

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The version of this text has not yet been defined or was untraceable and may differ from the publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/14155>

Please be advised that this information was generated on 2014-11-11 and may be subject to change.

# Infection with low numbers of the sea louse *Lepeophtheirus salmonis* induces stress-related effects in postsmolt Atlantic salmon (*Salmo salar*)

D.T. Nolan, P. Reilly, and S.E. Wendelaar Bonga

**Abstract:** Infection of postsmolt Atlantic salmon (*Salmo salar*) with three, six, or 10 preadult and adult sea lice (*Lepeophtheirus salmonis*) per fish resulted in changes to epithelial structure and at sites in the skin and gill, distant from lice attachment and feeding. In the skin, increased apoptosis and necrosis occurred in the superficial epithelial cells and numbers of mucous cells decreased. In the gill, where no lice were found, uplifting of the epithelium, intercellular swelling, and infiltration by leukocytes occurred in filaments and lamellae. High cell turnover of chloride cells was associated with significantly elevated gill  $\text{Na}^+/\text{K}^+$ -ATPase activities. Serum chloride levels were elevated in the 3 and 6 lice/fish groups, and the serum Na to Cl ratio was lower in all parasitized groups at 5 days. The results indicate that infection with low numbers of the preadult and adult parasite induced changes characteristic of a stress response. In the low- and medium-infested groups, homeostatic recovery had occurred by 10 days, but recovery was incomplete in the highly infected group. Thus, 10 lice per fish, which is a low infestation level in nature, is stressful and creates a long period during which the overall condition of the skin and gill epithelia may render the fish susceptible to secondary infections.

**Résumé :** L'infection de post-smolts de saumon atlantique (*Salmo salar*) par trois, six ou 10 poux de poisson (*Lepeophtheirus salmonis*) pré-adultes et adultes par poisson a donné lieu à des changements dans la structure épithéliale et en des endroits sur la peau et les branchies éloignés du site de fixation et d'alimentation des poux. Sur la peau, on a observé un accroissement d'apoptose et de nécrose dans les cellules épithéliales superficielles, ainsi qu'une réduction du nombre de cellules muqueuses. Dans les branchies, où aucun pou n'était présent, on a observé un soulèvement de l'épithélium, un oedème intercellulaire et une infiltration de leucocytes dans les filaments et les lamelles. On a aussi observé un fort taux de renouvellement des cellules à chlorures, associé à des activités significativement élevées de la  $\text{Na}^+/\text{K}^+$ -ATPase branchiale. Les niveaux de chlorure sérique étaient élevés chez les poissons infectés par trois ou six poux, et le ratio Na à Cl dans le sérum était réduit chez tous les groupes parasités après 5 jours. Les résultats montrent que l'infection par un petit nombre de parasites pré-adultes et adultes induit des changements caractéristiques d'une réponse à un stress. Dans les groupes faiblement et moyennement infectés, la récupération homéostatique s'est produite après 10 jours, mais la récupération a été incomplète dans le groupe fortement infecté. Ainsi, un parasitisme de 10 poux par poisson, qui représente un faible niveau d'infestation dans la nature, constitue un stress important qui affecte la condition générale des épithéliums de la peau et des branchies durant une longue période et rend ainsi le poisson vulnérable à des infections secondaires.

[Traduit par la Rédaction]

## Introduction

The sea louse *Lepeophtheirus salmonis* is a caligid copepod that is an ectoparasite of salmonids in seawater. This species is an economically important parasite of sea-farmed salmonids that frequently causes serious disease throughout the Northern Hemisphere (Wootten et al. 1982; Pike 1989).

In wild stocks, this species is normally present in low numbers (i.e., from 10 parasites/fish upwards) on wild fish (Berland 1993; Holst et al. 1993); however, under certain conditions, epizootics can occur (Wootten et al. 1982; Tully et al. 1993; Johnson et al. 1996).

*Lepeophtheirus salmonis* has a direct life cycle (i.e., no intermediate host) consisting of 10 stages. These include two planktonic naupliar stages, one infectious copepodid stage, and seven on-host stages (four chalimus, two preadult, and one adult) (Wootten et al. 1982; Johnson and Albright 1991b). Numerous studies have investigated aspects of the life cycle, population dynamics, and ecology of *L. salmonis* in relation to environmental factors (Ritchie et al. 1993; Heuch et al. 1995; Johnson et al. 1996). However, remarkably fewer studies have examined the nature of the host-parasite relationship. Tissue responses at the site of attachment and feeding, as well as copepodid distribution and load

Received June 17, 1998. Accepted January 22, 1999.  
J14655

D.T. Nolan<sup>1</sup> and S.E. Wendelaar Bonga. Department of Animal Physiology, University of Nijmegen, 6500 GL Nijmegen, The Netherlands.

P. Reilly. Aquaculture Development Center, University College Cork, Lee Maltings, Cork, Ireland.

<sup>1</sup>Author to whom all correspondence should be addressed.  
e-mail: dnolan@sci.kun.nl

Fig. 1. (A) SEM of the head skin of control *S. salar* uninfected with *L. salmonis*. The individual pavement cells form a continuous sheet. Occasional pores are the site of mucous cell discharge (arrows). Scale bar = 100  $\mu\text{m}$ . (B) SEM of head skin of *S. salar* at site where *L. salmonis* was attached. The pavement cells are mechanically damaged by the activities of the parasite and much residual mucus is visible around the lesion (asterisks). Many pores from discharging mucous cells can be seen (arrows). Scale bar = 100  $\mu\text{m}$ . (C) SEM of head skin of *S. salar* at 1 DPI with *L. salmonis* (6 parasites/fish) taken from an area where there was no parasite attachment. Although there are no direct signs of parasite feeding activity, swollen pavement cells can be seen sloughing off (asterisks), many mucous pores are visible, and the integrity of the pavement cell layer is disrupted. Scale bar = 50  $\mu\text{m}$ . (D) SEM of a mucous cell lined duct from the head of control *S. salar*. Scale bar = 50  $\mu\text{m}$ . (E) Light microscopy view through the scaleless skin from the head of control *S. salar*. The stain is Alcian blue (pH 2.5) to demonstrate mucous cells in the epithelium (e) and the mucous cells lining a mucous cell duct (d). p, pigment cells; m, muscular tissue. Scale bar = 100  $\mu\text{m}$ .

on the body of the fish, have been investigated (Jones et al. 1990; Johnson and Albright 1992a; Jonsdottir et al. 1992). Physiological effects following experimental infection of postsmolt Atlantic salmon (*Salmo salar*) with large numbers of *L. salmonis* have only recently been reported (Grimnes and Jakobsen 1996), and these effects include disturbance of hydromineral balance and mortality attributed to the development of subadult and adult stages of the lice.

One key factor that can affect the health status of fish and increase susceptibility to disease is stress (Pickering et al. 1989; Wedemeyer 1997). While the topic of stress in fish is complex and many of the mechanisms involved are not yet fully understood, cortisol and the catecholamines are crucial in mediating the integrated stress response (see review by Wendelaar Bonga 1997). Salmonids intensively raised in aquaculture are known to have high stress levels, and these can be compounded by aquaculture practices such as transport, grading, and routine maintenance (Barton and Iwama 1991), increasing susceptibility to disease, including ectoparasites. Johnson and Albright (1992b) have shown that intraperitoneal administration of cortisol influenced the salmonid host-parasite interaction and increased susceptibility of coho salmon (*Oncorhynchus kisutch*) to experimental infection with *L. salmonis* by suppressing inflammatory response and epithelial hyperplasia in skin and gills.

The skin and gills of a fish are covered by complex epithelia comprising several layers of living cells that are continuous over the body surface (Iger et al. 1995; Nolan et al. 1998). They form the first barrier between the external and the internal environment and are protected with a chemically and functionally complex mucus coat that is discharged by specialized mucous cells in the epidermis (Shephard 1994). The teleostean epidermis is apparently influenced by a number of endocrine factors (Pottinger and Pickering 1985; Iger et al. 1995) and shows characteristic changes in fish exposed to stressors. It has been proposed that salmonids probably die from osmoregulatory problems caused by adult lice mechanically disrupting the skin (Wootton et al. 1982). Recently, osmoregulatory failure and mortality by developing adult stages have been demonstrated in Atlantic salmon (Grimnes and Jakobsen 1996), yet possible indirect effects of the parasite on the epithelia have not been examined. Such effects will become expressed at locations distant from the feeding and attachment areas and may also affect osmoregulation adversely.

The skin epithelium of fish responds strongly to stressors (Iger et al. 1995; Nolan et al. 1998) and offers good possibilities for evaluating indirect stress effects of ectoparasites on the host fish. Several responses, such as increased apoptosis of branchial chloride cells and the pavement cells of the

skin, have been shown to be under control of the stress hormone cortisol and will therefore also occur in areas not affected directly by the parasites, including the gills. These stress parameters also allow any relationship between the effects of sea lice and susceptibility to secondary infection to be established through examination of the skin and gill epithelia. The direct tissue damage caused by parasite grazing and indirect epithelial effects and immunosuppression offer opportunities for invasion by secondary pathogens if the overall integrity of the epithelia is compromised during, or remains compromised beyond, this period. These effects may disrupt hydromineral balance and can be assessed by measuring blood ion levels and the activity of key ionoregulatory  $\text{Na}^+/\text{K}^+$ -ATPases, such as in the gill.

The objective of the present study was to determine whether infection with low numbers of adult and preadult *L. salmonis* induced a stress response in the Atlantic salmon. We studied parameters in the skin and gill (ultrastructure through light and electron microscopy, as well as gill  $\text{Na}^+/\text{K}^+$ -ATPase) coupled with blood parameters (serum sodium, chloride, calcium, protein, and urea), to reveal the presence of a stress response.

