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Cellular responses in the skin of the trout (*Oncorhynchus mykiss*) exposed to temperature elevation

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The skin of rainbow trout was examined at the ultrastructural and cytochemical level after a 3-h exposure to an elevation of the water temperature, from 15 to 22° C. Within 3 h, the thickness of the epidermis had significantly ($P < 0.05$) decreased when compared to control fish. After 24 h it was restored, and from day 4 onwards even increased above control levels. The thickening of the epidermis was associated with appearance of many mitotic cells, not observed in control fish. Within 24 h many apoptotic epidermal cells were found, indicating enhanced ageing of the cells. Filament cells from the outer epidermal layers synthesized vesicles with peroxidase activity within 3 h after temperature elevation. This enzyme was found also in apoptotic as well as in necrotic filament cells. Mucous cells became elongated and their mucosomes displayed peroxidase activity. Occasionally electrondense, probably serous, mucosomes appeared. In the epidermis rodlet cells were found. Both epidermis and dermis, became invaded by many lymphocytes and macrophages. The latter contained vesicles with peroxidase activity. Pigment-containing cytoplasmic extensions of melanocytes penetrated the epidermis while iridocytes disappeared from the dermis. The synthetic activity of dermal fibroblasts was stimulated. These results show that a moderate temperature elevation has pronounced and prolonged effects on the skin of the exposed fish. The effects are to a high extent comparable with those of stressors such as heavy metals, acid water or wounding.

Key words: epidermis; dermis; ultrastructure; peroxidase activity; thermal pollution; *Oncorhynchus mykiss*.

I. INTRODUCTION

Temperature is an environmental factor that has immediate and pervasive effects on cellular activity in fish and other poikilothermic vertebrates (Guderley & Blier, 1988). Thermal pollution, unlike many other stressors, has mainly local effects, e.g. in the outlet of power plant cooling systems (Dethlefsen & Tiews, 1985). The effects of thermal pollution are known to be cumulative with the effects of other stressors (Umminger & Gist, 1973). However a change in temperature alone, in particular an elevation, can markedly alter enzymatic activity (Guderley & Blier, 1988; Rady *et al.*, 1990) and may eventually lead to death (McGeer *et al.*, 1991).

External epithelial tissues, such as the skin, form the primary barrier between the internal tissues and the external environment, and thus are the first to experience environmental changes. In fish, changes in the environmental temperature are known to cause structural changes in the intestinal and branchial epithelium (Jacobs *et al.*, 1981; Mallatt, 1985; Lee & Cossins, 1988). Fish kept for a few days at just below their lethal temperature show degenerative changes

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in their superficial epidermal cells (Ferri, 1982). However, there is a lack of information about the structural changes and responses of the skin during and after a short period of moderate elevation of the temperature.

In this paper ultrastructural and cytochemical changes are studied in the epidermal and dermal tissues of rainbow trout (*Oncorhynchus mykiss* Walbaum) at different periods during and after a short-term exposure to a relatively mild elevation of water temperature. The results are also discussed in relation to the cellular responses of the skin to stressors in general.

II. MATERIALS AND METHODS

Thirty-six adult male and female trout weighing 276 ± 46 g ($x \pm$ s.d.; $n=36$) were kept in two groups for an acclimation period of 25 days in running tap water (pH 7.5, temperature 15°C). Each group was kept in a 400-l plastic tank, with a flow rate of 9–10 l min^{-1} . One group of trout served as a control. For the other group the temperature of the water was elevated to 22°C within 30 min, by continuously adding water of 30°C, until reaching the planned temperature. Fish were kept for 3 h (from the initial elevation of temperature) in water of 22°C with the same flow rate. Then the temperature was reduced to 15°C within 1 h. The water of both groups was continuously changed and well aerated.

For electron microscopy, skin biopsies (about 3×3 mm) were taken from the dorsal part of the head of fish by scalpel blade. Fish were sampled 2 h 55 min (3 h), 24 h and 4, 7 and 14 days after the initial increase in temperature. At each time point three fish, lightly anaesthetized with pH neutralized MS-222 (diluted 1 : 15 000) were sampled from each group, without killing the fish. The tissues were fixed in 3% glutaraldehyde buffered in sodium cacodylate (0.09 M, pH 7.3), washed in buffer, and post-fixed in 1% osmium tetroxide in the same buffer. Ethanol-dehydrated tissues were embedded in Spurr's resin. Ultrathin sections, collected on 150 mesh copper grid, were contrasted with uranyl acetate and lead citrate, and were examined in a Jeol 100 CXII transmission electron microscope. The detection of peroxidase activity was carried out with a conventional diaminobenzidine technique (see Iger & Abraham 1990), after fixation with glutaraldehyde. Samples incubated in the absence of H_2O_2 served as control for this method.

Morphometrical parameters were obtained from thin sections. For measuring the epidermal thickness, two to three micrographs at low magnification ($\times 100$ or $\times 150$) were taken from each fish. Negatives were magnified four times, and the thickness of the epidermis was calculated. The thickness was quite uniform within fish, apart from areas with taste buds or dermal papillae, that were excluded from this analysis. The data given represent the mean \pm s.d. of three experimental fish at each sampling time. The epidermal thickness of control fish was stable during the whole experiment, and therefore control values were pooled (control data represent the mean \pm s.d. of 10 fish, two from each sampling time). Relevance of the different s.d. was checked using the *F*-test. Thereafter differences between experimental and control fish were tested using Student's *t*-test. For some morphometrical parameters, data represent the range of the values measured.

III. RESULTS

CONTROLS

Epidermis

The trout epidermis was 120–140 μm thick and composed of several layers of filament-containing cells (filament cells) and mucous cells. Merkel cells and lymphocytes were occasionally observed. Chloride cells were rarely encountered, and rodlet cells were absent.

Filament cells. The basal layer of filament cells, adjusted to the slightly wavy basal lamina, were cylindrical and displayed 15–25 endocytotic vesicles per cell profile at their basal pole. Filament cells from the mid-epidermal zone were multiform, and interconnected by desmosomes. At the skin surface, pavement cells were characterized by microridges 0.3–0.4 μm in height. These cells contained a few electronlucent vesicles that were occasionally fused with the apical cell membrane. The intercellular space between pavement cells was bridged by many desmosomes and, near the surface, by tight junctions. Peroxidase activity was not found in pavement or inner filament cells, neither were mitotic figures.

Mucous cells. About 100–130 mucous goblet cells, mostly oval shaped, for each 1 mm section length of the epidermis were seen. Newly differentiated mucous cells were found in the third and fourth inner layers of the epidermis. Such cells contained a centrally located nucleus, surrounded by an extensive rough endoplasmic reticulum (rER) and several Golgi systems from which small vesicles were budded. In mature mucous cells, the number of desmosomes diminished and the cells migrated towards the epidermal surface. Before discharging their content the cells were 13–16 μm long and contained mucosomes of about $1.5 \times 0.75 \mu\text{m}$. After discharge, the cytoplasmic remnants of the mucous cell were shed without showing signs of apoptosis. Peroxidase activity was not found in mucous cells (Fig. 9).

Merkel cells. These cells were located in the three to four outermost layers of the epidermis. They were rounded, with one to three cellular extensions per cell, and contained about 10 densely-cored secretory granules per cell in section.

