagosomes	H:	=	+	+	=		
	L:	=	=	+	++	++	+
Number of chloride cells	H:	=	=	+	+		
	L:	=	=	+	+	+	+
Number of Merkel cell secretory granules	H:	=	−	−	−		
	L:	=	=	−−	−	−	=
Lymphocyte and basophil infiltration in epidermis	H:	++	++	+	+		
	L:	+	++	+	+	+	+
Macrophage infiltration in epidermis	H:	=	=	+	+		
	L:	=	=	=	+	++	++
Coupling of leucocytes	H:	=	+	+	++		
	L:	=	=	=	=	+	=
Number of Merkel cell secretory granules	H:	=	−	−	−		
	L:	=	=	−−	−	−	=
Dermis							
Secretion of collagen	H:	=	=	=	+		
	L:	=	=	=	+	++	++
Cytosis of endothelium	H:	−−	−	−	−		
	L:	=	−	−	−	=	=
Angiogenesis	H:	=	+	+	+		
	L:	=	+	+	+	+	+
Melanin dispersion	H:	+	+	+	+		
	L:	=	+	+	+	=	=
Melanocyte penetration in epidermis	H:	=	+	+	+		
	L:	=	+	+	+	+	+

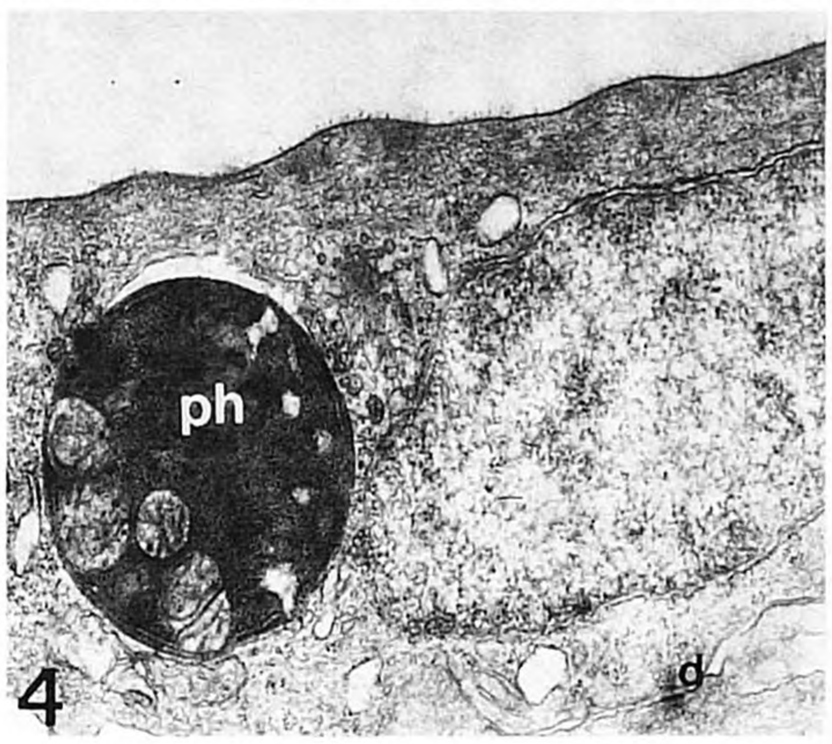
electron density, surrounded by a rim of low electron density. Many cells contained a fused mass of mucosomes with some remnants of their membranes.

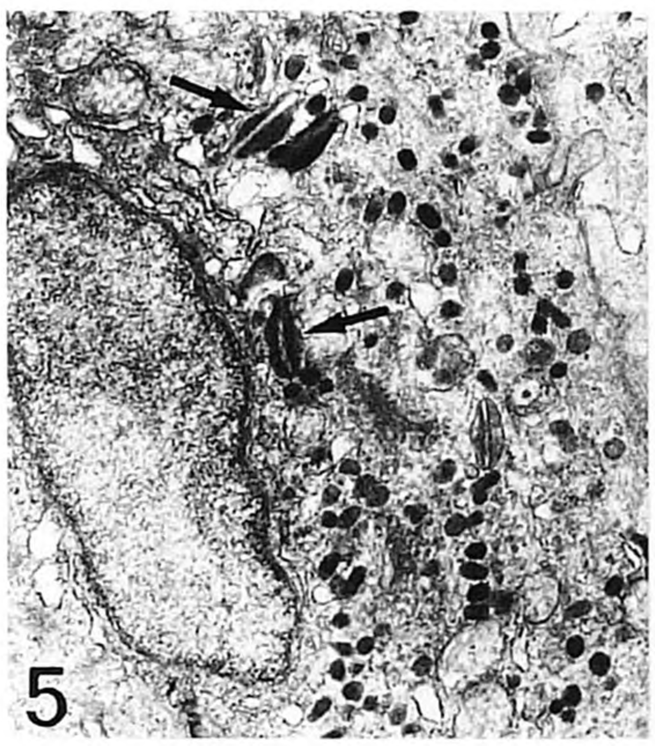
The nucleus of mucous cells was mostly multilobed rather than with smooth contours, as in controls. Occasionally cells were found in which the nucleus was located in the apical part, whereas in controls the nucleus was always basally located. Newly differentiated mucous cells were found mostly adjacent to the pavement cells (i.e., one layer from the surface; Figure 8). Several newly differentiated as well as some mature mucous cells were necrotic, whereas others were apoptotic (Figure 9). We could also recognize apoptotic mucous cells as apoptotic bodies inside macrophages (Figure 10). It is interesting to mention that mucous cells were also located adjacent to club cells and were in contact with axons. Such phenomena were not found in the controls.