## Materials and methods

### Experimental setup

Four groups of 40 seawater-adapted Atlantic salmon (200–250 g body weight) were placed in 400-L circular green plastic holding tanks at the Bantry research facility of the National Aquaculture Development Centre, Cork, Ireland. The tanks were provided with a constant supply of temperature-controlled seawater at 15°C under a 12 h light : 12 h dark photoperiod. After 3 weeks of acclimation, three groups were infected with 120, 240, or 400 subadult and adult *L. salmonis* of both sexes to achieve initial infection levels of 3, 6, and 10 lice/fish. Infection was carried out in the evening by pouring seawater containing the parasites into each tank. Seawater only was poured into the control tank. Seven fish from each group were sampled at 24 h, 5 days, and 10 days postinfection (1, 5, and 10 DPI).

### Sampling procedure

All fish were killed by concussion at the dorsal part of the head. Blood was collected by needle from the caudal blood vessels, allowed to clot for 24 h at 4°C, and centrifuged to obtain serum. Samples of skin from the rostral part of the head above the nostrils and small pieces from the second gill arches were fixed in Bouin's fixative for light microscopy and 3% glutaraldehyde buffered in sodium cacodylate (0.09 M, pH 7.3) with postfixation in 1% osmium tetroxide in the same buffer for electron microscopy (Iger et al. 1995). The samples were taken from areas where the parasites were not attached or where no indications of previous feeding activity could be observed. Another series of head skin samples were