Dermis

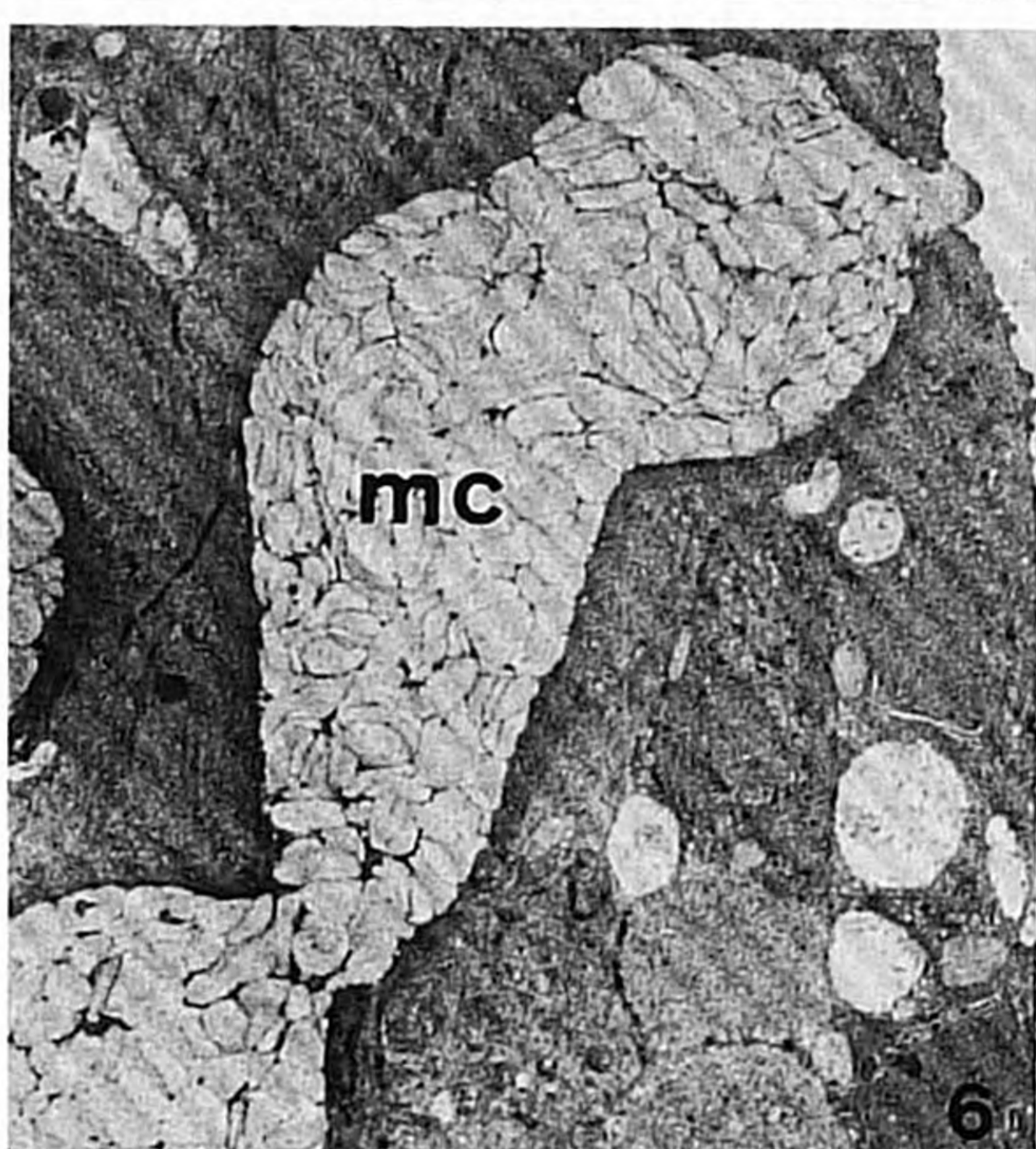
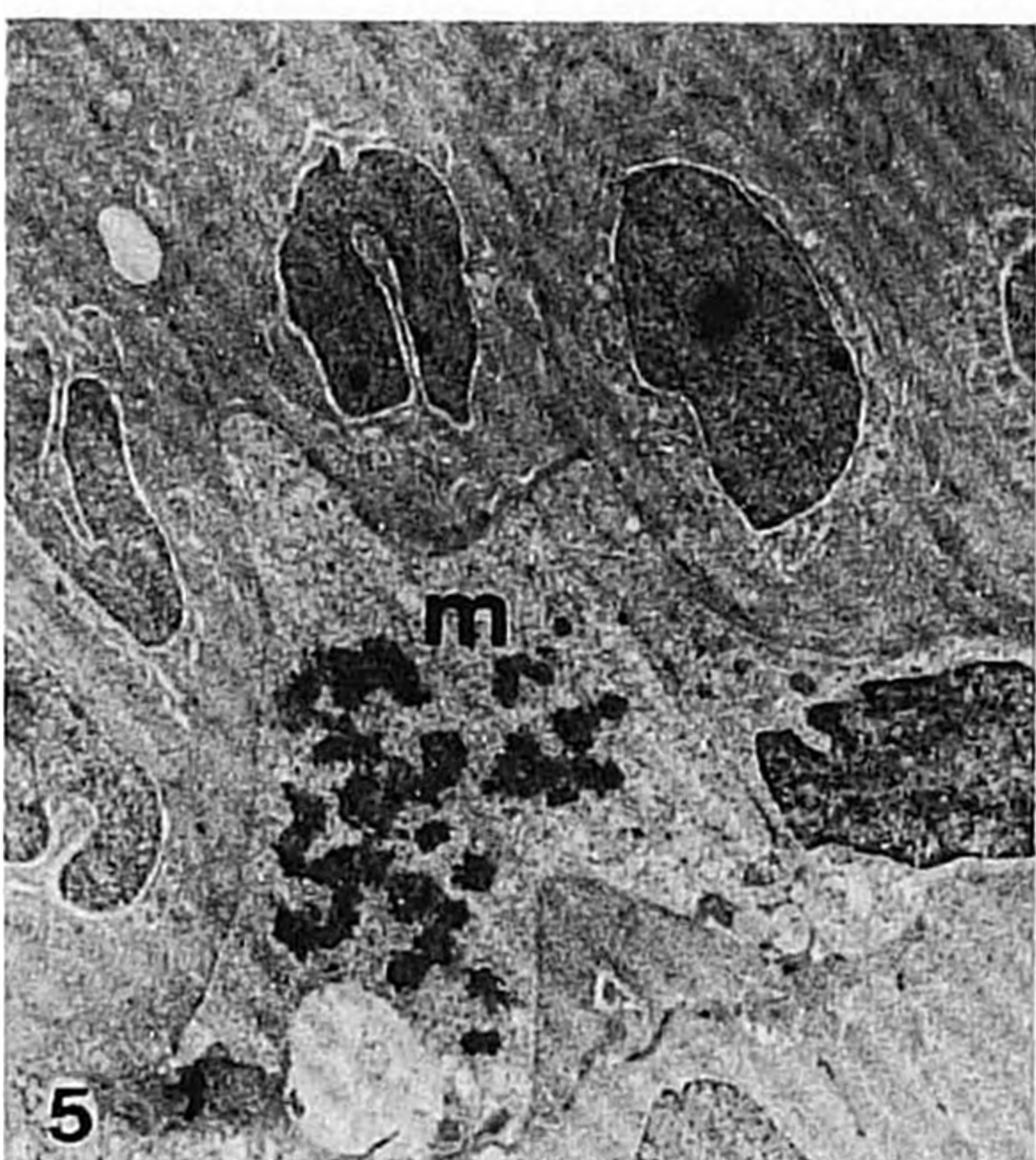
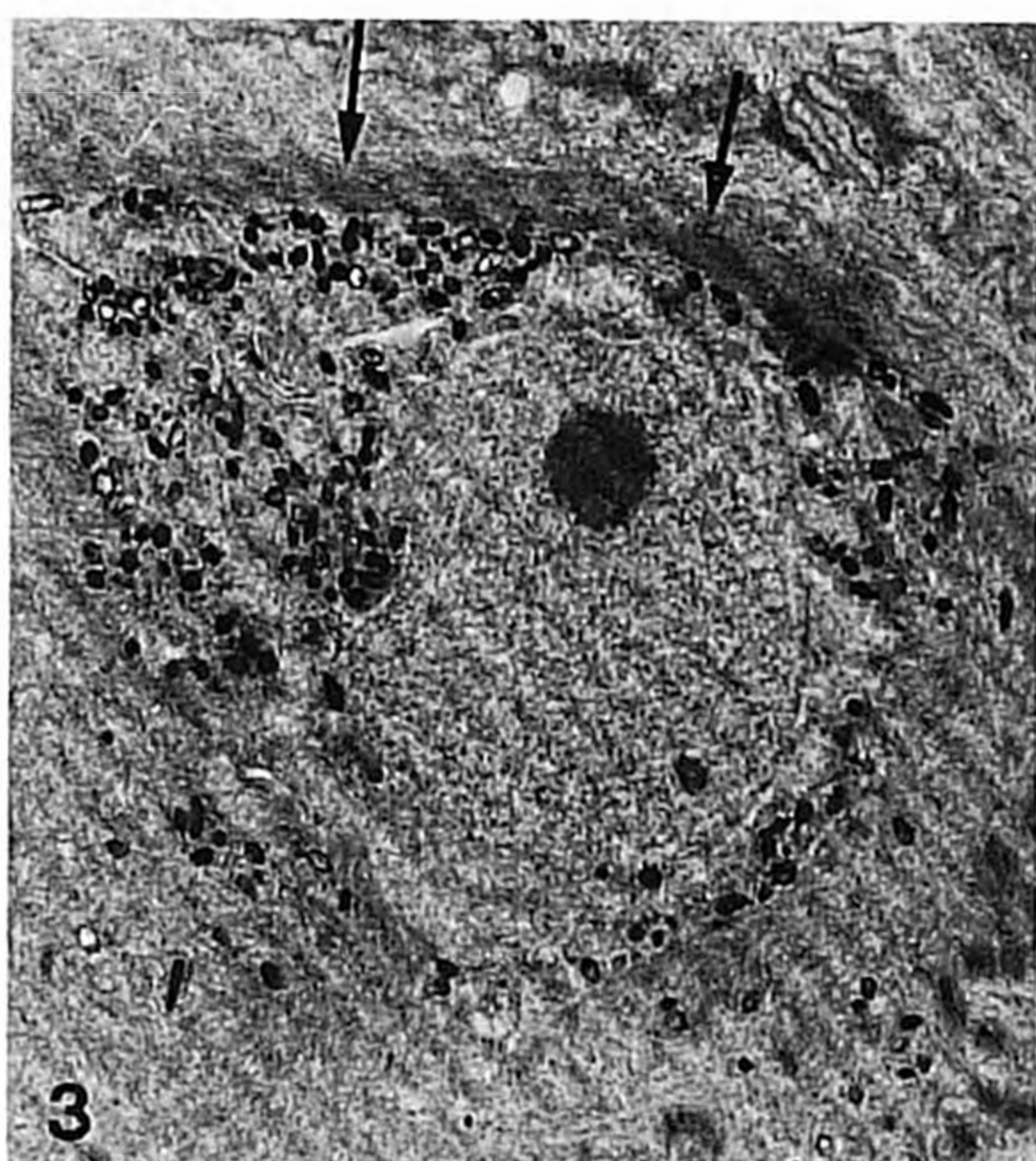
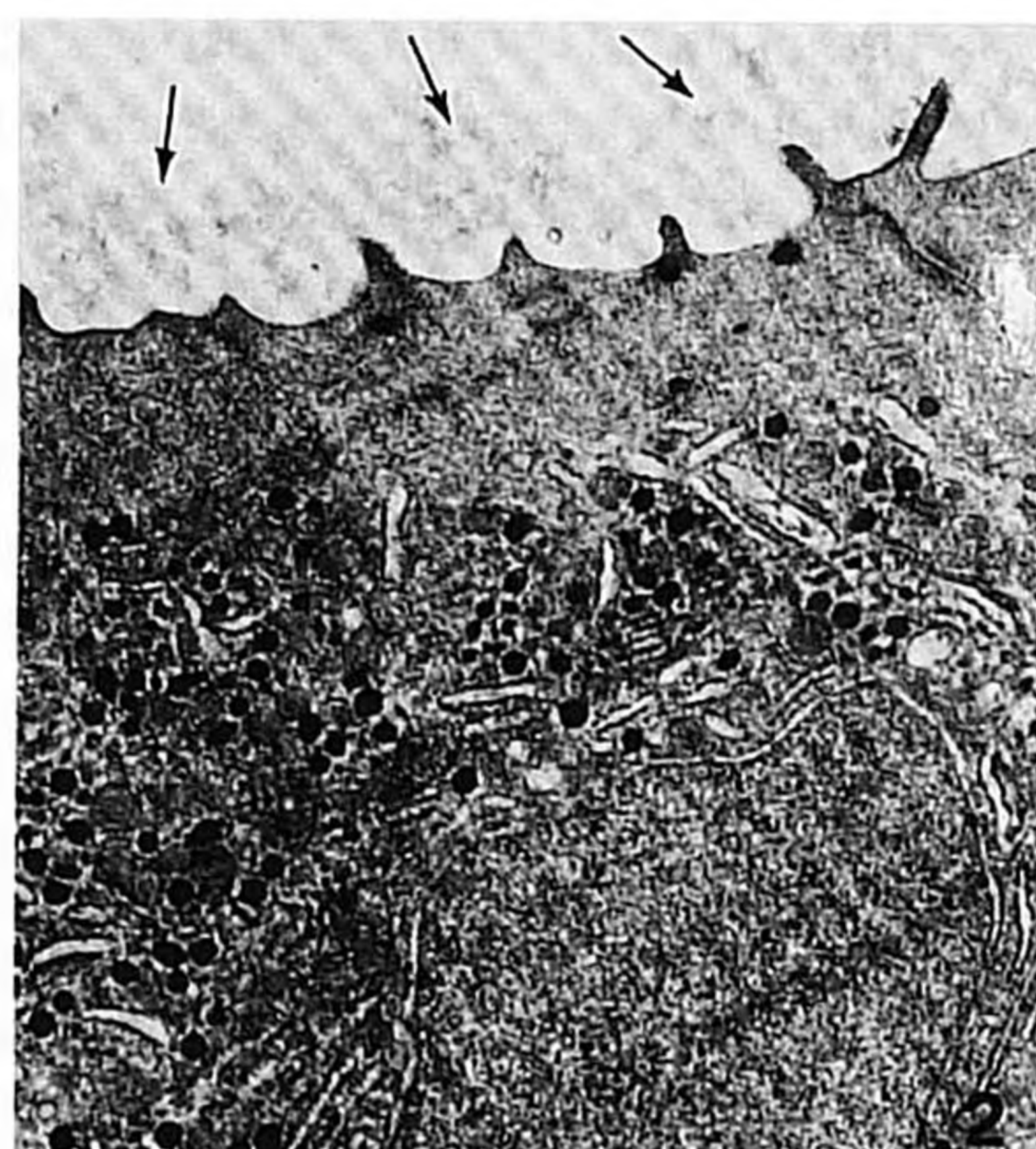
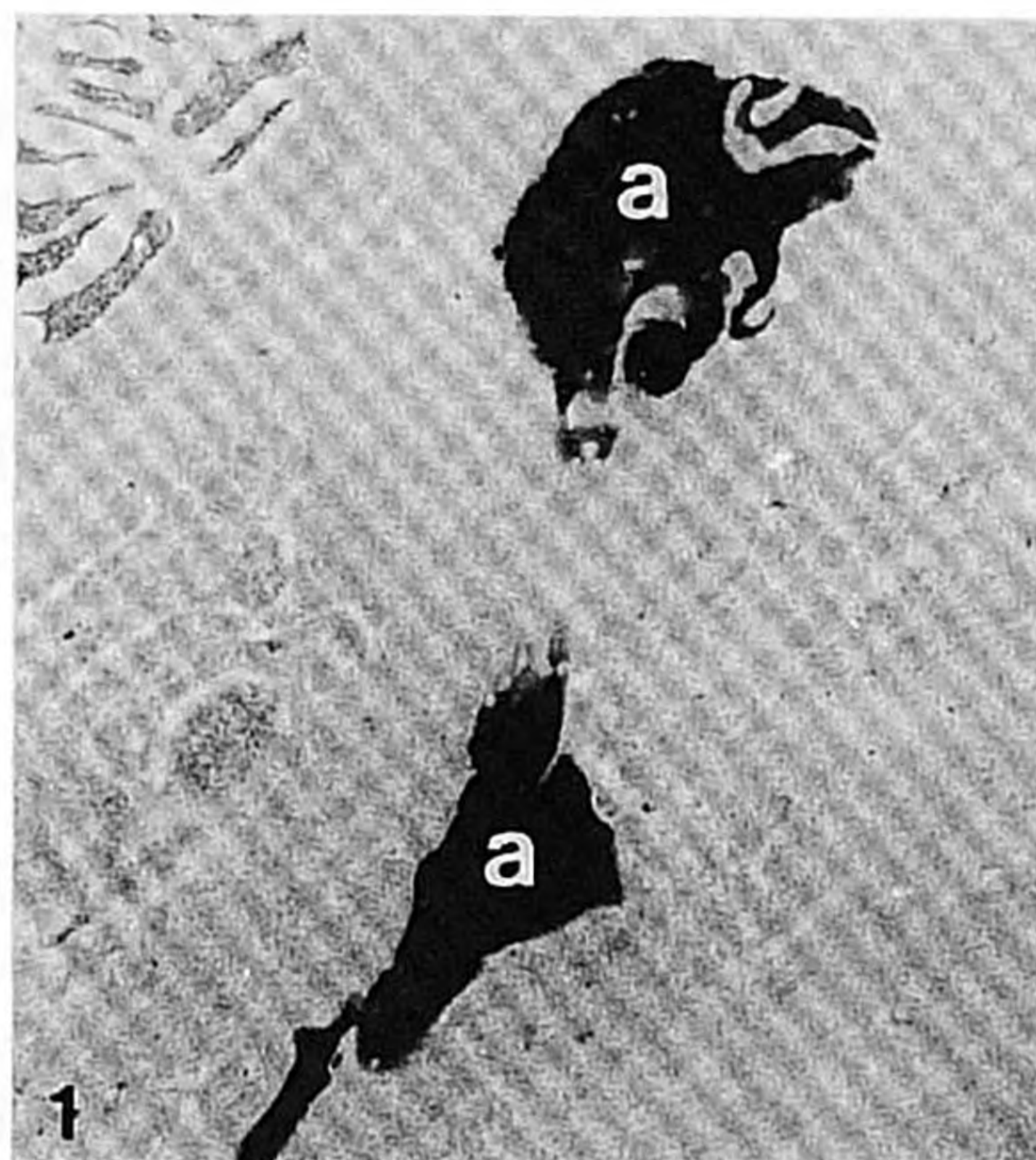
The dermis was composed of collagen fibres, which appear to be arranged randomly in the outer dermis (a zone of 40–60 μm thickness) and highly compressed in the inner dermis. The main cellular elements in the inner dermis were fibroblasts. Some capillaries were also present. Their endothelial cells were actively engaged in pinocytosis. In the outer dermis fibroblasts were less frequent and capillaries were rare.