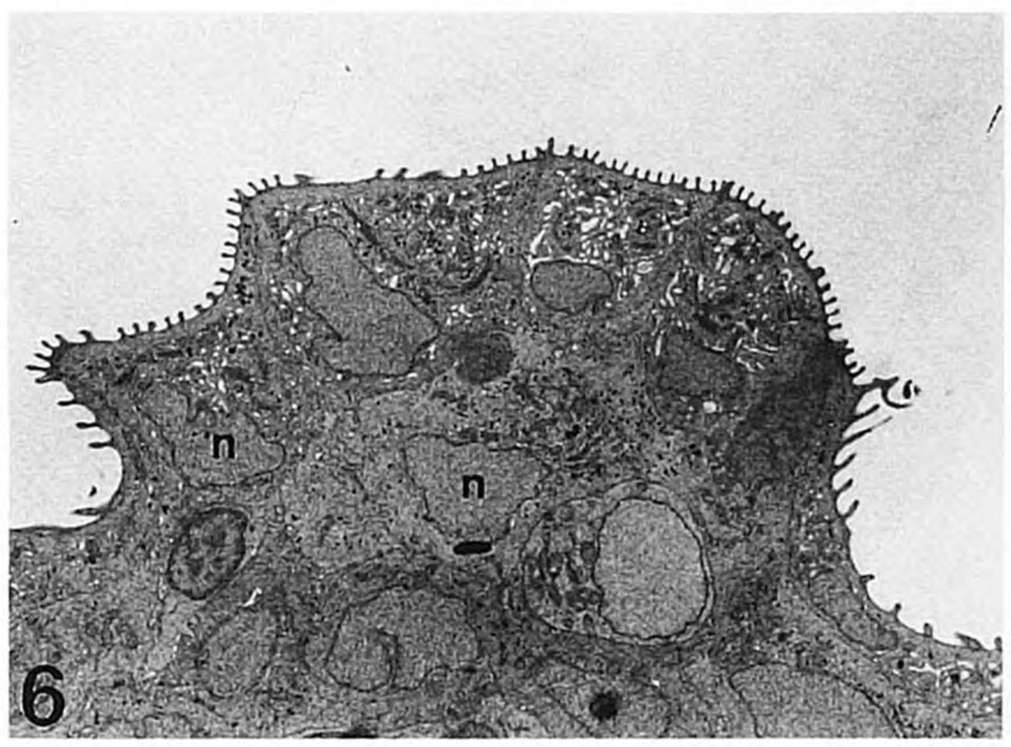


Fig. 1. Necrotic pavement cell (np) located close to the ridges of intact pavement cells (arrow heads); arrows, vesicles of high electron density in a pavement cell (upper) and deeper filament cell. Three days of exposure to 560 μg Cd/L; ×5,400

Fig. 2. Autophagosome (asterisk) inside a filament cell with numerous vesicles of high electron density. Three days of exposure to 22 μg Cd/L; ×6,300

Fig. 3. Osmiophilic vesicles (arrows) inside filament cells. 14 days of exposure to 22 μg Cd/L; ×6,300

Fig. 4. Phagosome (ph) containing pavement cell; d, desmosome. Twenty-four hours of exposure to 22 μg Cd/L; ×15,900

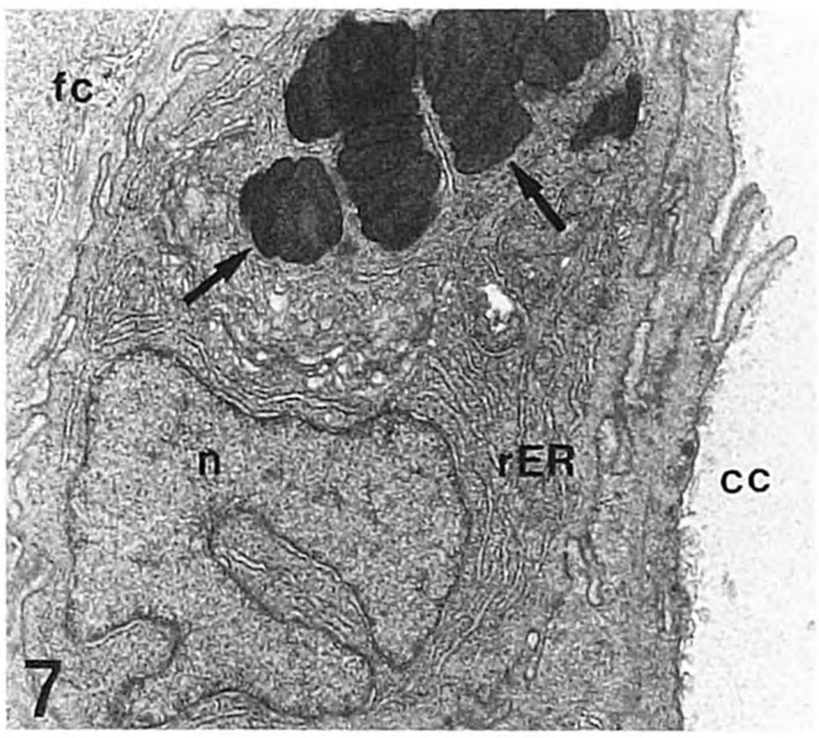
Fig. 5. Deeper filament cell with numerous vesicles of high electron density and several granules (arrows) with the shape of granules of eosinophilic granulocyte. Three days of exposure to 560 μg Cd/L; ×11,100

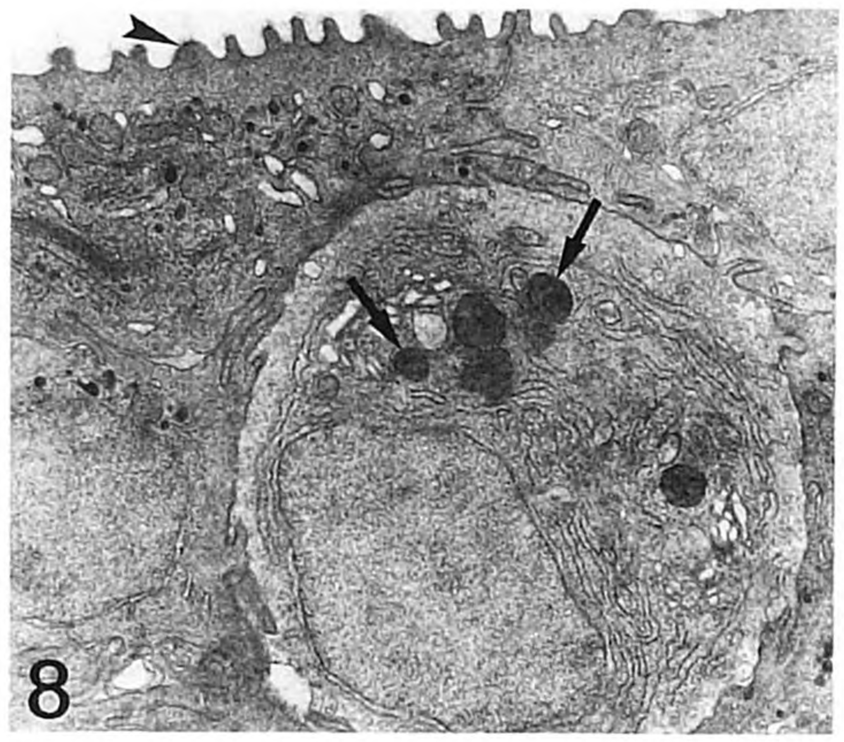
Fig. 6. Aggregate of filament cells forming a protrusion at the skin surface; n, nucleus. Twenty-one days of exposure to 22 μg Cd/L; ×2,100