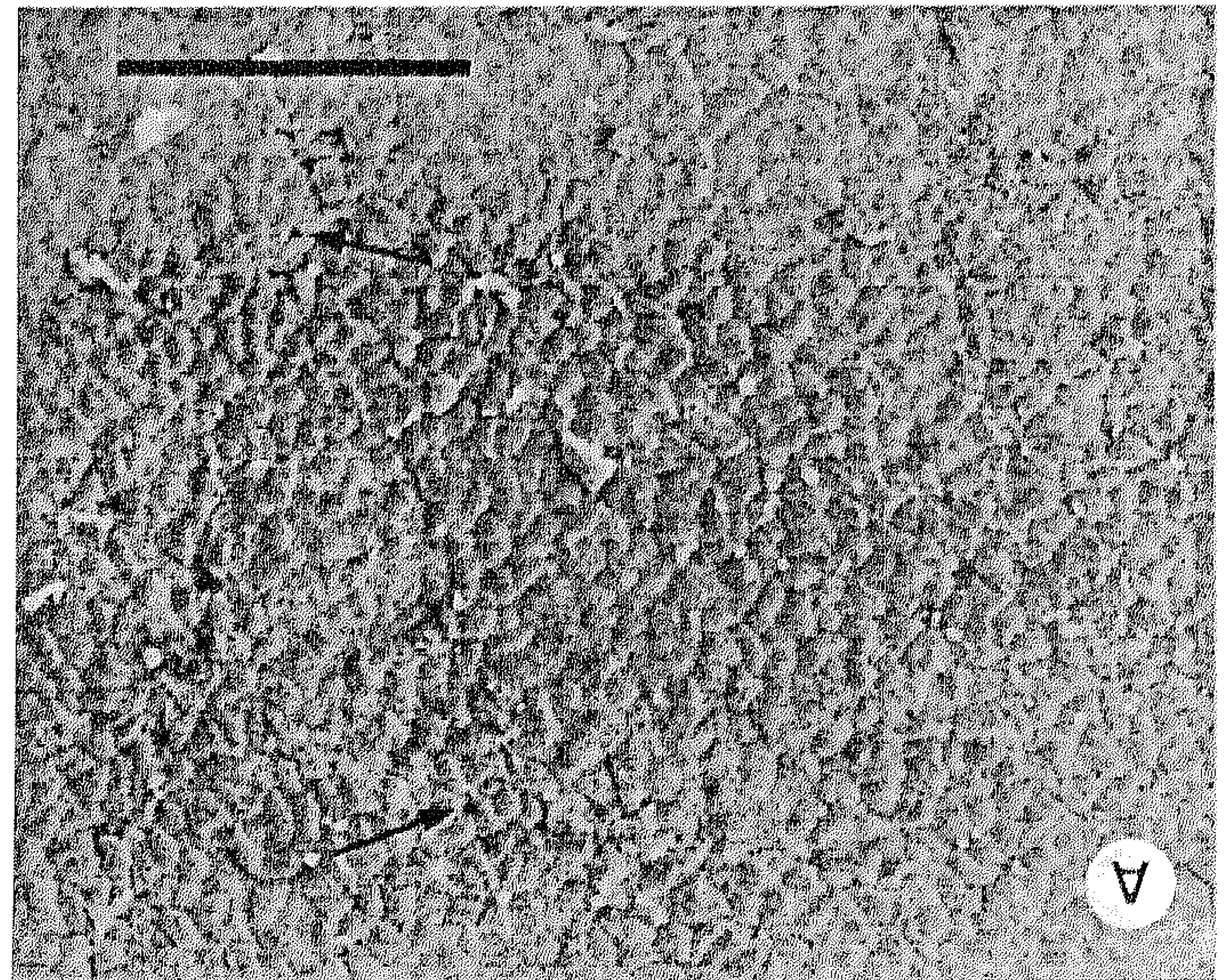
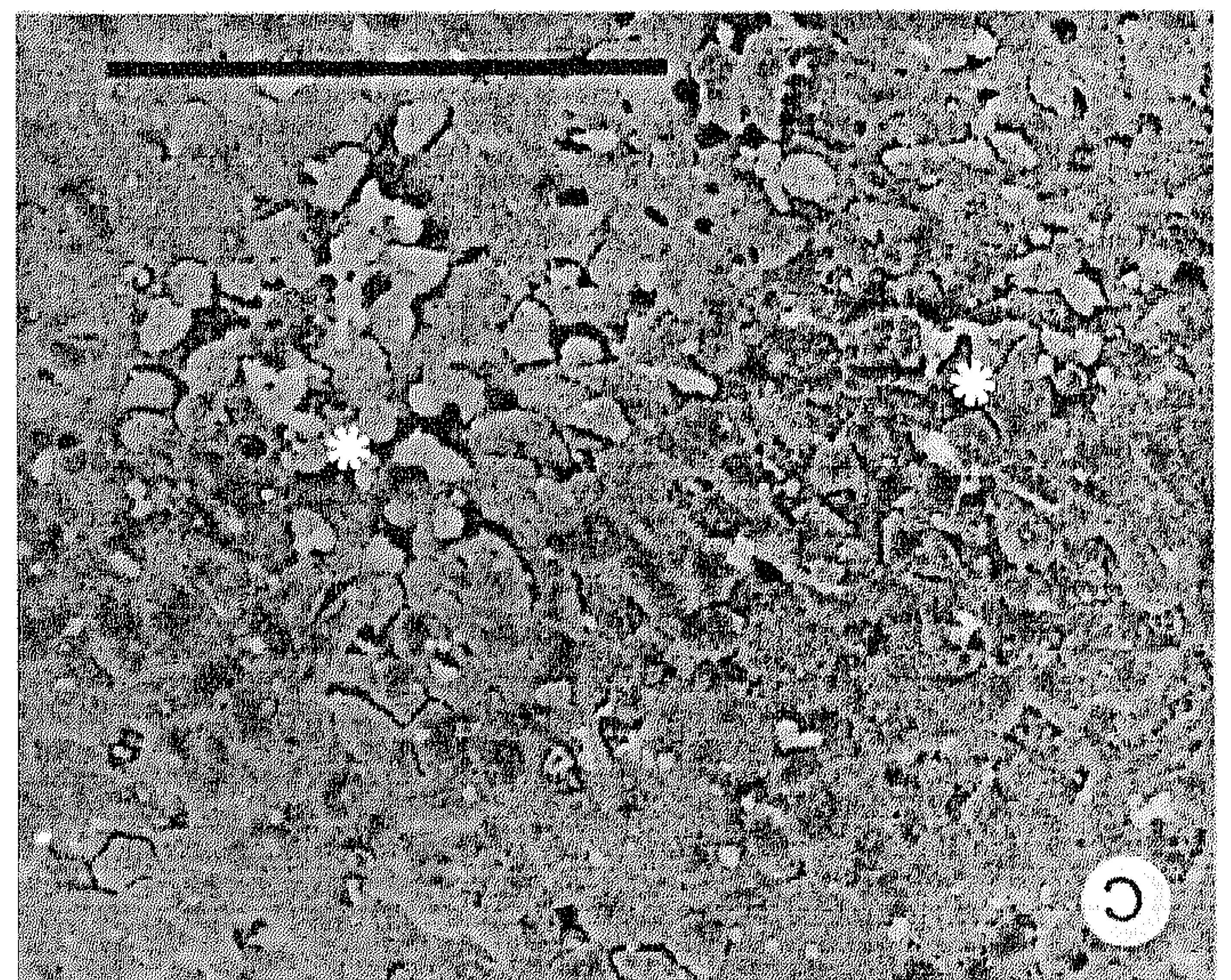
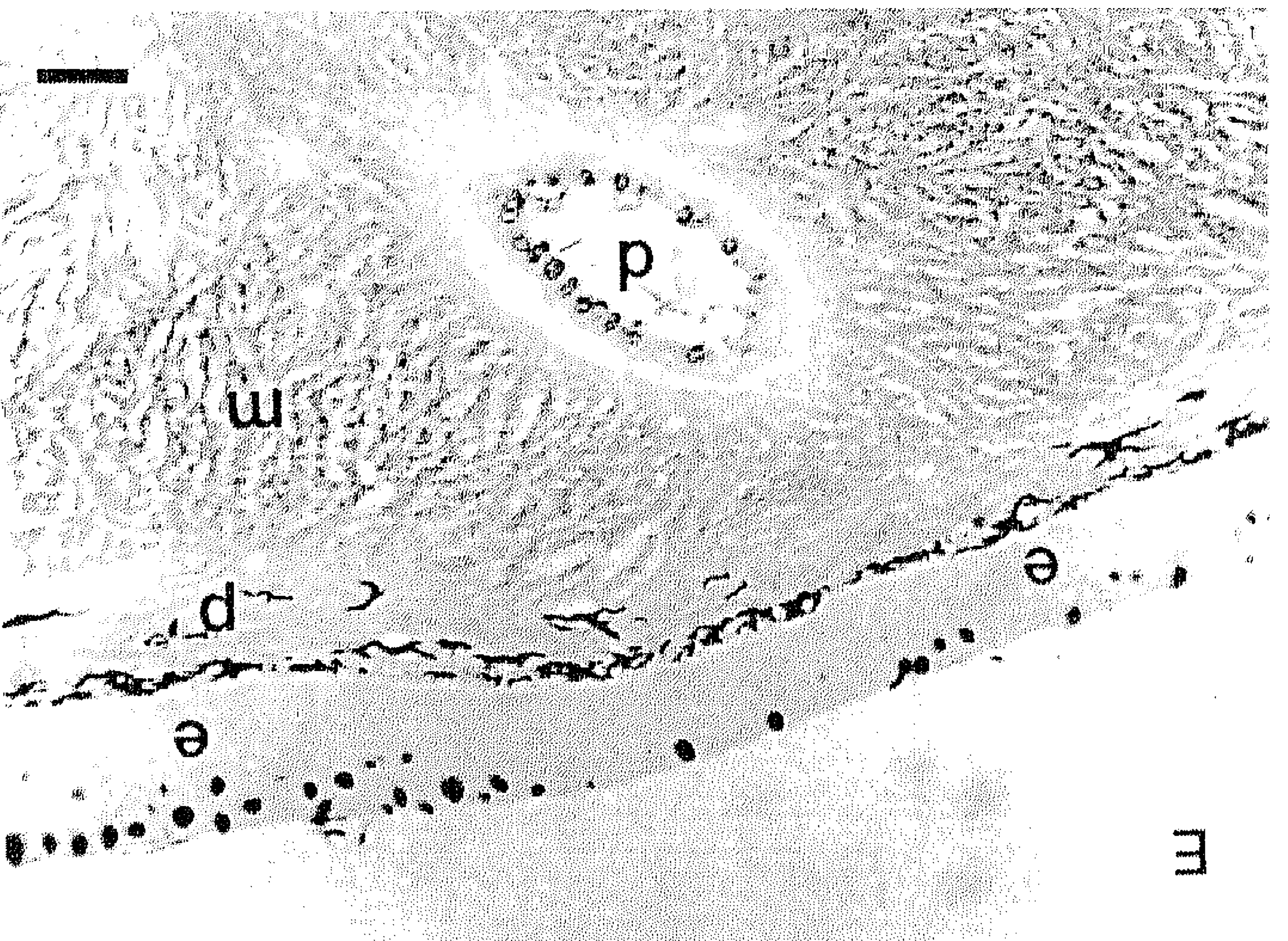
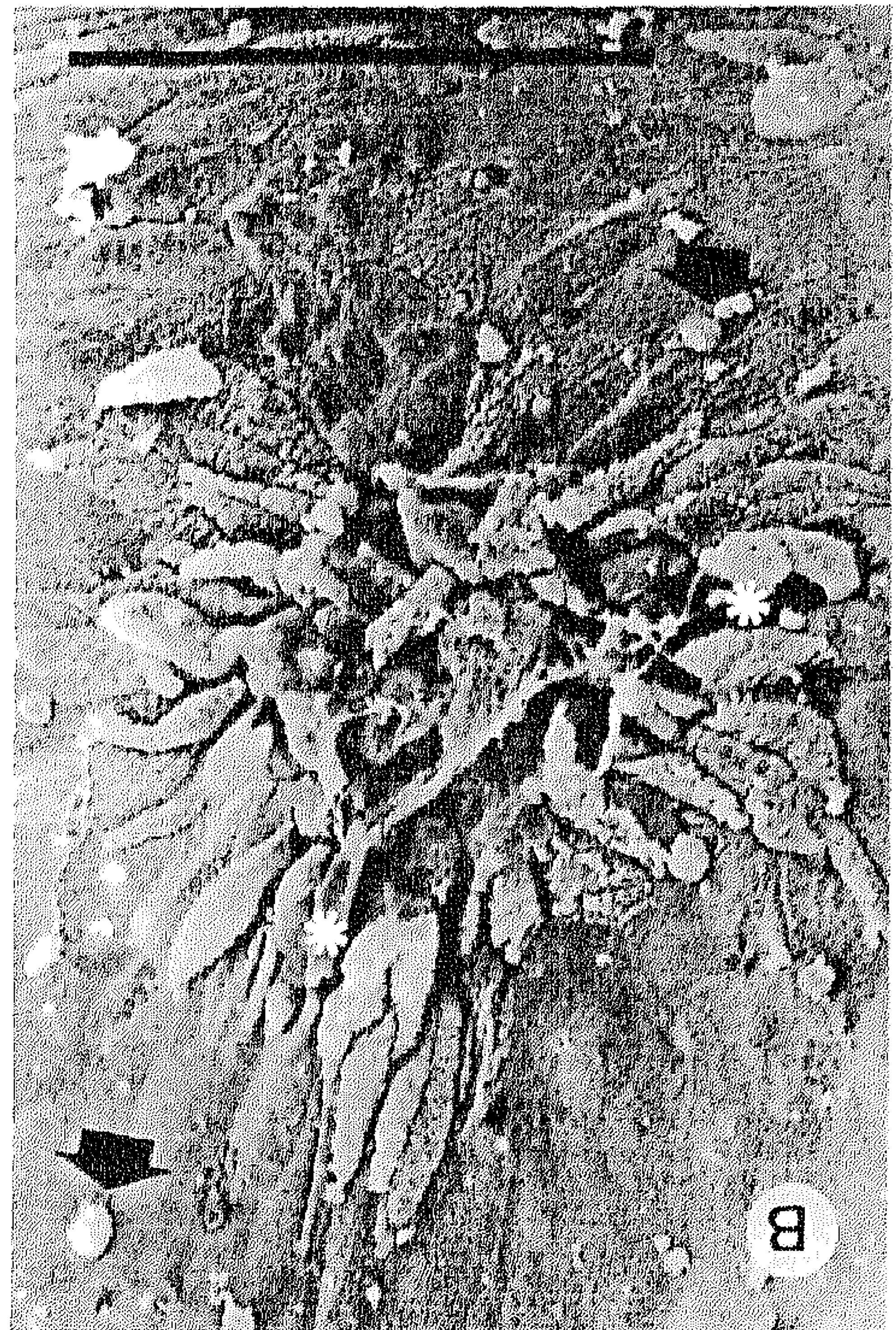
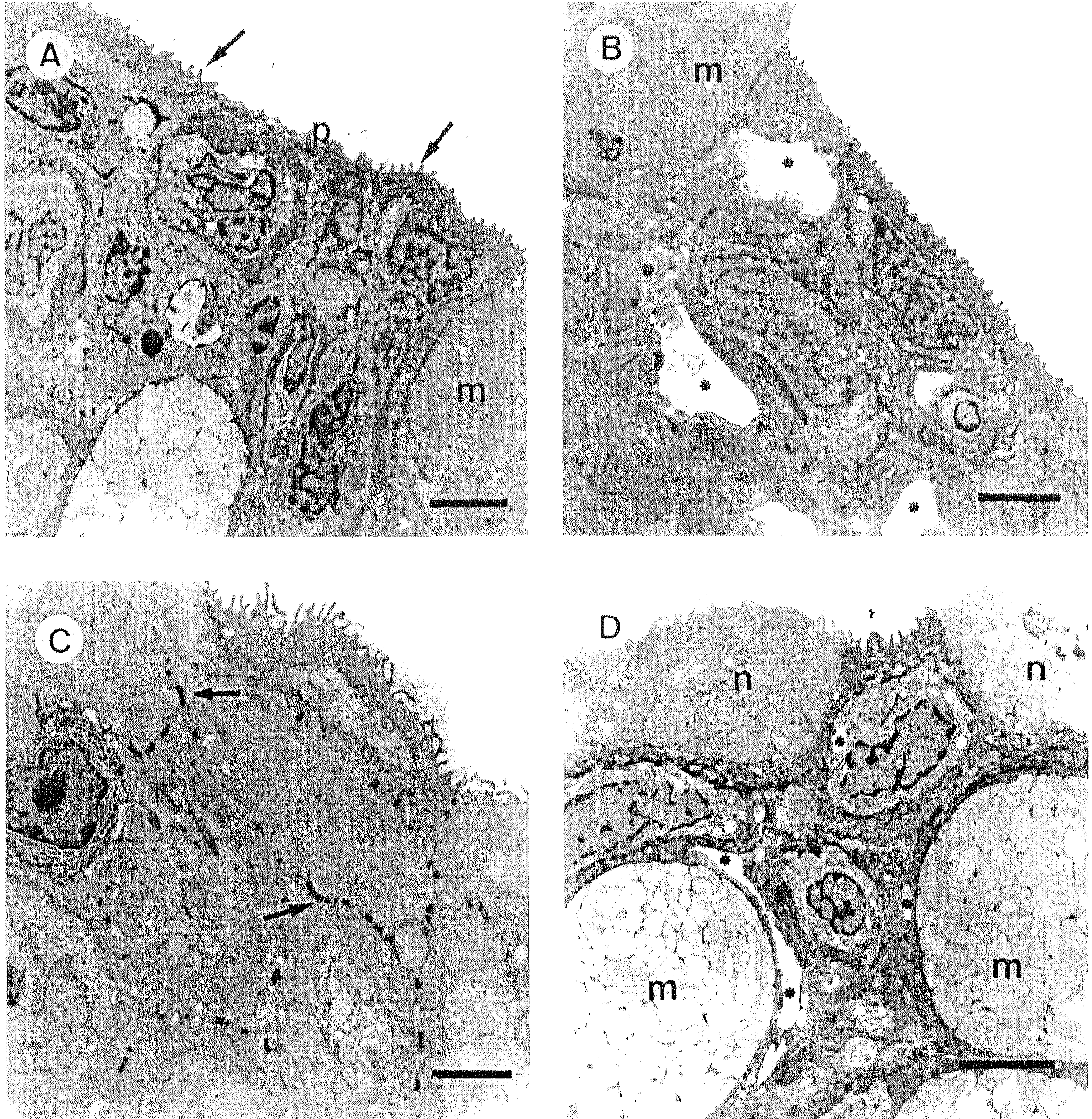