Pigment cells. Melanocytes, xanthophores and iridocytes were scarcely seen in the inner dermis but were common in the outer dermis. Melanosomes were located in the cell bodies and only rarely seen in the melanocyte extensions. Guanine platelets were located all over the iridocytes, mostly arranged parallel to the basal lamina. Leucocytes were rare.

ELEVATED TEMPERATURE

Epidermis

Epidermal thickness decreased significantly ($P < 0.05$) after elevation of temperature, from $128.2 \pm 9.76 \mu\text{m}$ in control fish to $111.6 \pm 12.2 \mu\text{m}$ at the end of the 3 h treatment. It returned to normal thickness ($119.4 \pm 14.35 \mu\text{m}$) after 24 h, and increased significantly ($P < 0.01$) above control values to $157.1 \pm 17.61 \mu\text{m}$ and $173.3 \pm 14.90 \mu\text{m}$ on days 4 and 7, respectively. After 14 days the epidermal



thickness ($148.4 \pm 19.55 \mu\text{m}$) was slightly, but still significantly ($P < 0.05$), above control values. At the skin surface, depressed areas were found after 24 h and 4 days. These areas probably represented sites of degenerate and shed pavement cells and exhausted mucous cells. The basal lamina (Fig. 15) was very wavy (10–15 waves per cell against four to seven in the control) throughout the experimental period.

Filament cells. Many necrotic pavement cells (swollen cells showing disrupted membranes and increased electron lucency of the cytoplasm) were detected 3 and 24 h after the start of the temperature elevation. From 24 h onwards, several pavement cells and many inner filament cells became apoptotic. These cells showed shrinkage and condensation of cellular elements, increased electron density of the cytoplasm, and loss of junctional complexes. The frequency of apoptotic cells appeared to be highest in the three fish sampled on day 7: 50–60 apoptotic cells were found per mm of epidermal section length. In necrotic cells several small vesicles reacted positively with diaminobenzidine, thus reflecting a content with peroxidase activity. In apoptotic cells (Fig. 1), the whole cytoplasm strongly reacted with diaminobenzidine.

The non-degenerative pavement cells were rather flattened and their tight junctions remained intact, although intercellular spaces increased slightly in width. This phenomenon was associated with internalization of desmosomes and a decrease in their number. After 3 h, pavement- and adjacent filament cells (the two outermost layers of the epidermis) exhibited signs of increased synthetic activity: well-developed rER, numerous Golgi areas and many secretory vesicles of high electron density (Fig. 2). After 24 h until day 14, increased synthetic activity was seen in the filament cells of the five to six outermost layers. These cells also contained many bundles of filaments (Fig. 3). Peroxidase activity (Fig. 4) was detected over the electron dense vesicles or occasionally only on their membranes. Peroxidase activity was also detected occasionally in the glycocalyx adhering to the microridges of the pavement cells.

Many of the nuclei of the filament cells contained two nucleoli. Mitotic filament cells (Fig. 5), not observed in control fish, were commonly seen after 4 and 7 days. Endocytotic vesicles were no longer observed in the basal filament cells after 3 h. This activity was restored after 24 h.

FIG. 1. Peroxidase activity in apoptotic bodies (a) of filament cell(s), 24 h after the start of the 3-h temperature elevation; DAB reaction, otherwise unstained; $\times 5200$.