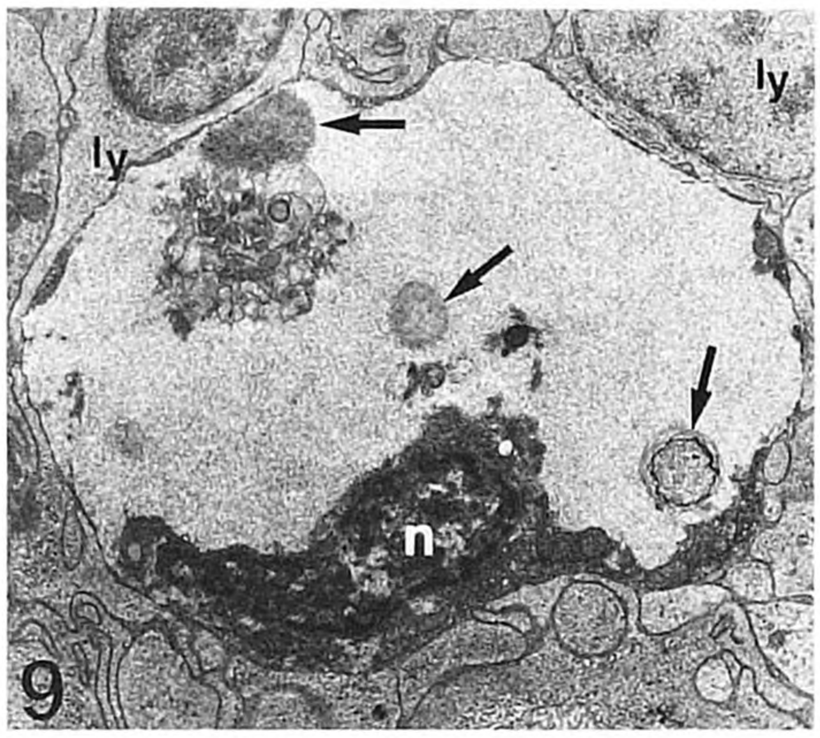
Club Cells: Several club cells contained large (about 8 × 10 μm) phagosomes. Such cells were very active. They contained extensive rER, Golgi systems, many free ribosomes, and small vesicles (probably primary lysosomes), mainly located around the nucleus. Ribosomes and vesicles appeared also at the cell periphery. Many phagosome-containing club cells as well as normal club cells lost their interdigitations with neighboring cells and were elongated and appeared close to the surface. Apparently, these cells migrated out of the epidermis, because at the higher concentration only few club cells remained in the epidermis. Fusion of club cells occurred during their migration (Figures 11 and 12). Many of the club cells were newly differentiated rather than mature ones.

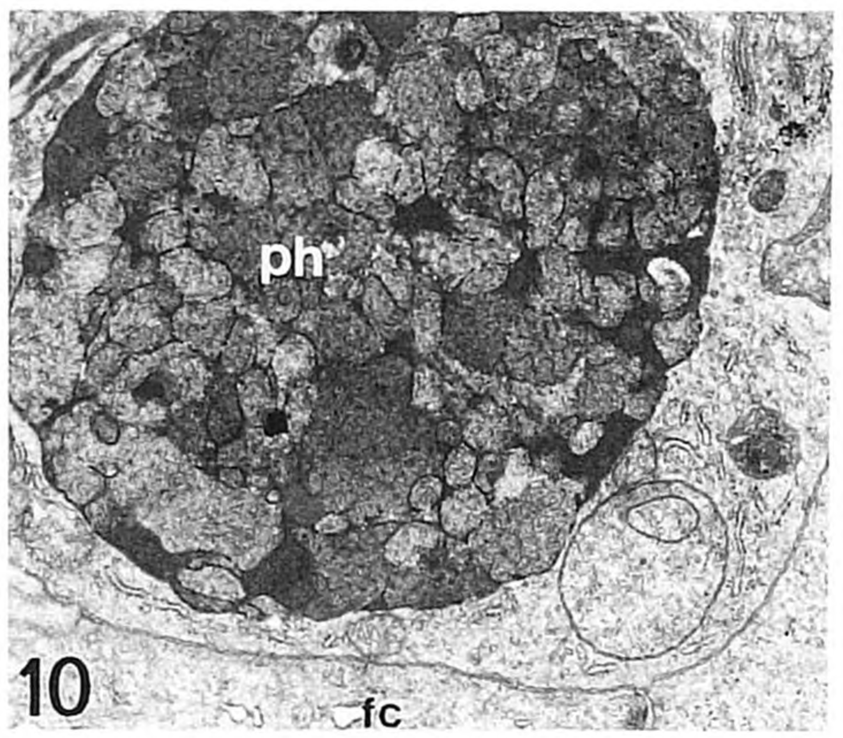
Chloride Cells: Single chloride cells were seen between the pavement cells. The chloride cells had the typical components of this cell type, i.e., an extensive tubular membrane system, many mitochondria, and a small apical crypt. Chloride cells were apically connected to adjacent cells by desmosomes and tight junctions. Occasionally necrotic chloride cells (Wendelaar Bonga and van der Meij 1989) were noted.