Fig. 2. (A) Upper epidermis of skin from control *S. salar*. The pavement cells (p), showing microridges at the upper cell limits (arrows), form the interface with the water. Little intercellular spacing occurs and the epithelium has good structural integrity. m, mucous cell. Scale bar = 5  $\mu$ m. (B) Upper epidermis of skin from *S. salar* at 1 DPI with *L. salmonis* (3 parasites/fish) taken from an area where there was no parasite attachment. Intercellular spaces are visible (asterisks) and the integrity of the epithelium is disrupted. A discharging mucous cell (m) can be seen. Scale bar = 5  $\mu$ m. (C) Upper epidermis of skin from *S. salar* at 5 DPI with 3 parasites/fish taken from an area where there was no parasite attachment. Intercellular spaces are not present and the integrity of the epithelium is good. Considerable numbers of electron-dense desmosomes (arrows) are seen at the borders of the filament cells. Scale bar = 5  $\mu$ m. (D) Upper epidermis of the skin from *S. salar* at 1 DPI with 6 parasites/fish taken from an area where there was no parasite attachment. Many necrotic pavement cells (n) can be seen and intercellular spaces are present (asterisks). Many predischARGE mature mucous cells (m) can be seen close to the surface. Scale bar = 5  $\mu$ m.



taken at locations where parasites were observed and fixed for electron microscopy in the same way. The remaining gills were dissected and the filaments trimmed from the gill arch, placed in 1 mL of ice-cold buffer (0.3 M sucrose, 20 mM Na<sub>2</sub>EDTA, 0.1 mM imidazole, pH 7.1 with HCl), and immediately frozen for measurement of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity.

### Sample processing

For quantification of mucous cells, Bouin-fixed skin samples from each fish were processed into paraffin blocks and 5- $\mu$ m sections were cut perpendicular to the plane of the epidermis. Replicate series of sections were stained with periodic acid - Schiff's (PAS) stain to identify total mucous cells and with the Alcian blue (pH 2.5) method to identify acidophilic mucous cells (Blackstock and Pickering 1982). Total numbers of mucous cells were counted in 10 independent 300- $\mu$ m views from each sample using a calibrated micrometer in the ocular lens of a light microscope, averaged for each fish, and the mean number of cells per millimetre of transverse epidermal section calculated. Gill samples from each fish were processed by sectioning perpendicular to the plane of the lamellae, stained with haematoxylin and eosin, and examined for general morphology by light microscopy.

For transmission electron microscopy (TEM), one replicate set of fixed skin and gill samples from four fish was stained for 2 h in 2% uranyl acetate, dehydrated through an ethanol series, and embedded in Spurr's resin. Ultrathin sections were cut by diamond knife, collected on 150-mesh copper grids, and contrasted with lead citrate before viewing in a Philips EM 300 transmission electron microscope at 40 kV. For skin, the overall ultrastructure was observed and attention was focused on cellular necrosis (characterized by nuclei with aggregations of chromatin, swelling of the cytoplasmic compartment, and loss of apical microridges) and apoptosis (characterized by progressive densification of the nucleus, organelles, and cytoplasm, leading to shrinkage and loss of contacts with surrounding cells), leukocyte infiltration, and epithelial integrity (evaluated by intercellular swelling and cell-cell contacts). For gills, the overall ultrastructure and the appearance of the respiratory lamellae were observed. In addition, the interlamellar chloride cell populations were studied and necrosis (defined above), apoptosis (characterized for chloride cells by progressive densification of the mitochondria, nucleus, and cytoplasm, as well as dilation of the tubular system), and leukocyte infiltration evaluated. From micrographs, the epithelia were evaluated and scored semiquantitatively against control using a graded evaluation system (-, +, ++, +++, +++++), representing parameter unaffected (-) to strongly affected (++++).

A second replicate set of skin and gill samples was examined by scanning electron microscopy (SEM). These samples were dehydrated through an ethanol series, critical point dried under liquid carbon dioxide, gold sputtered in a Balzers coating unit (CPD 020, Balzers, Switzerland), and viewed in a Jeol-JSM T 300 scanning electron microscope.

The sera were analyzed for sodium and chloride indirectly using ion-specific electrodes, calcium using *o*-cresolphthalein complexone, and urea using urease and glutamate dehydrogenase. Total protein was measured with the biuret reagent. All determinations were carried out on a Hitachi 747 automatic analyzer. The assay of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in the gill tissue was carried out on first homogenates according to the method of Flik et al. (1984).

### Statistical analysis

Data are expressed as means  $\pm$  SE of seven fish for each treatment at each timepoint. Differences between control groups over experimental time were assessed by ANOVA of log-transformed data (except for the Na to Cl ratio (Na:Cl)). Data sets for each timepoint (except Na:Cl) were log transformed, analyzed by

ANOVA, and significance between treatments assessed using the Bonferroni multiple comparisons test. For Na:Cl, data were tested using the Mann-Whitney *U* nonparametric test. Statistical significance was accepted at  $P < 0.05$ .

## Results

### Skin

Ultrastructure of head skin from control salmon by SEM showed the surface to be a continuous sheet of pavement cells (Fig. 1A). Examination by TEM at 1, 5, and 10 DPI revealed several distinct cell populations (Fig. 2A). The most numerous cell type is the filament cell, which is characterized by numbers of perinuclear microfilaments. The uppermost layer of cells is differentiated into specialized pavement cells that have apical microridges (Fig. 2A). Cell-cell contacts between pavement cells and filament cells are maintained by tight junctions and desmosomes. Little intercellular spacing occurs in the epithelium (Fig. 2A). Mucous cells differentiate in the middle layers of the epidermis and migrate through to the upper layer where they discharge their contents onto the body surface. Low numbers of leukocytes are also present (Table 1).