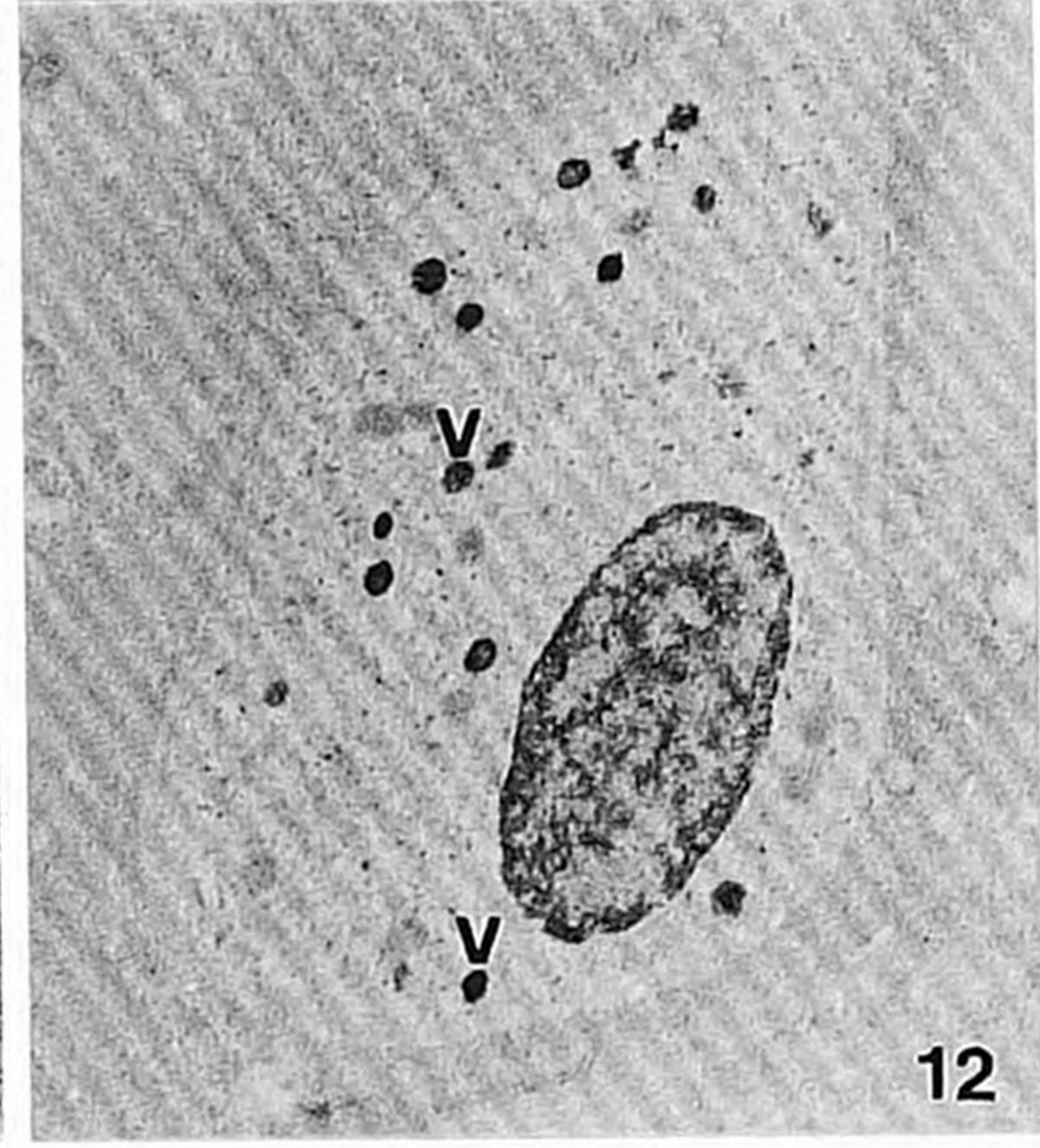
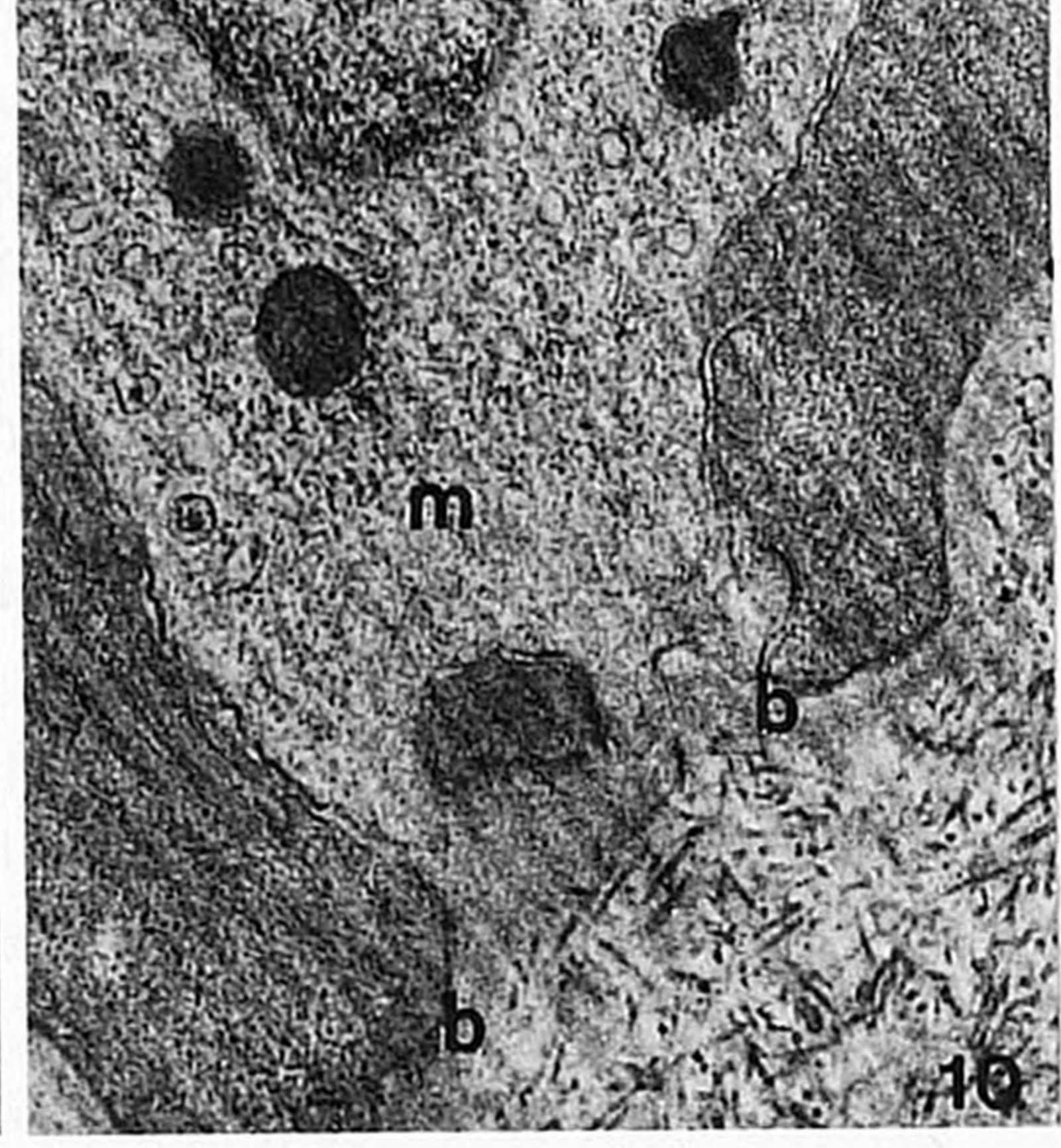
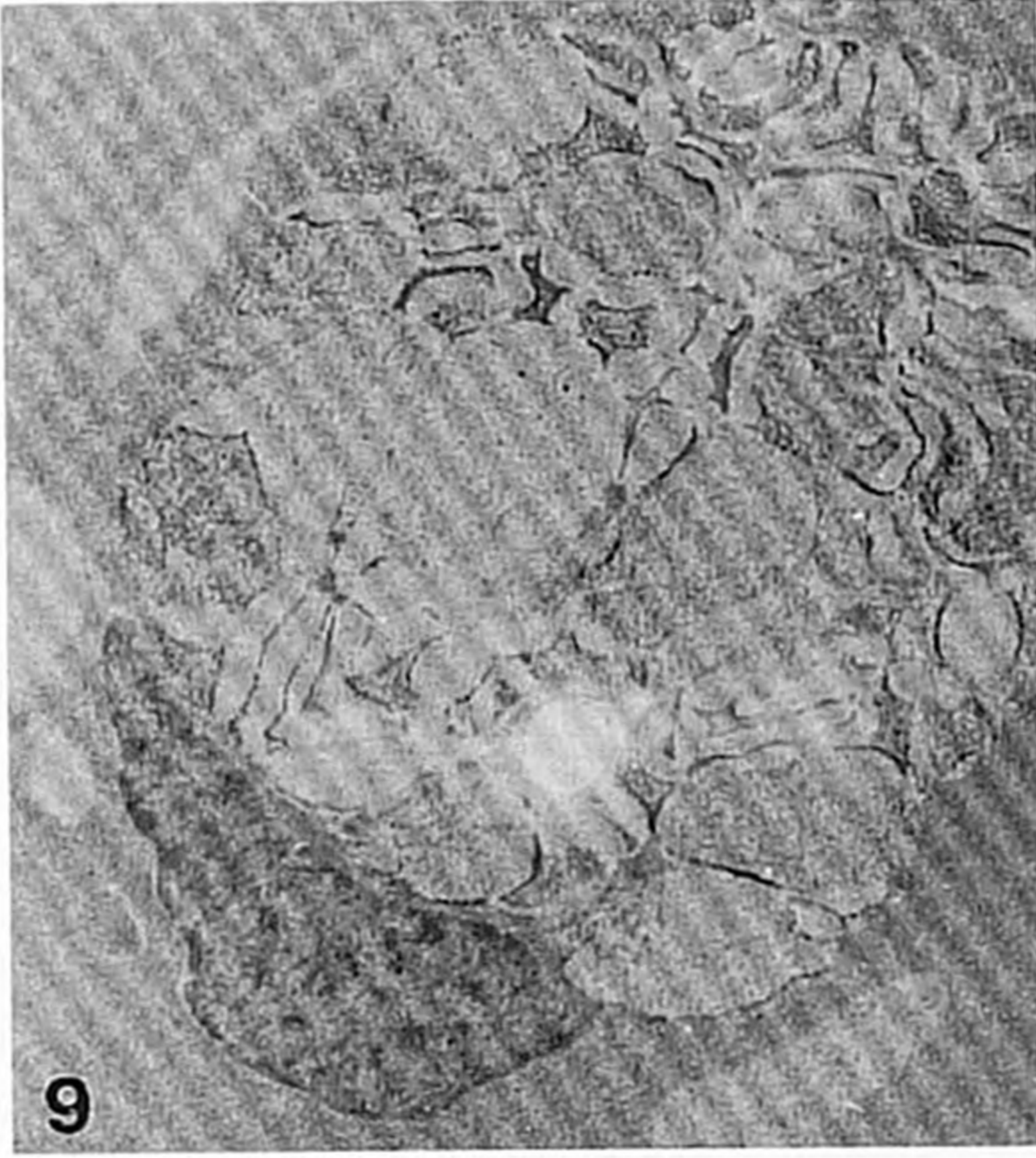
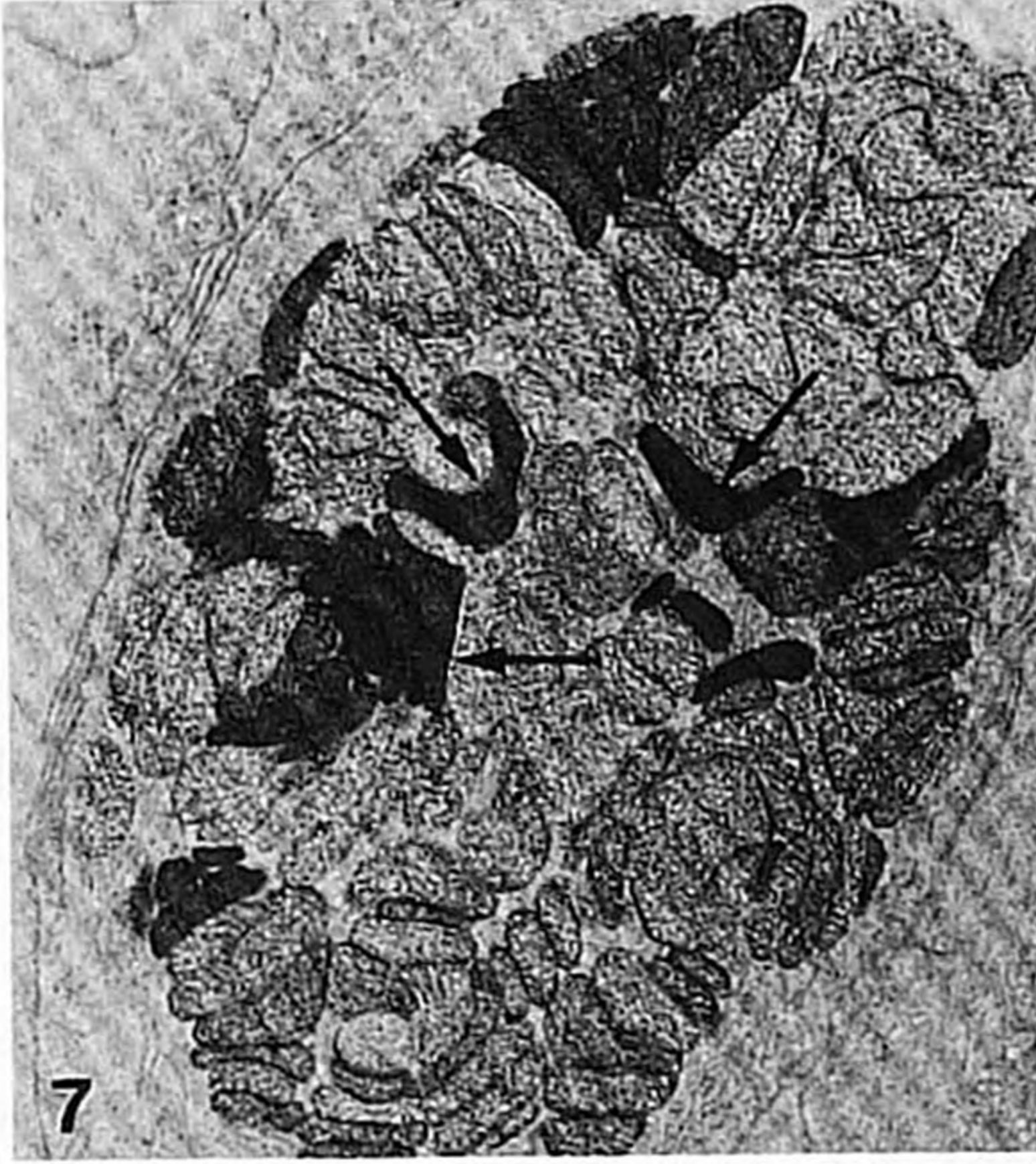
FIG. 2. Pavement cells showing electron-dense secretory vesicles; the microridges are covered by mucus (arrows); 7 days after temperature elevation; $\times 10\ 800$.