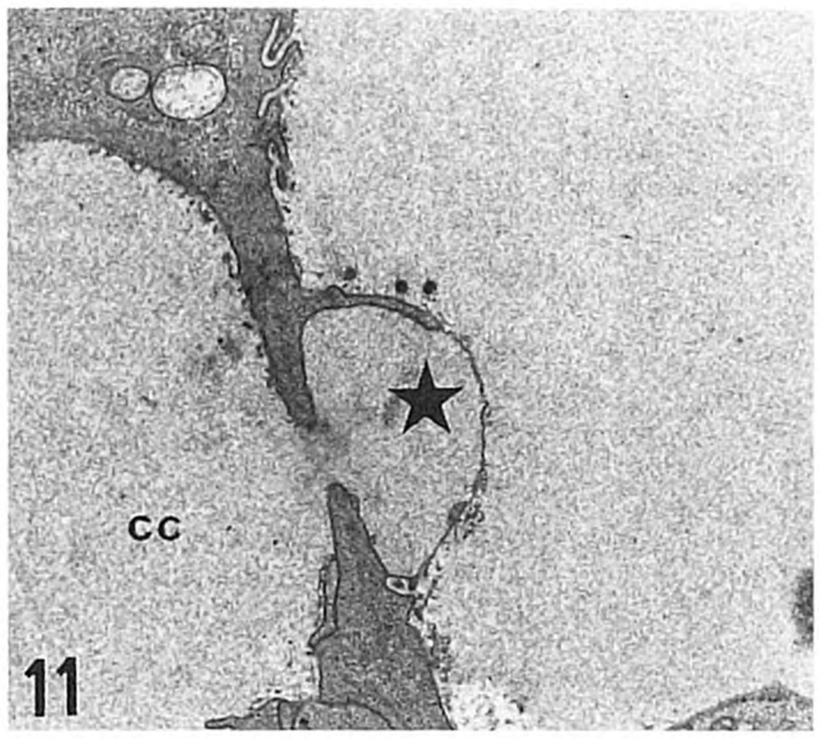
Leucocytes: Massive extravasation of leucocytes and penetration of these cells into the epidermis (Figure 13) were noted. First, most intruding leucocytes were lymphocytes and basophilic granulocytes. Later, macrophages and plasma cells were very common, and occasionally also eosinophils and neutrophils were seen. Many of the lymphocytes observed were located adjacent to club cells and occasionally found in phagosomes inside the club cells. The leucocytes, in particular macrophages and basophils, appeared to be very active. They contained well-developed rER and Golgi areas as well as many











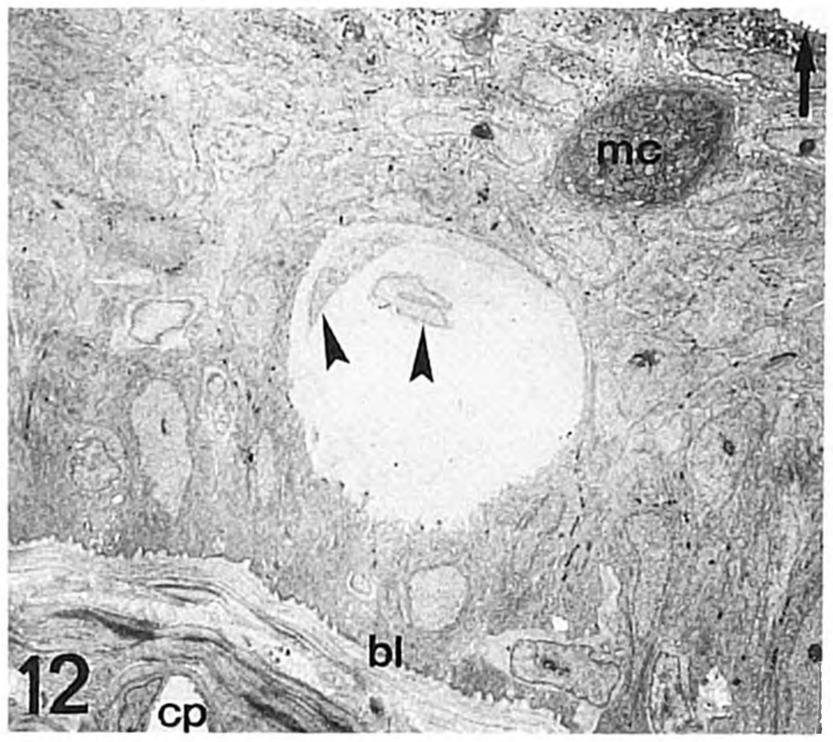


Fig. 7. Mucous cell with multilobed nucleus (n), extensive rough endoplasmic reticulum (rER) and numerous mucosomes of high electron density (arrows); fc, filament cell; cc, club cell. Three days of exposure to 560 μg Cd/L; ×8,100

Fig. 8. Newly differentiated mucous cell, with only several mucosomes (arrows) located close to the skin surface (arrow heads). Three days of exposure to 560 μg Cd/L; ×8,100

Fig. 9. Mucous cell showing degenerated nucleus (n) and amorphous matrix in which several intact mucosomes (arrows) can be found; ly, lymphocytes. Fourteen days of exposure to 22 μg Cd/L; ×6,300

Fig. 10. Part of a macrophage with phagosome (ph) composed of mucosomes; fc, filament cell. Seven days of exposure to 560 μg Cd/L; ×11,100

Fig. 11. Club cell (cc) with cellular process (asterisk) invaginating another club cell. Fourteen days of exposure to 22 μg Cd/L; ×6,300

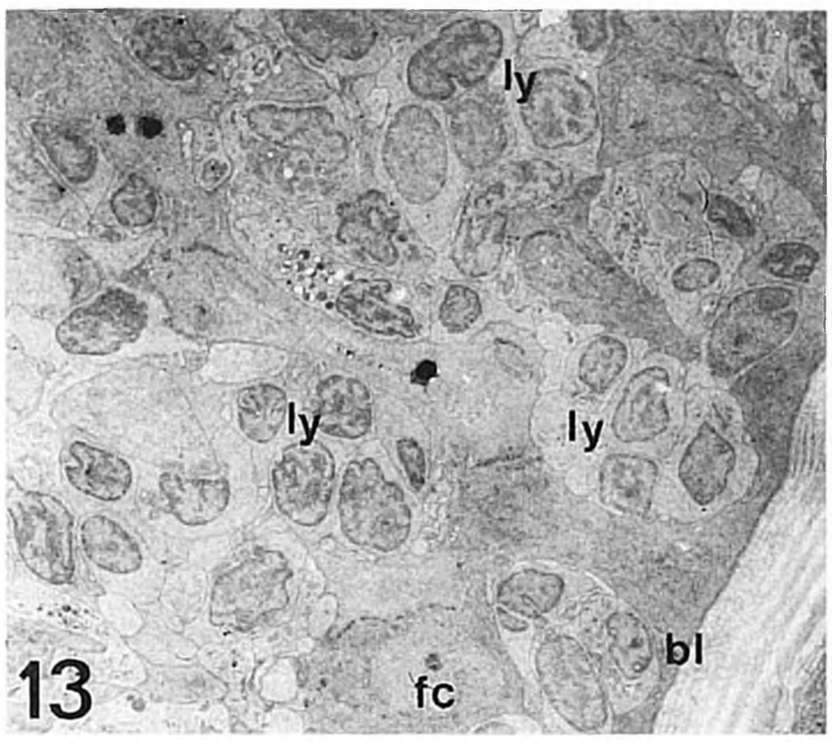
Fig. 12. Club cell with two nuclei (arrow heads). Capillary (cp) is located close to the undulating basal lamina (bl). Mucous cells (mc) are rare; arrow, skin surface. Seven days of exposure to 560 μg Cd/L; ×1,050