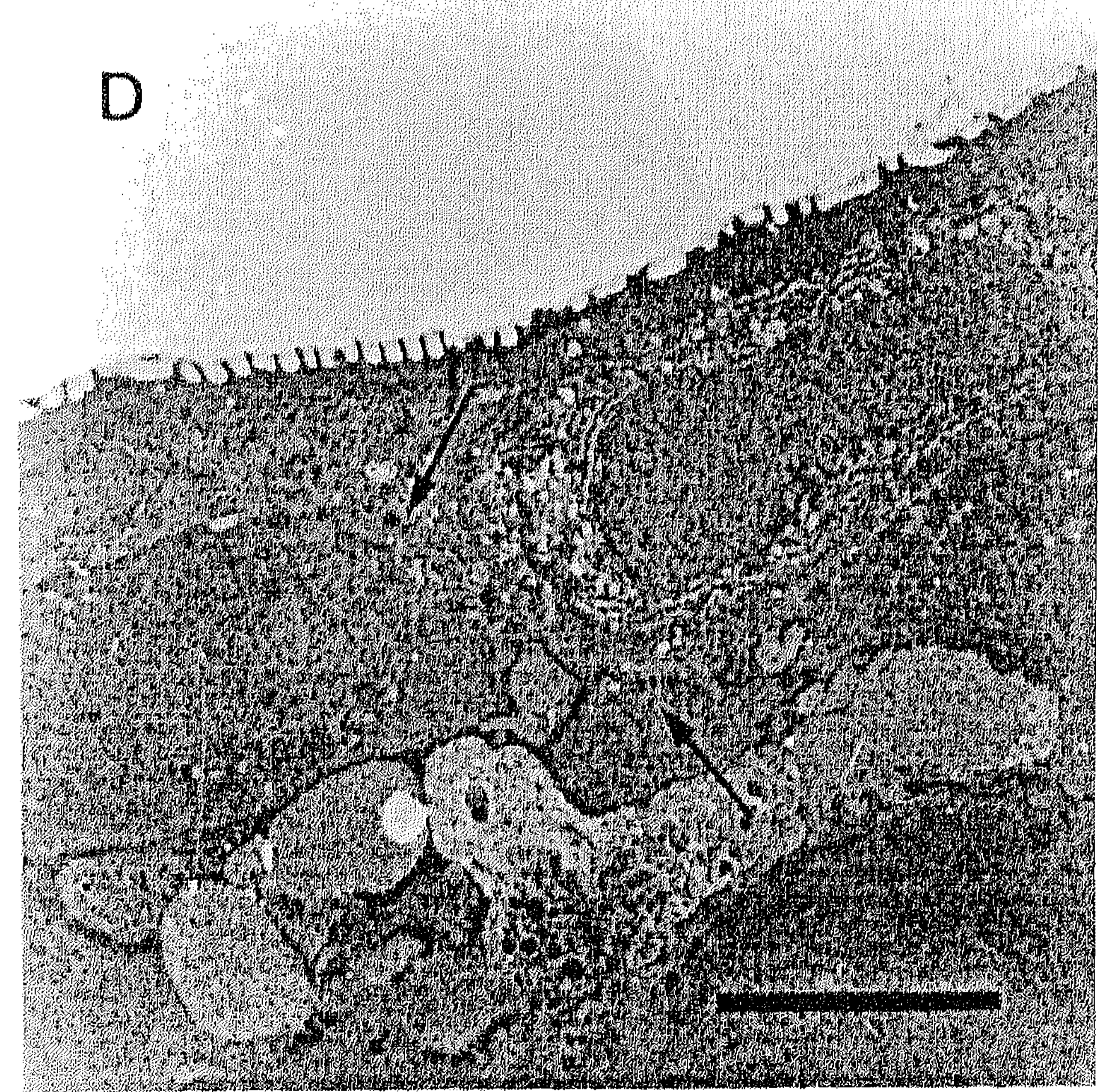
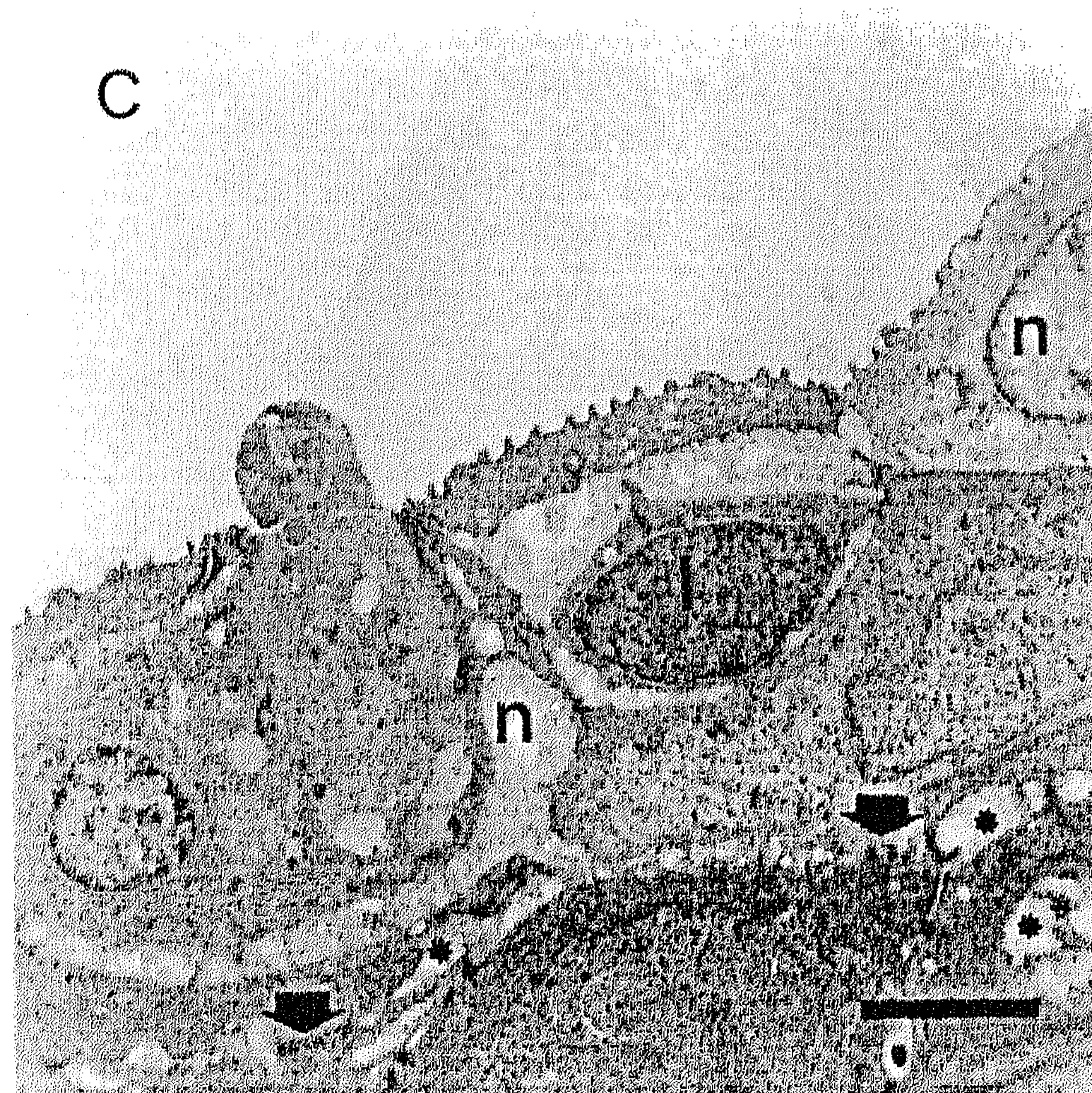
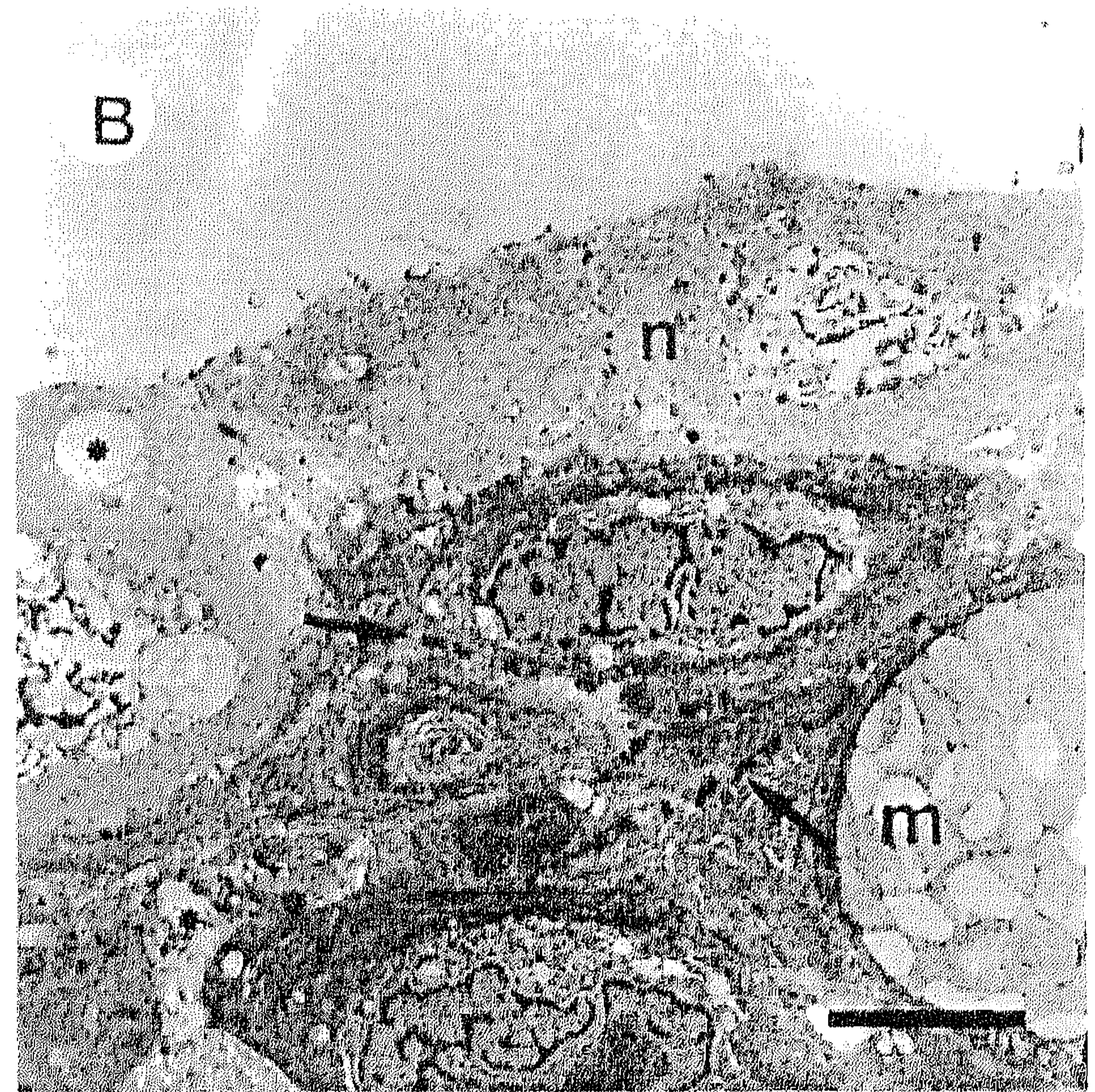
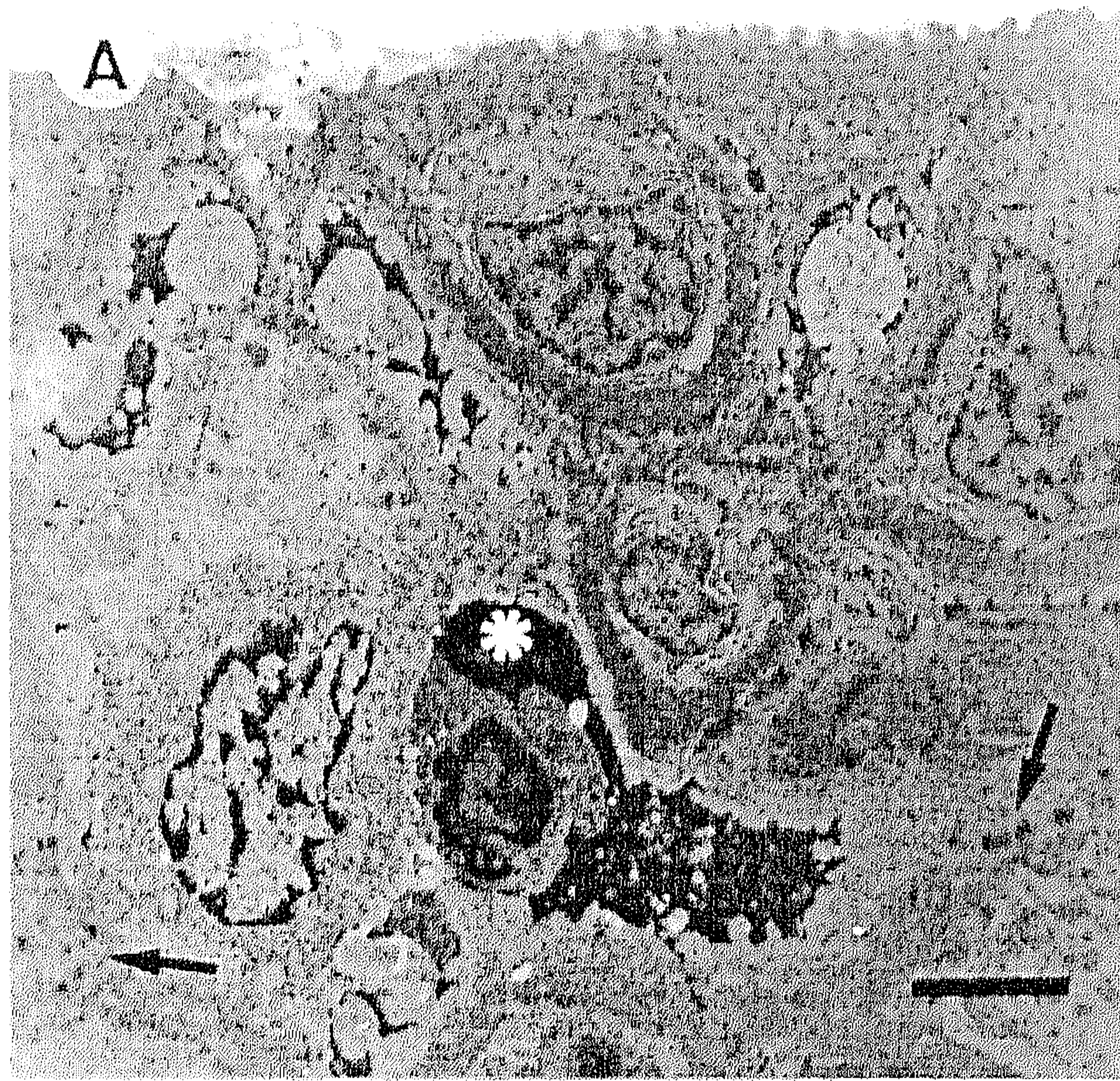
The skin ultrastructure from the head of parasitized fish by SEM in areas distant from the feeding sites of the parasites showed marked epithelial changes (Fig. 1C) compared with control fish (Fig. 1A), including swelling and shedding of the pavement cells (Fig. 1C). At sites of copepod feeding, gross damage was visible as lesions of disrupted and swollen pavement cells (Fig. 1B). In all fish examined, lines of ducts were observed in the scaleless region of the head (Fig. 1D) and, when examined by light microscopy, these ducts were richly lined with mucous cells (Fig. 1E).