FIG. 3. Filament cell, deep to the pavement cells, with many electron-dense secretory vesicles and bundles of microfilaments (arrows); 24 h after temperature elevation; $\times 10\ 800$.

FIG. 4. Normal (n)- and slightly necrotic (d) pavement cells showing peroxidase-positive secretory vesicles; 3 h after temperature elevation; DAB reaction, otherwise unstained; $\times 10\ 800$.

FIG. 5. Mitotic filament cell (m); 4 days after temperature elevation; $\times 4400$.

FIG. 6. An elongated mucous cell (mc) migrating towards the skin surface; 24 h after temperature elevation; $\times 2050$.



Mucous cells. Abundant mucous cells extruded their content at the epidermal surface. This was reflected by the large quantities of amorphous mucus (Fig. 2) that appeared at the skin surface within 3 h after the start of the temperature elevation. Until day 4, mucous cells were located in the outermost layers of the epidermis. Most of these mucous cells were elongated (Fig. 6) rather than oval shaped, and their mucosomes were smaller than in the controls (about $0.75 \times 0.5 \mu\text{m}$). Although the rate of secretion was enhanced, the epidermis did not lose its mucous cells. Until day 7, mucous cells containing several mucosomes of high electron density (Fig. 7) were common. The mucosomes showed a positive reaction in the peroxidase test after 3 h (Figs 8 and 9). This reactivity was enhanced after 24 h and was diminished slightly after 14 days. After 4 days the newly differentiated mucous cells appeared one to two cell layers from the pavement cells. Later they were found also at their normal location, i.e. close to the basal filament cells. Apoptotic mucous cells were occasionally observed throughout the epidermis.

Leucocytes. Macrophages (Fig. 10) and many lymphocytes (Fig. 13) extravasated and penetrated the epidermis. After 3 h, most leucocytes were found in the lower layers of the epidermis while later on they were located throughout the epidermis. Macrophages were very active with well developed rER, many Golgi systems and vesicles, and several phagosomes (Fig. 11). The latter appeared mainly during the first 7 days. The vesicles (Fig. 12) and phagosomes reacted strongly in the endogenous peroxidase test. Leucocytes (in particular lymphocytes, Fig. 13) were occasionally found adjacent to mucous cells, a phenomenon not observed in control fish.

Rodlet cells. Rodlet cells were found in the outer epidermal layers of all the fish sampled at 7 and 14 days after the start of the rise in temperature. These cells were elongated, about $10 \times 5 \mu\text{m}$, with their long axis perpendicular to the epidermal surface. The nucleus of the cells was located at their deep or lateral side, and tubular bodies were found at the apical pole. Each cell contained 8–15 rodlets composed of an electron dense core surrounded by a moderately electron dense mantle. The cores of the rodlets showed peroxidase activity. The cytoplasm was surrounded by a fibrillar capsule. Occasionally, desmosomes were found between rodlet cells and neighbouring filament cells.

FIG. 7. Mucous cell with several mucosomes of high electron density (arrows); 24 h after temperature elevation; $\times 7200$.