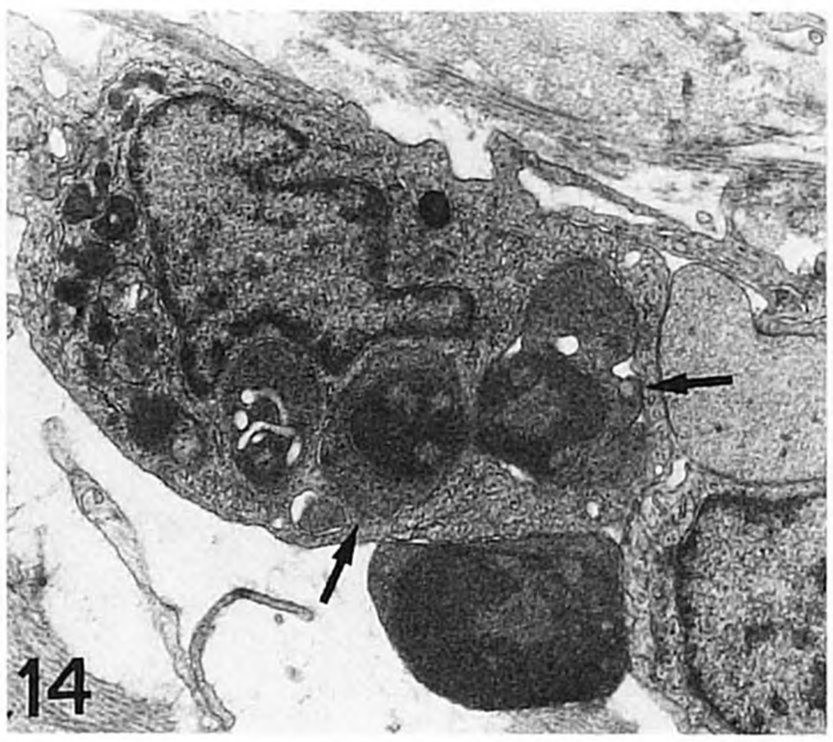
lysosomes and phagosomes (Figures 14, 17, and 20). Mast cells were also seen in the epidermis. Associations of two basophils or of basophil and mast cell were commonly seen (Figure 15). Also couples of eosinophils were observed (Figure 16). In both epidermis and dermis, several basophils and mast cells were necrotic. In such cells many of the granules were found outside the disrupted membrane of the cell. These granules were phagocytosized by neighboring cells, as was concluded from their presence in macrophages and fibroblasts. As the experiment proceeded, many melanomacrophages were seen in the epidermis and the dermis. The phagosomes in these cells contained cellular remnants, in particular of granulocytes (Figure 17).

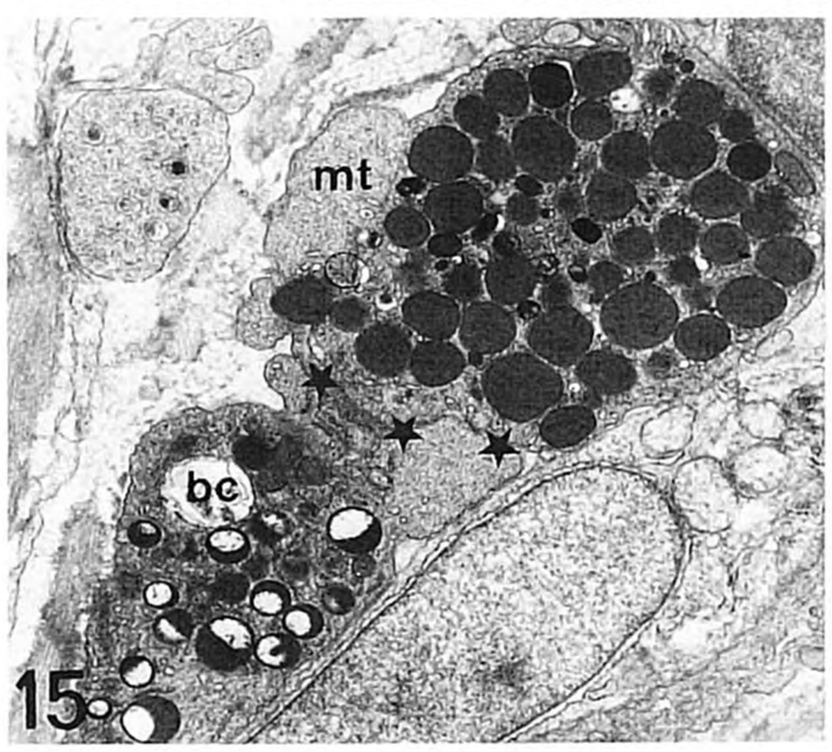
Sensory Elements: Merkel cells, normally located in the outer layers of the epidermis, were numerous and were found also in the inner layers of the epidermis. The number of secretory granules in these cells was reduced and remained low during the whole experiment. In the dermis many axons were found close to the basal lamina. Axons were also common in the epidermis and so were dermal papillae and taste buds.