There was no statistical difference between the counts of cells stained with PAS or with the Alcian blue (pH 2.5) method at any sample point; therefore the total numbers of cells counted from PAS staining are given. There were significantly fewer mucous cells in the nonfeeding areas of the epidermis of infected fish when compared with controls at 1, 5, and 10 DPI (see Table 3).

There were strong effects in the epidermis of fish infected with 3 parasites/fish at nonfeeding sites at 1 DPI (Fig. 2B). Necrotic pavement cells were commonly observed. Increased intercellular spaces and increased incidence of heavily stained desmosomes were typical (Table 1; Fig. 2B). Leukocytes were seen throughout the epidermis and within the intercellular spaces. At 5 DPI, heavily stained desmosomes were very abundant (Fig. 2C). Apoptotic cells were occasionally encountered, but necrosis in the pavement cell layer was reduced. At 10 DPI, the condition of the skin of these fish was slightly recovered compared with 5 DPI (Table 1).

At 1 DPI, the epidermal condition of fish infected with 6 parasites/fish was similar to that of the group infected with 3 parasites/fish (Table 1; Fig. 2D). However, necrosis in the pavement cell layer was more severe, disruption of the underlying cell layers was greater and many predischarge mucous cells were located in the upper epidermis (Fig. 2D). At 5 DPI, there was an increase in the staining intensity of the desmosomes and the intercellular spacing was reduced (Table 1; Fig. 3A). Various stages of filament cell apoptosis

Fig. 3. (A) Upper epidermis of skin from *S. salar* at 5 DPI with *L. salmonis* (6 parasites/fish) taken from an area where there was no parasite attachment. A macrophage (asterisk) engulfs an apoptotic filament cell. Intercellular spaces are absent and numbers of electron-dense desmosomes (arrows) are visible at the filament cell borders. Scale bar = 5  $\mu$ m. (B) Upper epidermis of skin from *S. salar* at 1 DPI with 10 parasites/fish taken from an area where there was no parasite attachment. Many necrotic pavement cells (n) can be seen and intercellular spaces are present (asterisks). Numbers of electron-dense desmosomes (arrows) are visible at the filament cell borders and a mature predischarge mucous cell (m) can be seen. Scale bar = 5  $\mu$ m. (C) Upper epidermis of skin from *S. salar* at 5 DPI with 10 parasites/fish taken from an area where there was no parasite attachment. Necrotic pavement cells and intercellular spaces (asterisks) still occur. A lymphocyte (l) is present in the pavement cell layer. Numbers of electron-dense desmosomes are visible and a discharging immature mucous cell can be seen. Scale bar = 5  $\mu$ m. (D) Upper epidermis of skin from *S. salar* at 10 DPI with 10 parasites/fish taken from an area where there was no parasite attachment. The integrity of the epithelium is restored in this fish. Few electron-dense desmosomes (arrows) are visible. Scale bar = 5  $\mu$ m.



**Table 1.** Semiquantitative evaluation of the epidermal responses of head skin of postsmolt *S. salar* to three infection levels of preadult and adult *L. salmonis*.

| Skin parameter             | Control | 3 lice/<br>fish | 6 lice/<br>fish | 10 lice/<br>fish |
|----------------------------|---------|-----------------|-----------------|------------------|
| <b>1 and 5 DPI</b>         |         |                 |                 |                  |
| Pavement cell necrosis     | -       | ++              | +++             | ++++             |
| Heavily stained desmosomes | -       | +++             | +++             | ++++             |
| Intercellular space        | -       | +++             | +++             | ++++             |
| Mucous cell discharge      | -       | +++             | +++             | +++              |
| Apoptotic cells            | -       | ++              | +++             | +++              |
| Leukocyte infiltration     | -       | ++              | ++              | ++               |
| <b>10 DPI</b>              |         |                 |                 |                  |
| Pavement cell necrosis     | -       | -               | ++              | +++/+            |
| Heavily stained desmosomes | -       | ++              | ++              | +++              |
| Intercellular spaces       | -       | +               | ++++            | +++/+            |
| Mucous cell discharge      | -       | ++              | ++              | ++               |
| Apoptotic cells            | -       | +               | ++              | ++               |
| Leukocyte infiltration     | -       | ++              | ++              | +++/+            |

Note: The samples are taken away from areas of parasite attachment and feeding. - = unaffected to ++++ = strongly affected.

were observed (Fig. 3A). At 10 DPI, the overall situation was comparable with 5 DPI, with the exception that intercellular spaces were more extensive and necrosis of the pavement cell layer was reduced (Table 1).

The 10 parasites/fish group showed the strongest epidermal effects at all samplings (Table 1). At 1 DPI, there was extensive necrosis of the pavement cell layer, heavily stained desmosomes, and intercellular spaces present (Fig. 3B). At 5 DPI, immature mucous cells could be seen discharging at the surface, and apoptosis and necrosis of the pavement cell were common (Fig. 3C). At 10 DPI, there was some variation in the condition of the epidermis of individual fish. Some individuals still had extensive pavement cell necrosis and intercellular swelling, while others showed good recovery with a well-differentiated and active pavement cell layer and good epithelial integrity (Fig. 3D).

### Gill

The external gill structure of uninfected salmon inspected by SEM and TEM was typical for salmonids that we have sampled from aquaculture conditions (personal observation). The filaments had leaflike lamellae arranged alternately on both sides (Fig. 4A). The respiratory epithelium was one cell thick and leukocytes were commonly in the intercellular spaces (Fig. 4B). In the interlamellar areas, populations of mitochondria-rich chloride cells and mucous cells were present (Fig. 4E). Apoptotic chloride cells were seen occasionally, whereas apoptosis and necrosis were uncommon in other cell types (Table 2). The epithelium of the filaments, with the exception of the interlamellar areas, resembled that of the skin, being composed of filament cells, mucous cells, leukocytes, and an apical pavement cell layer with micro-ridges.

In lice-infected groups, SEM observations showed that the lamellae were swollen and wrinkled (Table 2; Fig. 4C). In TEM observations, the lamellar respiratory epithelium was

**Table 2.** Semiquantitative evaluation of the responses of the gill of postsmolt *S. salar* to three infection levels of preadult and adult *L. salmonis*.

| Gill parameter                               | Control | 3 lice<br>/fish | 6 lice/<br>fish | 10 lice/<br>fish |
|--|---------|-----------------|-----------------|------------------|
| <b>1 and 5 DPI</b>                           |         |                 |                 |                  |
| Lamellar swelling                            | -       | +++             | +++             | +++              |
| Chloride cells: dilated tubular system       | -       | +++             | +++             | ++++             |
| Chloride cells: apoptosis                    | -       | +++             | +++             | ++++             |
| Gill Na <sup>+</sup> /K <sup>+</sup> -ATPase | -       | +++             | +++             | +++              |
| Leukocytes                                   | -       | ++              | +++             | +++              |
| <b>10 DPI</b>                                |         |                 |                 |                  |
| Lamellar swelling                            | -       | -               | +               | +++/+            |
| Chloride cells: dilated tubular system       | -       | +               | +               | +++/+            |
| Chloride cells: apoptosis                    | -       | +               | +               | +++/+            |
| Gill Na <sup>+</sup> /K <sup>+</sup> -ATPase | -       | +               | +               | +++              |
| Leukocytes                                   | -       | ++              | ++              | +++/+            |

Note: - = unaffected to ++++ = strongly affected.

uplifted and detached from the endothelium of the central blood sinus in many places (Fig. 4D). Both mucous and chloride cells were found on the filaments, as well as on the lamellae. In lice-infected fish, the chloride cells showed an extensive dilated tubular system (Fig. 4F) not observed in control fish (Fig. 4E; Table 2).