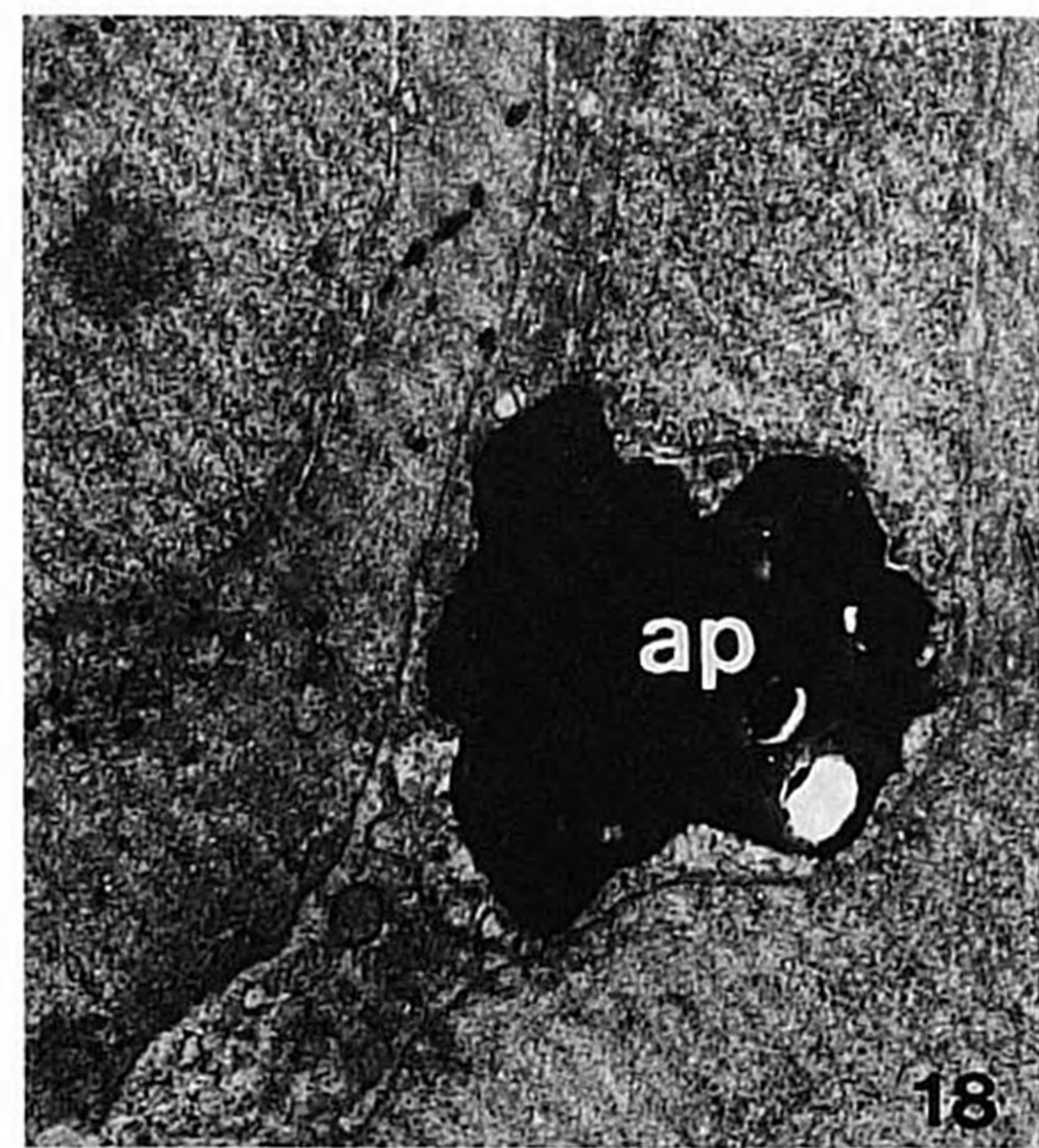
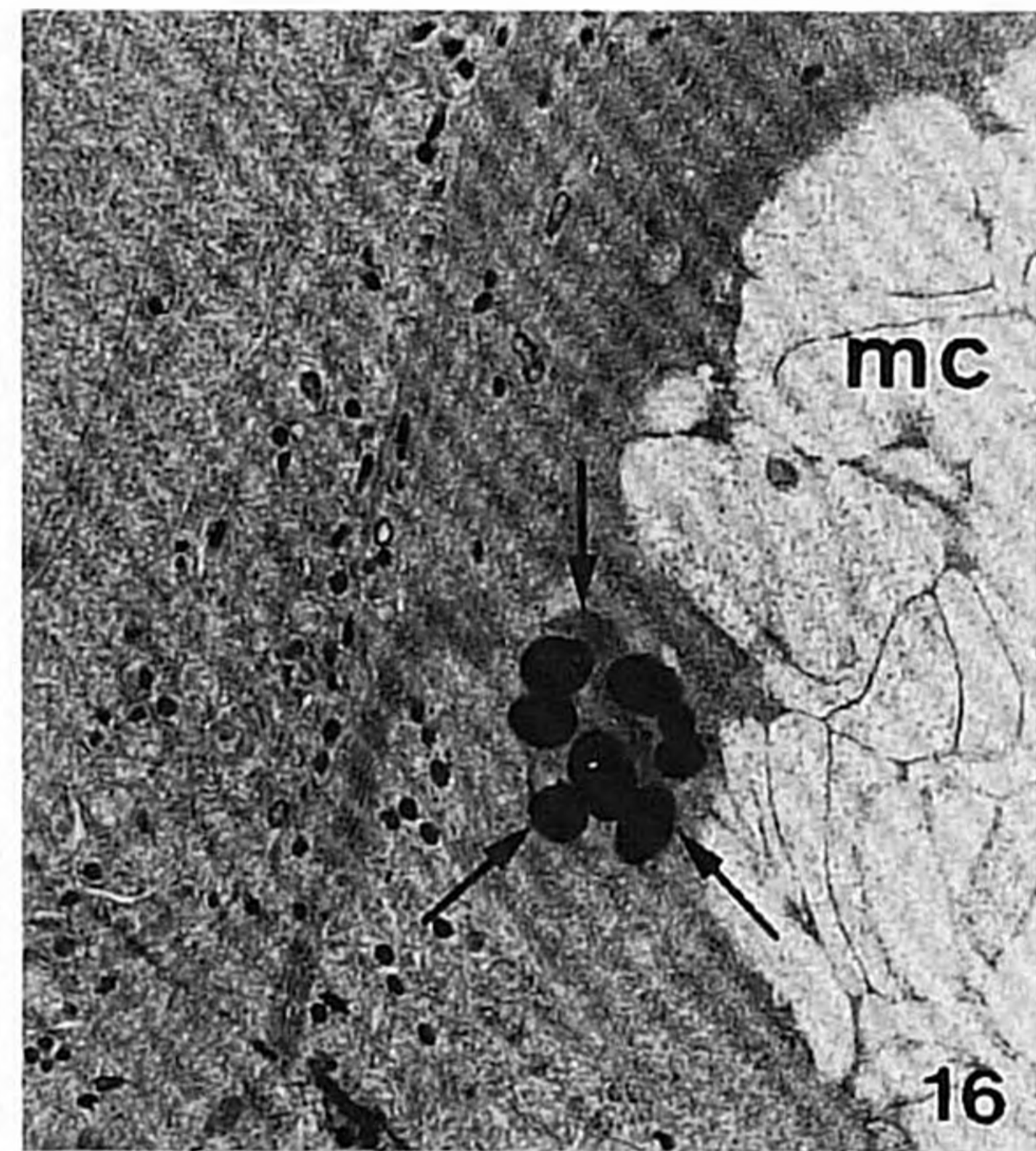
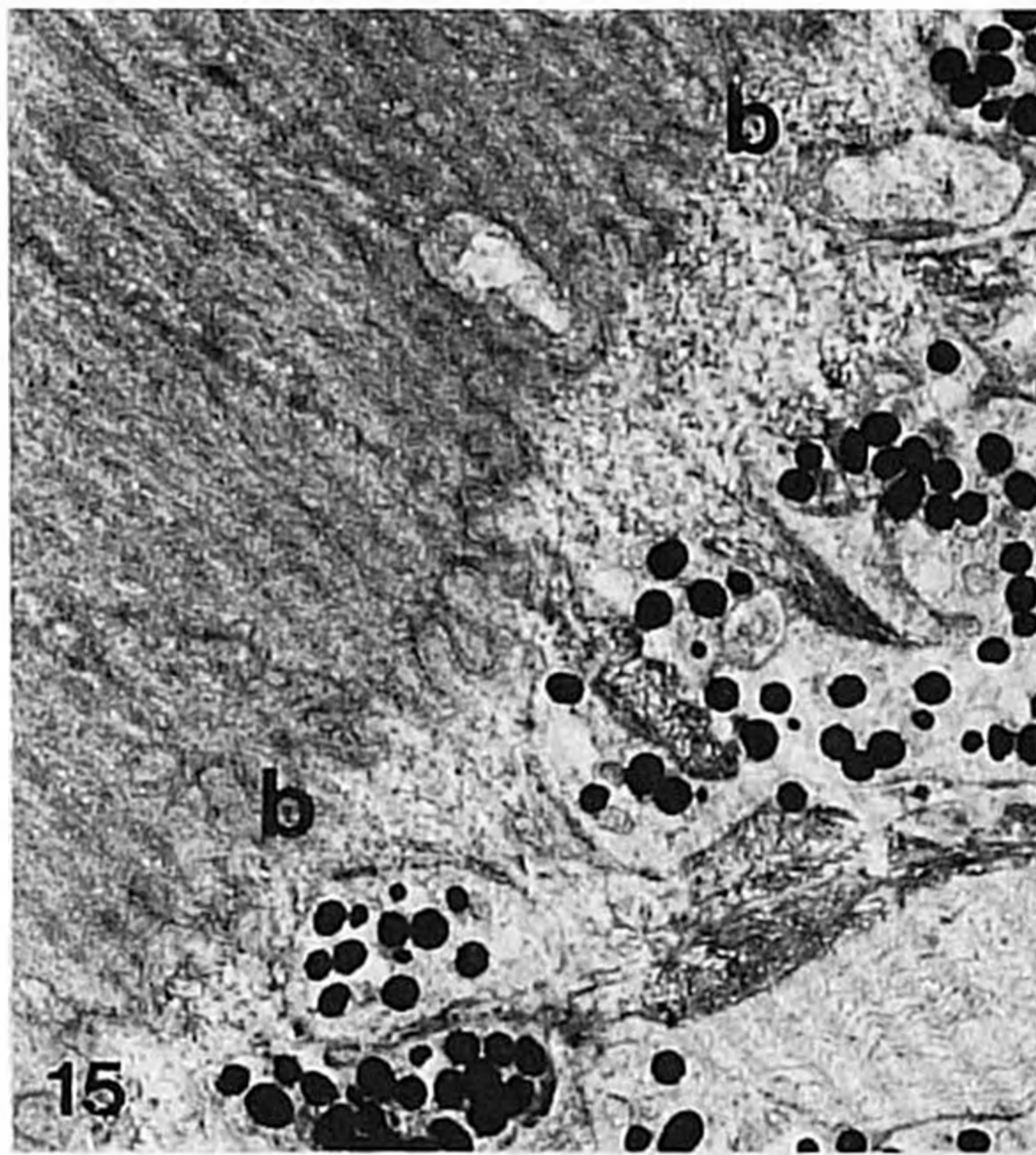
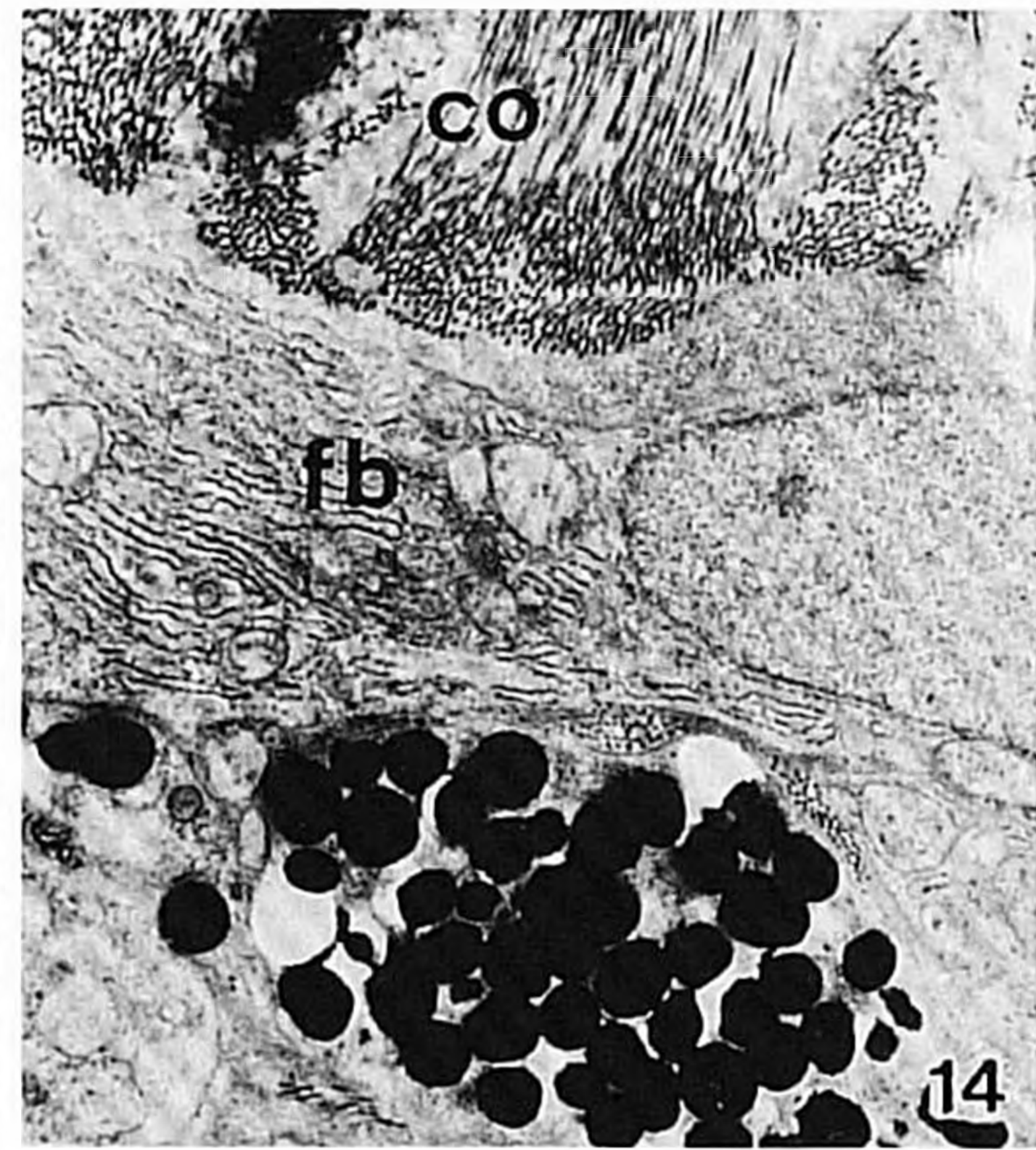
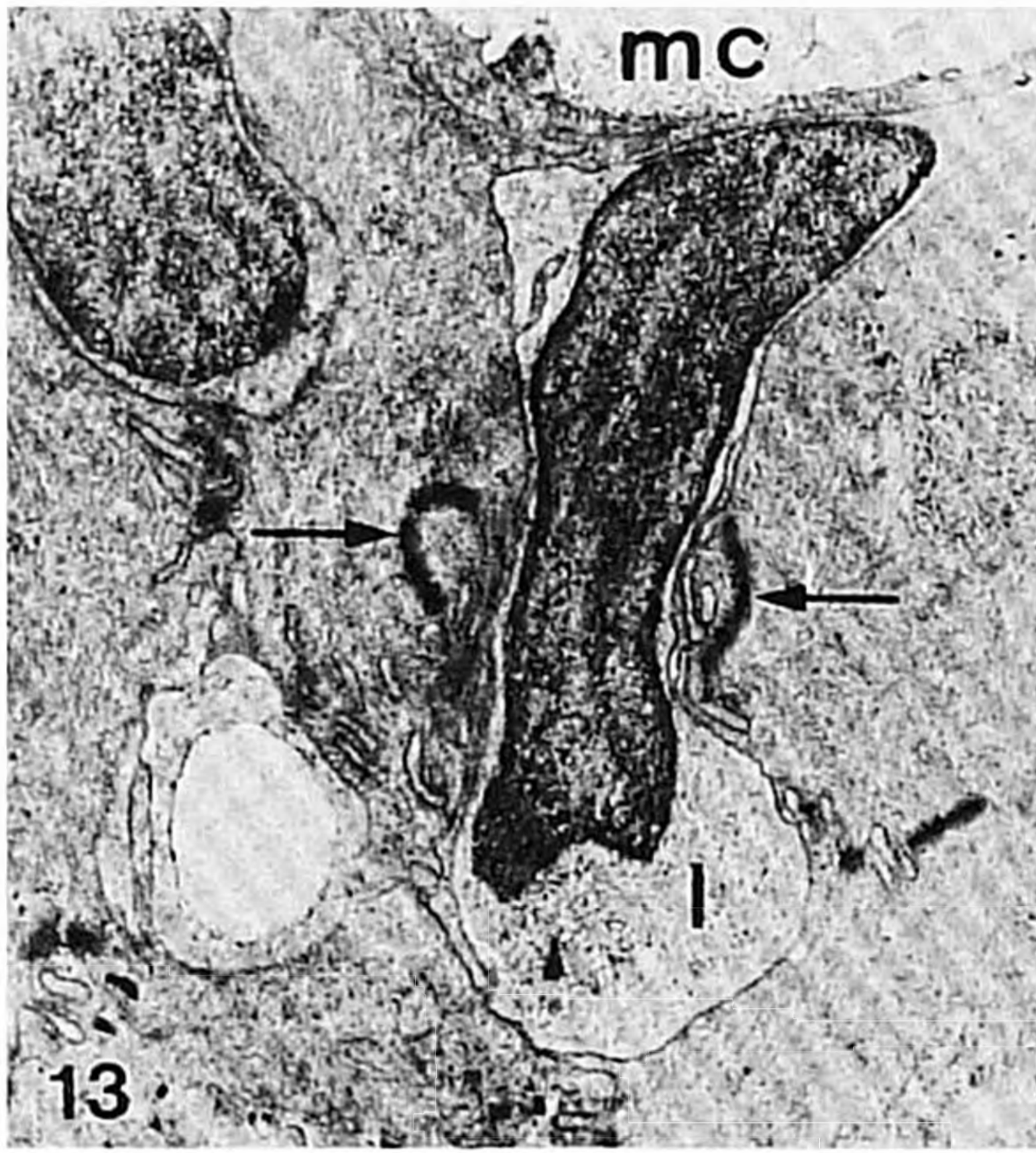
FIG. 8. Superficial mucous cell showing peroxidase activity; 14 days after temperature elevation; DAB reaction, otherwise unstained; $\times 10\ 800$.