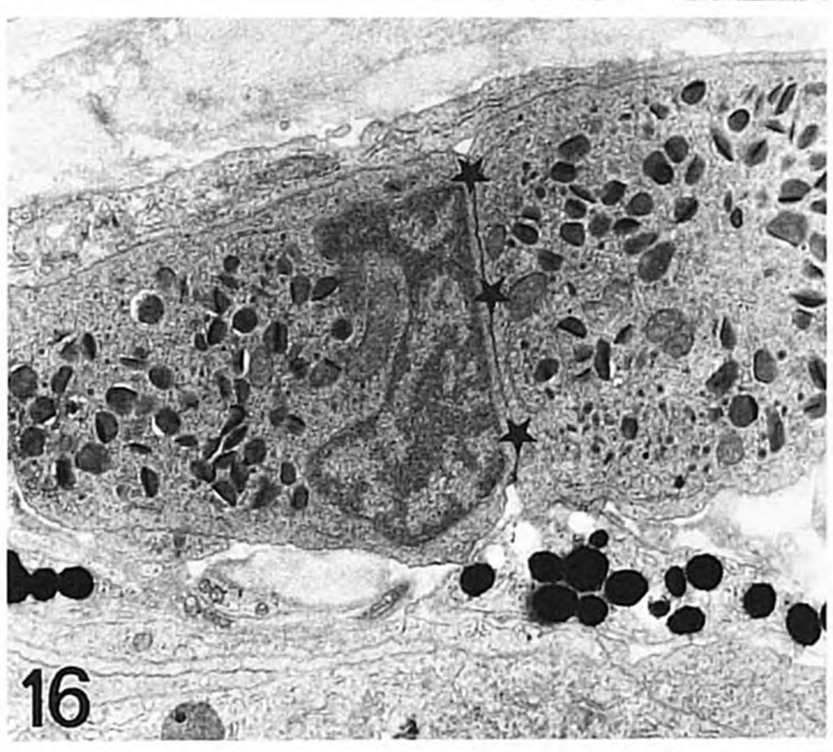
Dermis

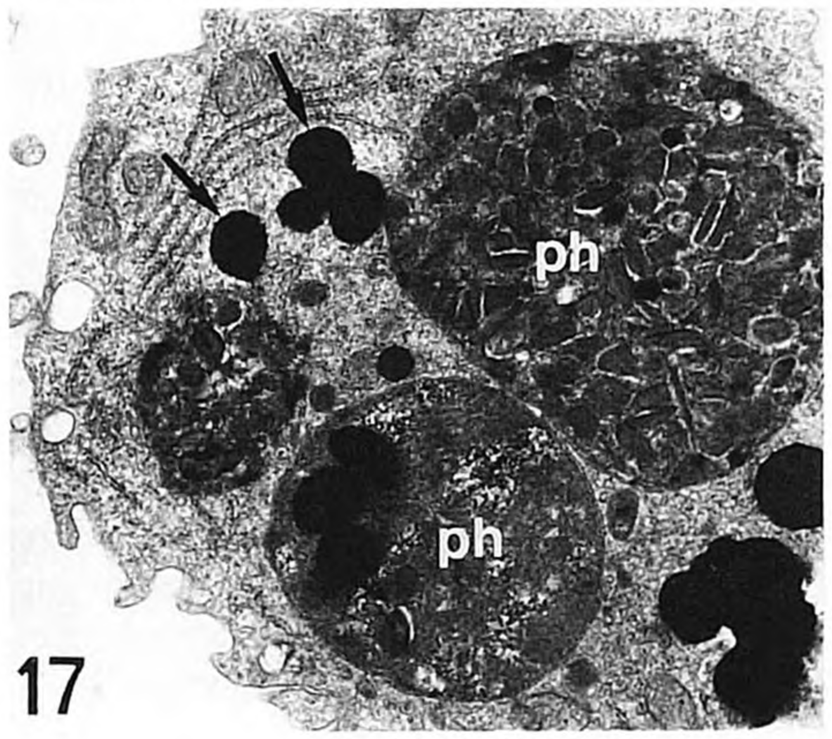
Fibroblasts: Activated fibroblasts, *i.e.*, cells with abundant rER and peripheral vesicles, were commonly found close to the basal lamina. Occasionally, the density of the collagenous matrix of the outer dermis increased markedly by the deposition of new, randomly arranged, collagen fibers. Also dermal "her-











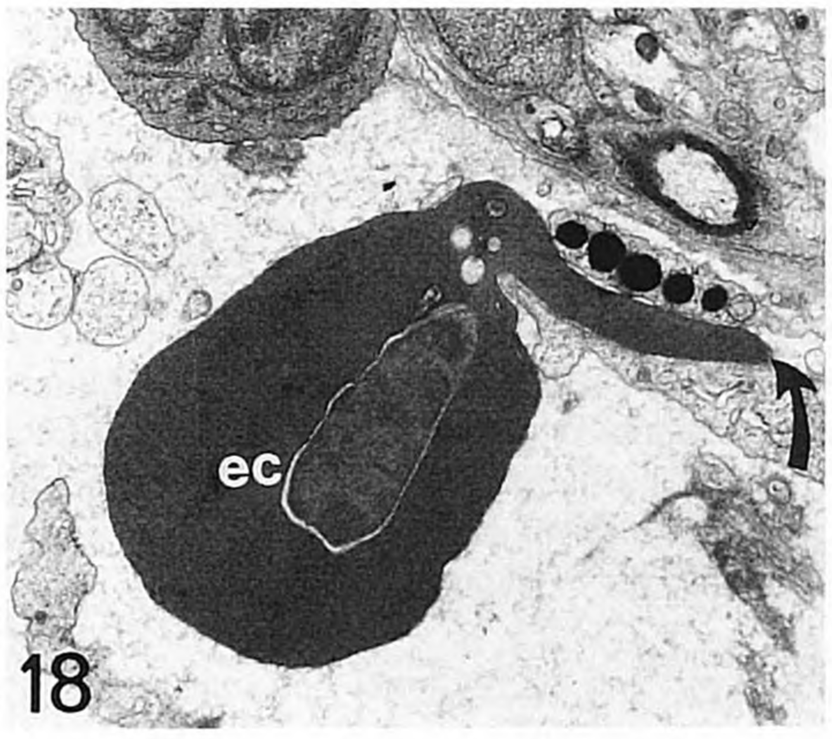


Fig. 13. Clusters of leucocytes, mainly lymphocytes (ly), in inner layers of the epidermis; bl, basal lamina; fc, filament cell. Seven days of exposure to 22 μg Cd/L; ×1,538

Fig. 14. Macrophage with several phagosomes (arrows), probably of lymphocytes. Three days of exposure to 560 μg Cd/L; ×8,100

Fig. 15. Coupling (asterisks) between mast cell (mt) and basophilic granulocyte (bc). 24 h of exposure to 560 μg Cd/L; ×6,300

Fig. 16. Coupling (asterisks) between two eosinophilic granulocytes. Three days of exposure to 560 μg Cd/L; ×6,300

Fig. 17. Melanomacrophage with phagosomes (ph). The upper phagosome is composed of remnants of an eosinophilic granulocyte; arrows, free melanosomes. Three days of exposure to 560 μg Cd/L; ×11,100

Fig. 18. Erythrocyte (ec) with cellular extension (arrow) apparently migrating in the dermal matrix. One hour of exposure to 560 μg Cd/L; ×6,300

rings," *i.e.*, bundles of collagen fibers oriented perpendicular to the basal lamina, were common.