At 1 DPI, the lamellae of fish infected with 3 parasites/fish were swollen (Table 2). Mucus discharge was stimulated in the filament, and apoptotic chloride cells were frequently seen in the interlamellar area (Table 2). Apoptotic chloride cells in the interlamellar lost apical contact with the water and were apparently replaced by neighboring new chloride cells. At 5 DPI, apoptotic bodies, the last stage of apoptotic chloride cells, could be observed (Fig. 5A) in addition to other apoptotic stages (Fig. 5B).

The lamellae sampled from fish infected with 6 parasites/fish at 1 DPI were comparable with those of the 3 lice/fish group (Table 2). Mucus discharge was stimulated in the filament epithelium, and intercellular spaces occurred with heavy leukocyte infiltration (Fig. 5C). The chloride cell population and pavement cells in the interlamellar area were also strongly affected, as desmosomes were reduced and apoptotic cells atrophied (Fig. 5D). At 10 DPI, the gill structure in this group was comparable with the 3 lice/fish group, with the exception of lamellar swelling, which still occurred (Table 2).

The group infected with 10 parasites/fish showed the strongest lamellar effects (Table 2). Cellular shrinkage and swelling of the lamellar respiratory epithelium were severe (Fig. 5E). At 1 DPI, newly differentiated cells could be seen at the base of the lamella (Fig. 5F). At 5 DPI, many macrophages containing cellular debris (composed mainly of apoptotic bodies) were observed. The high turnover of chloride cells was continuous and observed at 5 and 10 DPI in most fish examined, although there was some variation between individuals at 10 DPI (Table 2).

### Blood parameters

Compared with control values, serum sodium was signifi-



**Fig. 4.** (A) Representative SEM of gill from control *S. salar* uninfected with *L. salmonis*. The filament (f) and lamellae (arrows) are clearly seen. Scale bar = 100  $\mu\text{m}$ . (B) TEM view of the lamellae of control *S. salar* gills. The blood space (b) and the water (w) are separated by a thin respiratory epithelium that is one cell layer thick. Leukocytes (l) can be seen in the intercellular spaces of this epithelium. Scale bar = 5  $\mu\text{m}$ . (C) SEM view of the gills of *S. salar* at 1 DPI with 10 lice/fish. The lamellae are wrinkled and swollen (arrows). Scale bar = 50  $\mu\text{m}$ . (D) Lamellae of gill from *S. salar* at 1 DPI with 6 lice/fish. The distance between the blood space and the water is increased by epithelial uplifting. Numbers of leukocytes can be seen in the epithelial intercellular spaces (asterisks). Mucous cells (m) and chloride cells (lc) are present on the lamella. Scale bar = 5  $\mu\text{m}$ . (E) Representative TEM of the interlamellar region of gill from control *S. salar* uninfected with lice. Many mitochondria-rich chloride cells (c) are present here, as well as mucous cells (m). There are few enlarged intercellular spaces, epithelial integrity is good, and some leukocytes (arrows) and mast cells (mc) can be seen. Scale bar = 10  $\mu\text{m}$ . (F) TEM view of the interlamellar region of gill from *S. salar* at 1 DPI with 10 lice/fish. The tubular systems of the chloride cells are dilated (arrows) and apoptotic chloride cells (a) are present. Scale bar = 5  $\mu\text{m}$ .

cantly lower in the 10 lice/fish group sampled at 1 DPI and serum chloride significantly higher at 5 DPI in both the 3 and 6 lice/fish groups (Table 3). Although the serum sodium and chloride levels did not differ significantly in any other group at any other time, the serum Na:Cl in all lice-infected groups was significantly reduced at 5 DPI (Table 3). Serum total calcium levels, protein, and urea were significantly elevated in the 10 lice/fish group sampled at 1 DPI (Table 4) but not at any other time point or in any of the other groups.

#### Gill $\text{Na}^+/\text{K}^+$ -ATPase

The gill  $\text{Na}^+/\text{K}^+$ -ATPase activity was significantly higher in all infected fish at 5 DPI and remained elevated in the 10 lice/fish group sampled at 10 DPI (Table 3).