FIG. 9. Mucous cell not showing peroxidase activity; control fish; DAB reaction, otherwise unstained; $\times 8400$.

FIG. 10. Part of a macrophage (m), probably shortly after penetrating the epidermis; b, basal lamina; 7 days after temperature elevation; $\times 21\ 200$.

FIG. 11. Macrophage with several phagosomes (arrows) in the epidermis; 7 days after temperature elevation; $\times 5200$.

FIG. 12. Vesicles (v) of macrophage showing peroxidase activity; 7 days after temperature elevation; DAB reaction, otherwise unstained; $\times 8400$.



Dermis

Fibroblasts. After 24 h and onwards, many fibroblasts were observed close to the basal lamina. Fibroblasts (Fig. 14) were very active: the cells contained abundant rER and small peripheral vesicles, and secreted large amounts of collagen. The outer dermis was filled up with dense aggregates of collagen fibres, which seemed to be arranged randomly.

Capillaries. The pinocytotic activity of the endothelial cells diminished within 3 h and was restored after 24 h. Later on (from day 4) the number of profiles of capillaries was about 25% higher than in the controls. However, migration of capillaries via elongation of endothelial cells, or true angiogenesis towards the basal lamina, were not found.

Melanocytes. Pigment granules were distributed in the cellular processes of the melanocytes rather than in the cell bodies after 3 h. From this time onwards, many of these processes were located close to, and perpendicular to, the basal lamina (Fig. 15). After 24 h the melanocyte processes penetrated the epidermis and were found in the deep layers of this tissue. Later on (at days 4 and 7) they were located over the entire epidermis (Fig. 16). After 7 days this process reversed, and the melanosomes became more and more restricted to the cell bodies of the melanocytes. After 14 days pigment granules were only scarcely seen in the epidermis. On days 7 and 14, several melanocyte processes in the epidermis were apoptotic. These apoptotic bodies were located in filament cells (Fig. 17) and in macrophages (Fig. 18).

Iridocytes. Twenty-four hours after temperature elevation, guanine platelets disappeared from the apical parts of the iridocytes and were found only in the lateral and inner sides of the iridocytes. The platelets were oriented in the direction of the nucleus rather than the basal lamina. After 4 days the cells contained many mitochondria and Golgi systems. Later iridocytes were seen only rarely.

IV. DISCUSSION

A 3-h period of moderate elevation in water temperature had pronounced and prolonged effects on the skin of fish. No recovery of this tissue was observed

FIG. 13. An elongated, probably migrating, lymphocyte (l) between epidermal cells; arrows, desmosomes between filament cells; mc, part of a mucous cell; 4 days after temperature elevation; $\times 8400$.

FIG. 14. An active fibroblast (fb) near densely packed collagen fibres (co) in the outer dermis, close to a melanocyte process; 24 h after temperature elevation; $\times 9500$.

FIG. 15. Processes of melanocytes close to the highly undulating basal lamina (b); 4 days after temperature elevation; $\times 5200$.

FIG. 16. Process of melanocyte (arrows) in the epidermis; mc, part of a mucous cell; 4 days after temperature elevation; $\times 8400$.

FIG. 17. Filament cell containing part of a cytoplasmic process of a melanocyte (arrows); 7 days after temperature elevation; $\times 4400$.

FIG. 18. An apoptotic remnant of a melanocyte (ap) within a macrophage in the epidermis, 7 days after temperature elevation; $\times 10\ 800$.

within the 14 days of the experiment. The effects of this temperature elevation were monitored ultrastructurally as well as cytochemically. Most of the observed cellular changes represent adaptive responses rather than degenerative effects. These responses have been found also in the skin of carp (*Cyprinus carpio* L.) exposed to other stressors, i.e. heavy metals, acid water, brackish water or wounding (Iger & Abraham, 1990; Iger, 1992), and therefore should be considered as general responses of the skin to stressors.