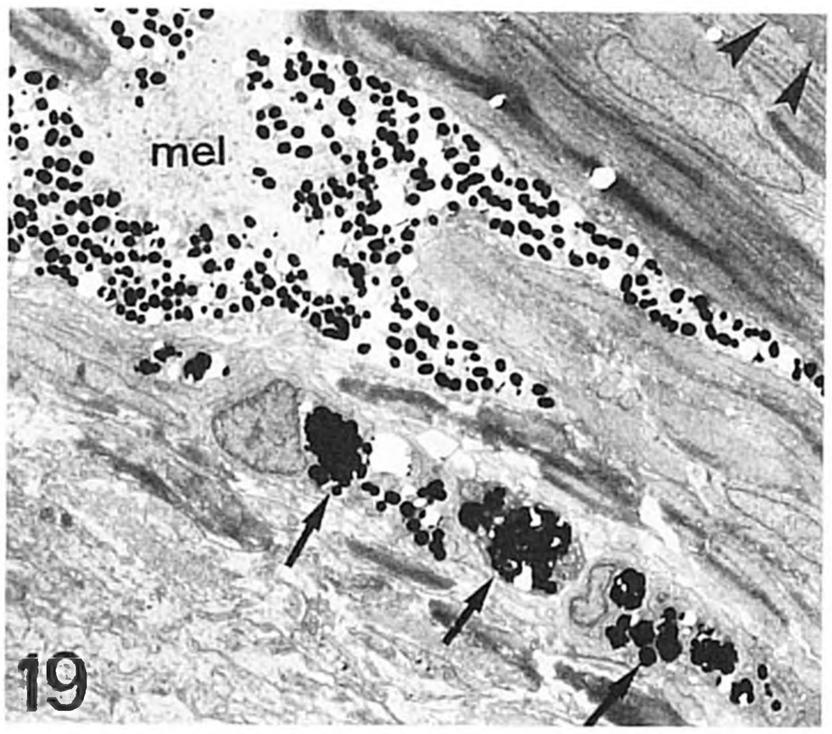
Capillaries: The endo- and exocytotic activity (cytosis) of the endothelial cells diminished shortly after the start of the exposure. Endothelial cells were elongated in such a way that many of the capillary profiles in tissue sections became elongated, perpendicular to the basal lamina, rather than rounded. The distance between the capillaries and the basal lamina decreased markedly, indicating the formation of new capillaries (angiogenesis). Cytosis was resumed later in the rounded capillaries, but the elongated endothelial cells hardly displayed this activity. Occasionally the tight junctions between endothelial cells were open; erythrocytes had moved out of capillaries, and some were found in the dermal matrix (Figure 18).

Melanocytes: Melanosomes were located in the cytoplasmic processes of the melanocytes, rather than in the cell bodies. These processes also penetrated in the epidermis, where aggregates of melanosomes were common. Many of the melanocyte processes were apoptotic (Figure 19). Several of these were confined within macrophages (Figure 20).

Discussion

Epidermis

Filament Cells and Pavement Cells: The appearance of necrotic pavement cells may reflect direct toxic effects of cad-



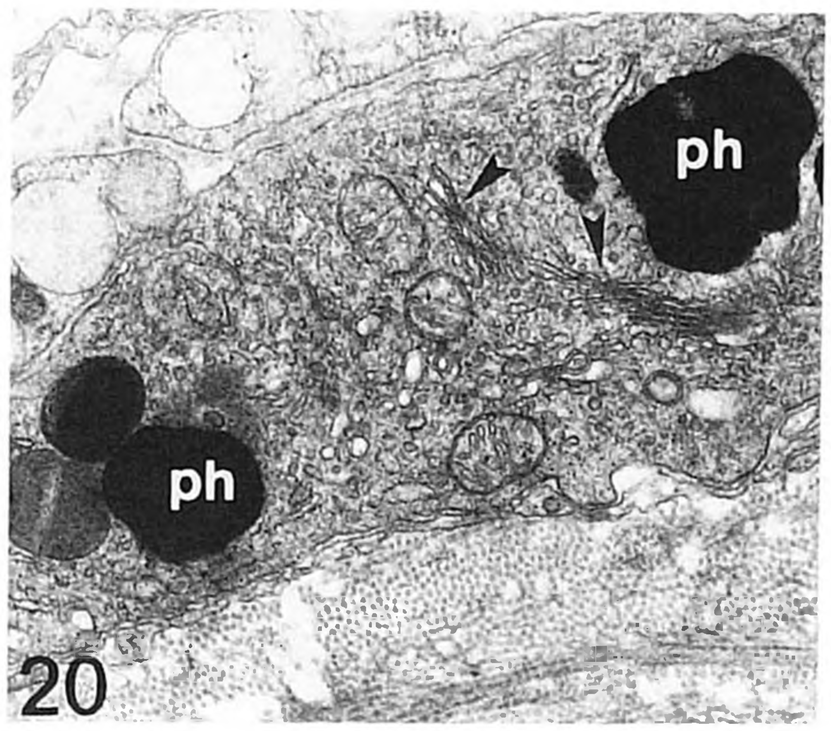


Fig. 19. Normal melanocyte (mel) close to several apoptotic processes of melanocytes (arrows); arrow heads, basal lamina. Seven days of exposure to 560 μg Cd/L; ×2,100

Fig. 20. Part of melanocyte extension as apoptotic body in phagosomes (ph) of macrophage; arrow heads, Golgi systems. Seven days of exposure to 560 μg Cd/L; ×8,100

mium on these superficial cells. Increased apoptosis reflects accelerated aging and exhaustion of cells (Wyllie 1981), and thus the appearance of apoptotic pavement cells might be a direct effect of cadmium on the aging of cells or an indirect effect via the observed increased synthetic activity. Necrosis followed by apoptosis, as observed in this study, has been previously reported in the skin of carp exposed to water-borne lead (Iger 1992) and to acid water (Iger and Wendelaar Bonga 1993). Similar degeneration of pavement cells was prominent in the gill epithelium of *Oreochromis mossambicus* exposed to cadmium or acidified water (Wendelaar Bonga *et al.* 1990; Pratap and Wendelaar Bonga 1993). The production of the small electron dense vesicles by the pavement cells of the cadmium-exposed carp was observed earlier in carp exposed to acid water, water polluted with manure or lead, and wounding of the skin (Iger and Abraham 1990; Iger 1992; Iger and Wendelaar Bonga 1993). They contain a peroxidase-positive mucous substance that, upon release, contributes to the glycocalyx covering these cells (Iger and Wendelaar Bonga 1993). Some of the larger vesicles of high electron density that we found in the cadmium-exposed pavement cells resembled granules of eosinophilic granulocytes. For the other type of large electron-dense vesicles found in these cells, we have shown, in a separate study using X-ray microanalysis, that they contain cadmium (Iger 1992).

The appearance of numerous mitotic cells and the high incidence of necrosis and apoptosis indicate that cadmium accelerates the turnover of the filament cells. In our earlier studies on the effects of stressors on the skin of carp, we mostly found mitotic filament cells adjacent to club cells (Iger 1992; Iger and Wendelaar Bonga 1992). The present finding of mitotic filament cells at other locations, *e.g.*, close to the skin surface, might be ascribed to the mitogenic effect reported for cadmium (Eaton 1974). Hyperplasia is commonly seen in the gill epithelium of fish exposed to toxicants (Wendelaar Bonga and Lock 1992). The aggregates of filament cells at the skin surface of carp from the high cadmium group probably represent initial stages in the development of papillomas (Lamas *et al.* 1990). Papillomas have been observed in the skin of fish from industrially polluted water (Smith and Zaidlik 1987). Their occurrence indicates that cadmium is carcinogenic at high concentrations (Couch 1985).

The intercellular spaces between epidermal cells slightly widened, as we have reported earlier for the gill epithelium of cadmium exposed tilapia (Pratap and Wendelaar Bonga 1993). In contrast to the gills of these fish, where some tight junctions were opened, the tight junctions at the skin surface of carp remained intact. Their apparent stability was associated with densification of the terminal web.

Endocytotic activity at the basal pole of basal filament cells stopped at the beginning of the exposure to cadmium but was restored later. This was also seen after wounding (Iger and Abraham 1990) and after exposure to lead (Iger *et al.* 1992) and to acid water (Iger and Wendelaar Bonga 1993), and thus may reflect a general response to stressors.

Mucous Cells: Secretion of mucus was stimulated in cadmium contaminated water. In skin of fish exposed to 560 μg Cd/L, this activity was apparently not compensated for by the differentiation of new mucous cells, as indicated by the scarcity of mucous cells after 3 days. A notable reduction in mucous cell numbers in the epidermis was reported shortly after exposure to copper (Benedetti *et al.* 1989), water fertilized with manure (Iger *et al.* 1988), and acid water (Iger and Wendelaar Bonga 1993). Mucus proteins have the capacity of binding cadmium (Pärt and Lock 1983). Secreted mucus, therefore, may reduce metal penetration and could enhance its elimination.

In our experiment, we found a change in mucus composition, as was indicated by the appearance of mucosomes of high electron density. Cells with such mucosomes, termed serous mucous cells (Whitear 1986), have been reported earlier for stressed fish (Blackstock and Pickering 1980; Iger and Wendelaar Bonga 1993; Iger 1992).

Club Cells: Cadmium exposure initiated migration of club cells towards the surface of the epidermis and the formation of phagosomes in these cells, as we have reported for other stressors (Iger 1992; Iger and Wendelaar Bonga 1993). The appearance of club cells close to the skin surface may facilitate the release of their alarm substances (Pfeiffer 1977).

Chloride Cells: Chloride cells, absent in controls, appeared in the skin of cadmium exposed fish already after 3 days. They also appeared in the skin of carp after exposure of the fish to water contaminated with manure or lead, or in fish kept in acidified water (Iger 1992; Iger and Wendelaar Bonga 1993), and their appearance may represent a general response to stressors. The appearance of chloride cells in the epidermis likely is a compensatory response to the disturbing effects of cadmium

on ion transport, in particular calcium uptake in the gills (Verbost *et al.* 1989).

Leucocytes: Cadmium induced infiltration of leucocytes into the epidermis as we have reported for fish exposed to other pollutants (Iger *et al.* 1988; Iger 1992; Iger and Wendelaar Bonga 1993). This phenomenon is well known for the epithelium covering the gills of fish exposed to toxicants (Mallatt 1985). For cadmium, it was reported for the branchial epithelium of *Oncorhynchus mykiss* and *Oreochromis mossambicus* (Karlsson-Norrgren *et al.* 1985; Pratap and Wendelaar Bonga 1993). This infiltration may partially explain the leucopenia commonly seen in stressed fish (Pickering and Pottinger 1987), including fish exposed to cadmium (Murad and Houston 1988; Tort and Torres 1988).

Infiltration of the epidermis by mast cells and coupling of leucocytes, as were described in this study, were not found under the influence of the other stressors studied, and thus probably represent a specific effect of cadmium.

Sensory Elements: Degeneration of sensory elements of the skin, as reported in response to environmental changes such as water acidification or the presence of heavy metals such as copper or mercury (Benedetti *et al.* 1989; Pevzner *et al.* 1986; Iger and Wendelaar Bonga 1993), was not observed. We detected changes in the distribution of Merkel cells, which appeared throughout the epidermis in the cadmium-exposed fish.

Dermis

Fibroblasts: Exposure to cadmium induced an increase in numbers and collagen-synthesizing activity of fibroblasts in the outer dermal zone. This was earlier reported for carp exposed to lead (Iger 1992) and for flatfish exposed to water contaminated with sewage sludge (Bucke *et al.* 1983).

Capillaries: Exposure to cadmium decreased the cytosis of the endothelial cells and induced angiogenesis. Similar responses were detected in carp skin after wounding (Iger and Abraham 1990) or lead contamination (Iger 1992), and in the oesophageal epithelium of tilapia during adaptation to salinity changes (Cataldi *et al.* 1988), and this indicates that it may be a general response to stressful conditions. Angiogenesis might be connected with the increased metabolic requirements of the epidermis as a result of enhanced cellular activity and increased cellular turnover rate.

Melanocytes: The penetration of pigment-containing cell processes of the melanosomes into the epidermis was observed before in the skin of carp exposed to water with lead (Iger *et al.* 1992). Dispersion of melanosomes was suggested to represent a defense mechanism to stressors (Iger *et al.* 1988; Iger and Wendelaar Bonga 1993). Melanin can scavenge free radicals (Sarna *et al.* 1986).

In conclusion, exposure of fish to 22 μg/L or 560 μg/L cadmium in the water markedly affected the structure of the skin. The effects of the low cadmium concentration were qualitatively similar to those of the higher concentration, but, in general, effects of the low concentration were less pronounced and appeared later than those of the high concentration. Most of the effects observed are not specific for cadmium and have been observed before under the impact of other stressors. Many of these effects may be mediated by stress hormones such as cortisol. Cortisol levels become elevated after exposure to stressors, including cadmium (Pratap and Wendelaar Bonga 1990; Fu *et al.* 1990). Only a few of the responses observed, such as the coupling of leucocytes, the appearance of Merkel cells close to the basal lamina, and the appearance of tumorlike aggregates of filament cells, may be specific for cadmium.

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