Epidermis

The decrease in epidermal thickness within 3 h after the start of the rise in temperature was probably caused by shedding of necrotic cells and enhanced secretion of mucous cells, and should be therefore, at least partially, considered as a direct negative effect of this treatment. Prolonged high temperatures have been shown to lead eventually to a thinner epidermis in the minnow, *Phoxinus phoxinus* L. (Bolognani-Fantin *et al.*, 1984). In our trout, the thickness of the epidermis was restored and even increased above control values. It was associated with enlargement of the intercellular spaces, a phenomenon more widely observed in stressed fish (Whitaker, 1986; Wendelaar Bonga & Lock, 1992), and with penetration of the epidermis by many leucocytes. The increase of intercellular spaces was associated with internalization of desmosomes, as has been described earlier for stressed fish (Whitaker, 1989). From day 4 onwards, the proliferation of filament cells, as reflected by the appearance of mitotic cells, almost certainly contributed to the increase in epidermal thickness. This phenomenon might be considered as an adaptive response. Apparently, from this time onwards cell proliferation surpassed cell shedding, although the incidence of apoptosis remained high. An increase in epidermal thickness has been reported earlier, e.g. in our studies on the effect of manure on carp or low water pH on the tilapia *Oreochromis mosambicus* Peters (Iger *et al.*, 1988; Wendelaar Bonga *et al.*, 1990). Thus, it may represent a general response to a stressor, and may be mediated via cortisol and/or prolactin (Wendelaar Bonga & Meis, 1981; Iger, 1992).

Filament cells. The most prominent effects of temperature elevation on the trout epidermis were the increased secretory activity of the upper layers of filament cells and the high incidence of apoptosis in the upper as well as in the inner layers of filament cells. The secretory vesicles of the upper layers contained peroxidase activity. In earlier studies we reported similar peroxidase activity in the upper layers of epidermal filament cells in carp exposed to manure-containing water and carp stressed by wounding (Iger, 1992). This activity has further been demonstrated in chlorine-exposed trout as well as in carp exposed to acidified water (Y. Iger & S. E. Wendelaar Bonga, unpublished). Thus, this phenomenon, absent in our control fish as well as in untreated *Blennius sanguinolentus* Pallas studied by Zaccone *et al.* (1985), appears to be a general response to stressors. In our carp studies we also found an increased incidence of apoptotic filament cells. However, apoptosis was mainly associated with the upper layers of filament cells, the same layers that showed increased secretory activity. A more elaborate cytoskeleton was also found, which indicates high cellular motility (Theriot & Mitchison, 1991). Increased apoptosis reflects

accelerated ageing of cells (Wyllie, 1981), and this may have been affected by increased cellular activity. In the present study, the apoptotic filament cells were present in both the upper and inner layers of epidermal filament cells. Normally the cells in the inner epidermal layers do not die before they reach the upper layer and have been transformed into pavement cells. The accelerated ageing and cell death of the inner filament cells may therefore be caused by the elevation in the temperature.

Fish are poikilothermic vertebrates and thus have a rapid thermal equilibration with the environment (Guderely & Blier, 1988). Whether or not the different sites of apoptotic and necrotic cells in the epidermis are related to differential effects of the temperature on different stages of cell maturity is not clear. Maturation and cell death from the gut epithelium in carp are also known to be temperature dependent (Lee & Cossins, 1988). In the epidermis of *Pimelodus maculatus* Bloch exposed to prolonged high but sublethal temperatures, degenerative changes (keratinization) of the epidermal pavement cells were found (Ferri, 1982).

Peroxidase activity was not only found in secretory vesicles of the upper layers of filament cells, but also throughout the cytoplasm of apoptotic filament cells. The presence of hydrolases has been described before for apoptotic cells in general (Bowen, 1981). Peroxidase activity in apoptotic cells was found earlier in our studies on carp skin (Iger, 1992; Y. Iger & S. E. Wendelaar Bonga, unpublished).

Mucous cells. Migration and secretion of mucous cells were stimulated by elevated temperature. The mucous cells were elongate rather than oval, possibly reflecting rapid migration. This elongation is consistent with the hypertrophy reported earlier for mucous cells of thermally stressed *Phoxinus phoxinus* (Bolagnani-Fantin *et al.*, 1984). We have previously reported that in carp kept in manured water massive mucus secretion depleted the epidermis of its mucous cells (Iger *et al.*, 1988). In our temperature-stressed trout the number of mucous cells declined only progressively. The appearance of newly-differentiated mucous cells after 4 days, at least partially compensated for losses of mucous cells caused by the intense secretion. This period (4 days) probably reflects the generation time for mucous cells under these conditions. Bullock *et al.* (1978) concluded that the generation time of the epidermal cells of the plaice, *Pleuronectes platessa* L., is in excess of 108 h. To our knowledge, changes in differentiation sites of mucous cells, i.e. the appearance of the newly differentiated cells close to the skin surface in stressed fish has not been published before.

The mucous cells of temperature-stressed trout contained mucosomes, which were smaller and of higher electron density than the controls. Electron dense trout mucosomes may contain a basic proteinaceous material absent in the electron transparent mucosomes (Blackstock & Pickering, 1980). Both changes in mucous composition and the smaller size of the mucosomes may reflect a higher viscosity of the mucus in stressed fish (Lewis, 1976; Whitear, 1986). This may explain the large amounts of mucus found at the skin surface in our experiment and on the surface of the branchial epithelium of thermally stressed rainbow trout (Jacobs *et al.*, 1981).

Under the influence of increased temperature the mucous cells of our trout showed a positive reaction to the peroxidase test. The peroxidase activity in these cells was mainly restricted to the mucosomes. This distribution is in contrast to our observations on acid-exposed carp, where peroxidase activity was found in particular in the rest of the cytoplasm, and only occasionally in mucosomes (Y. Iger & S. E. Wendelaar Bonga, unpublished). The peroxidase activity in mucosomes may represent part of the general stress response, as mentioned above.

Leucocytes. The thermal shock rapidly initiated extravasation of leucocytes and the penetration of these cells into the epidermis. We have reported massive infiltration of leucocytes into carp epidermis after contamination of water by manure (Iger *et al.*, 1988), lead (Iger, 1992), and after water acidification (Y. Iger & S. E. Wendelaar Bonga, submitted). However, while in stressed carp granulocytes are the first cells to appear in the epidermis (Iger *et al.*, 1988; Iger & Abraham, 1990), in the present experiment on trout lymphocytes and macrophages dominated, whereas granulocytes were found only rarely. Whether this different distribution is species-specific or stressor-specific remains to be elucidated.

Migration of leucocytes is also seen commonly in the gill epithelium in response to different water pollutants and low water pH (Mallatt, 1985; Karlsson-Norrgren *et al.*, 1985; Wendelaar Bonga & Lock, 1992). The migration of leucocytes into external epithelia may, at least partially, explain the leucopenia observed in stressed fish (Pickering & Pottinger, 1987; Maule & Schreck, 1990). We found peroxidase activity in vesicles and phagosomes of macrophages. Previously we reported this enzyme in basophilic granulocytes of carp (Iger & Abraham, 1990). It was also observed in neutrophils of *Ictalurus punctatus* Rafinesque (Cannon *et al.*, 1980). This enzyme was further detected in epidermal cells which contained phagosomes (Iger & Abraham, 1990). In fish, it may serve as an indicator for phagocytosis in leucocytes and epidermal cells. Interestingly, lymphocytes were found very close to mucous cells. The significance of this phenomenon is not clear.

Rodlet cells. Rodlet cells appeared between 4 and 7 days. In the epidermis of trout stressed by acid water their appearance was already noticed within 4 days (Y. Iger, P. H. M. Balm & S. E. Wendelaar Bonga, unpublished). In carp epidermis these cells were found 1 h after wounding (Iger & Abraham, 1990), and 3 h after contaminating the water with 1 mg l^{-1} cadmium (Iger, 1992). The origin of rodlet cells is not clear. They have been interpreted as parasites (Barber *et al.*, 1979), granular leucocytes (Cenini, 1984), or migrating secretory cells (Leino, 1982). Here we present further evidence for their appearance in the epidermis of stressed fish. Our tests reveal strong peroxidase activity in the rodlet cores of these cells, which supports previous suggestions that rodlet cores contain enzymes surrounded by a protective mantle (Leino, 1982).

Dermis

Fibroblasts. The synthesis and secretion of collagen by fibroblasts was greatly stimulated by temperature elevation. Increased secretory activity of fibroblasts

has been reported earlier for wounded carp (Iger & Abraham, 1990), carp exposed to heavy metals (Iger, 1992) or for *Solea solea* L. exposed to pollutants such as sewage sludge (Bucke *et al.*, 1983). In carp this activity was stimulated by ACTH, probably via cortisol (Iger, 1992), and thus may reflect a general response to stressors. It has been demonstrated (Whitear, 1990) that during rapid synthesis in regeneration of the barbels of a catfish, the newly synthesized collagen fibres had a random arrangement. We assume that in our experiment the random arrangement of the collagen fibres is also associated with their rapid synthesis, under the influence of the thermal stress.

Pigment cells. After temperature elevation, iridocytes exhibited changes in the orientation of guanine platelets. This was eventually followed by disappearance of these cells. We could not find apoptotic iridocytes, thus we assume that their disappearance was associated with migration to the inner dermal layers. In contrast with this, the melanocytes became activated, and their pigment granules distributed into the cytoplasmic processes of the cells. The latter were no longer restricted to the dermal area but also penetrated into the epidermis. We have previously observed this phenomena in stressed carp and suggest that this may represent a defence mechanism (Iger *et al.*, 1988), because intermediates of the melanin synthetic pathway have bactericidal capacities (Edelstein, 1971). Within 7 days the melanosomes were again found mainly in the cell bodies. Apoptotic remnants of their processes remained in the epidermis and were phagocytosed by macrophages and filament cells. The phagocytotic activity of the latter has been demonstrated previously (Iger & Abraham, 1990).

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