## Discussion

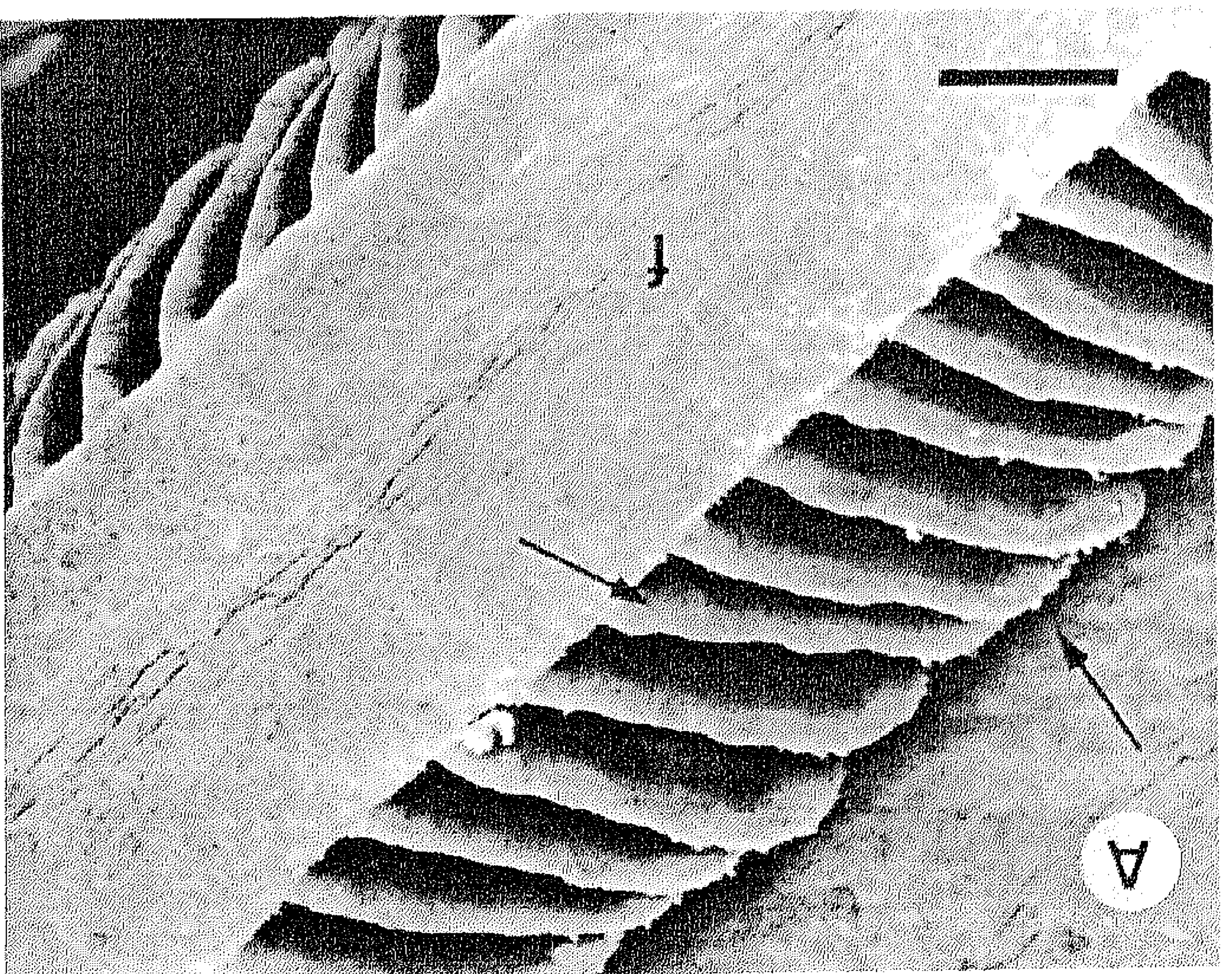
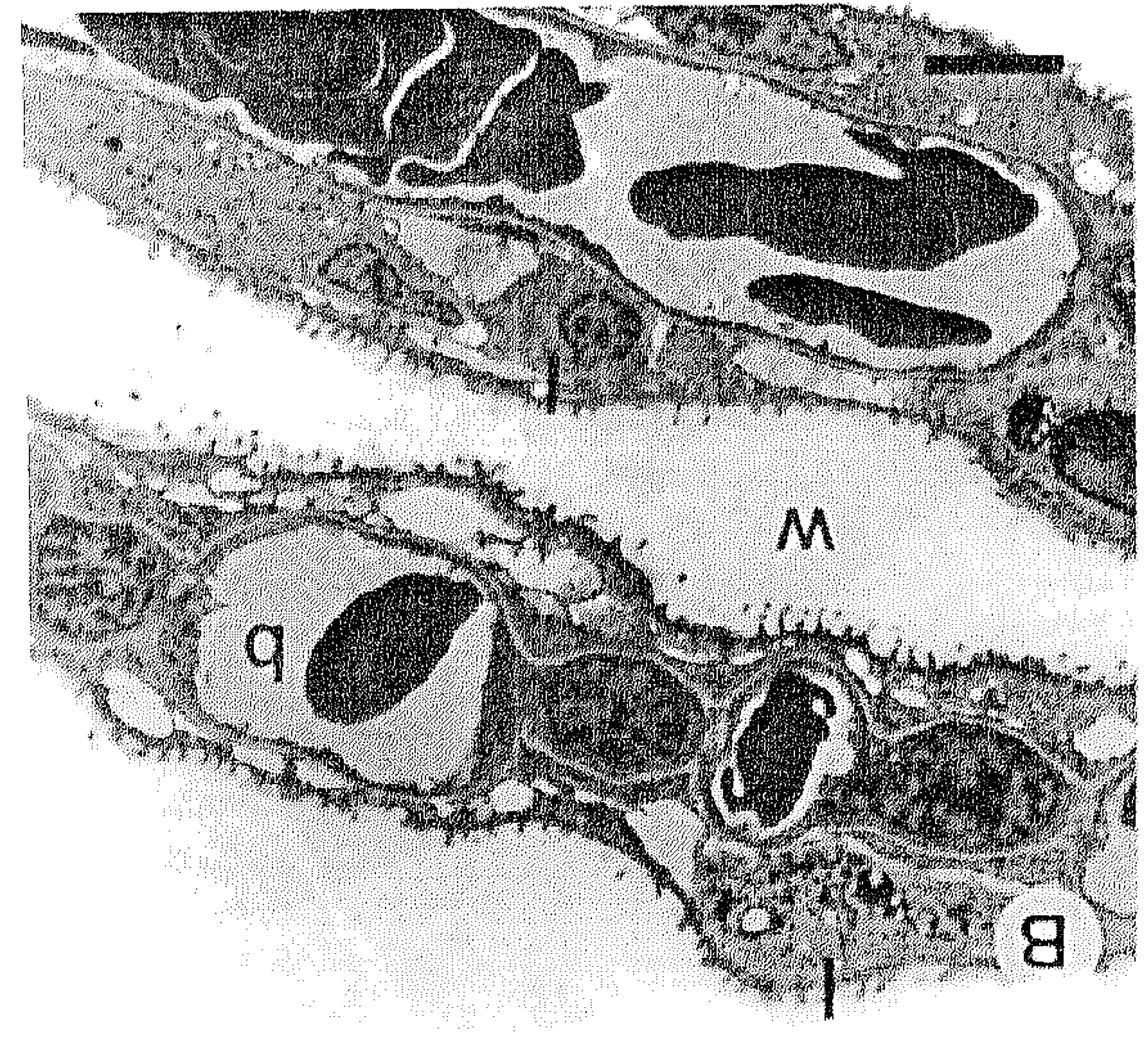
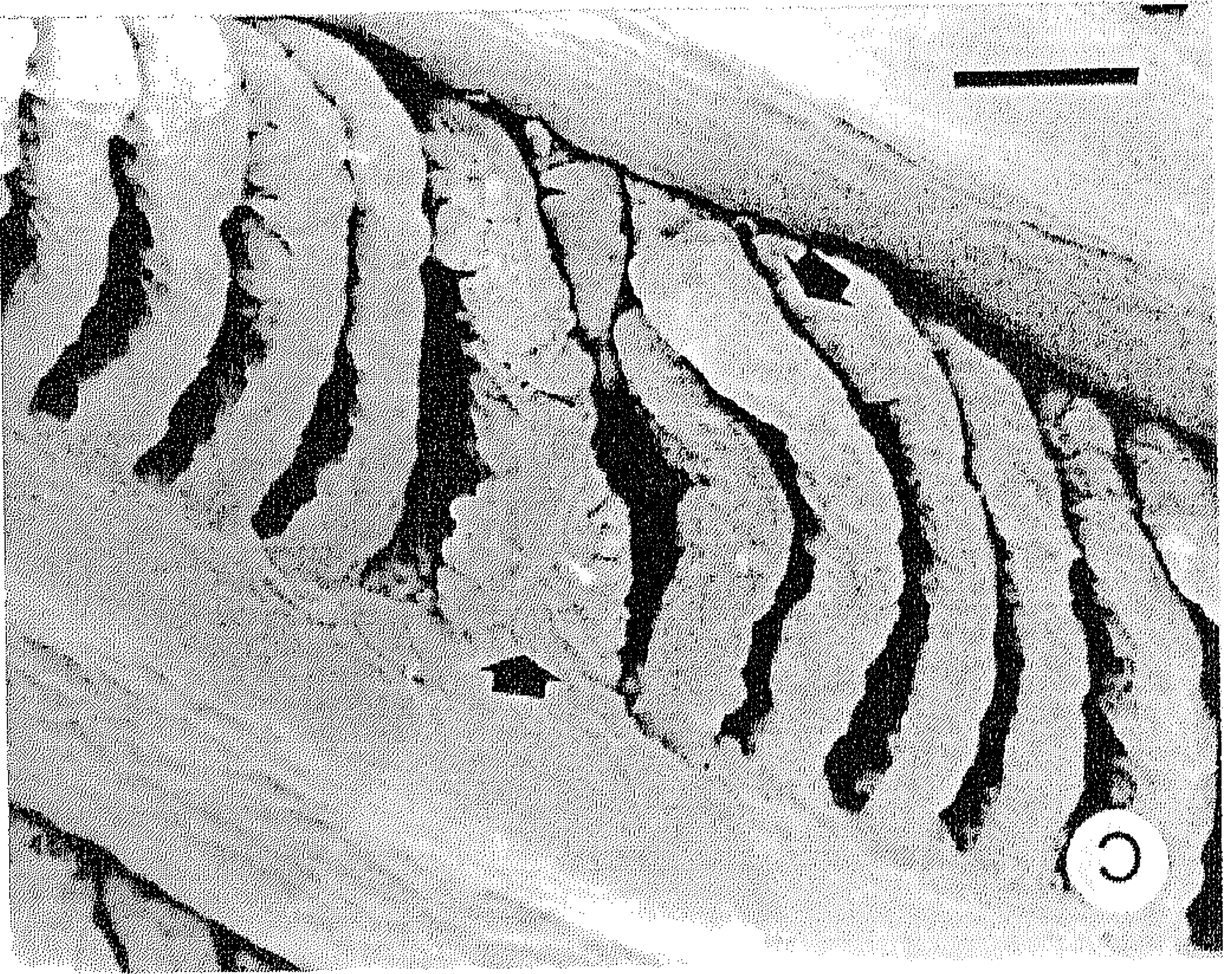
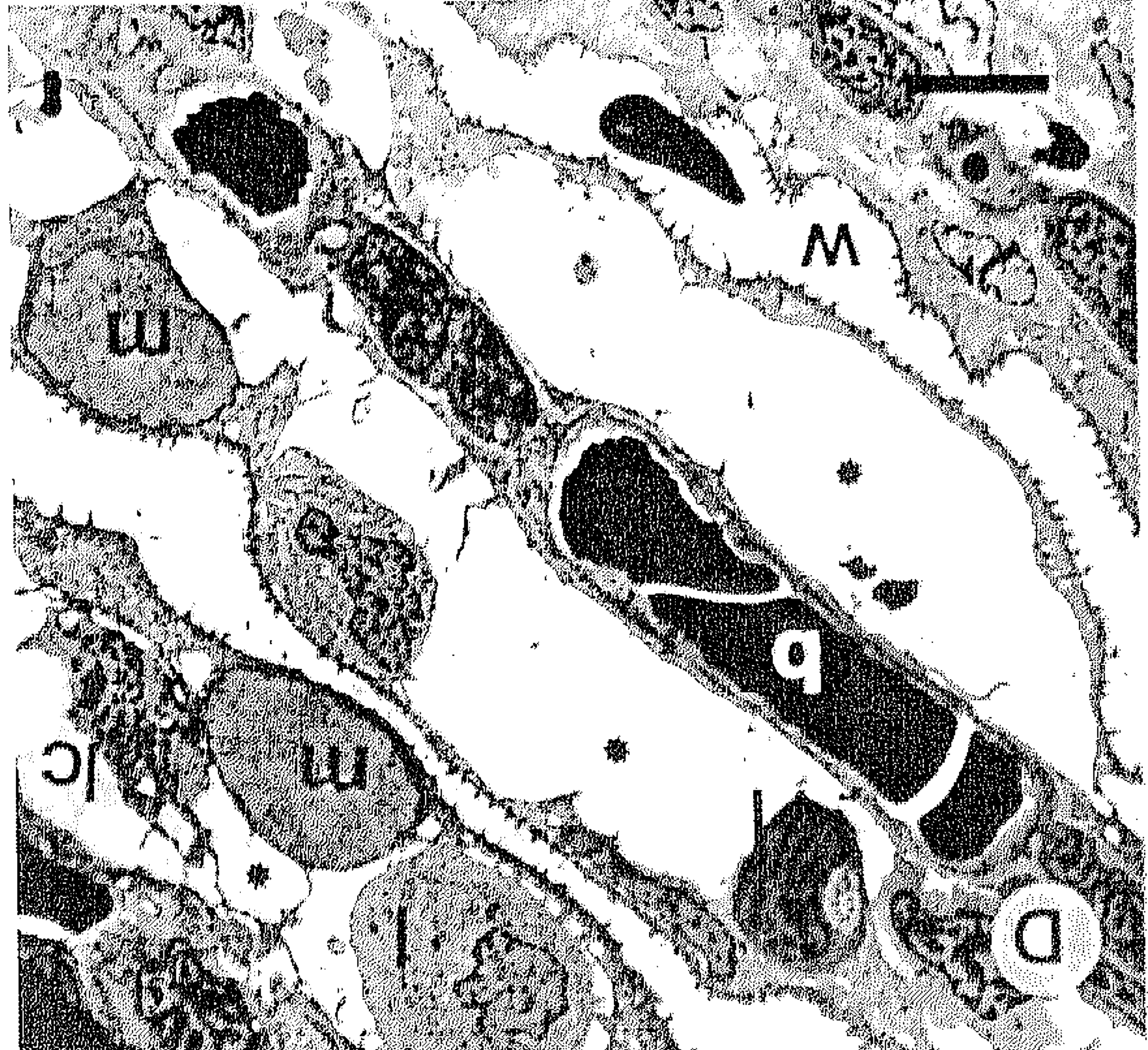
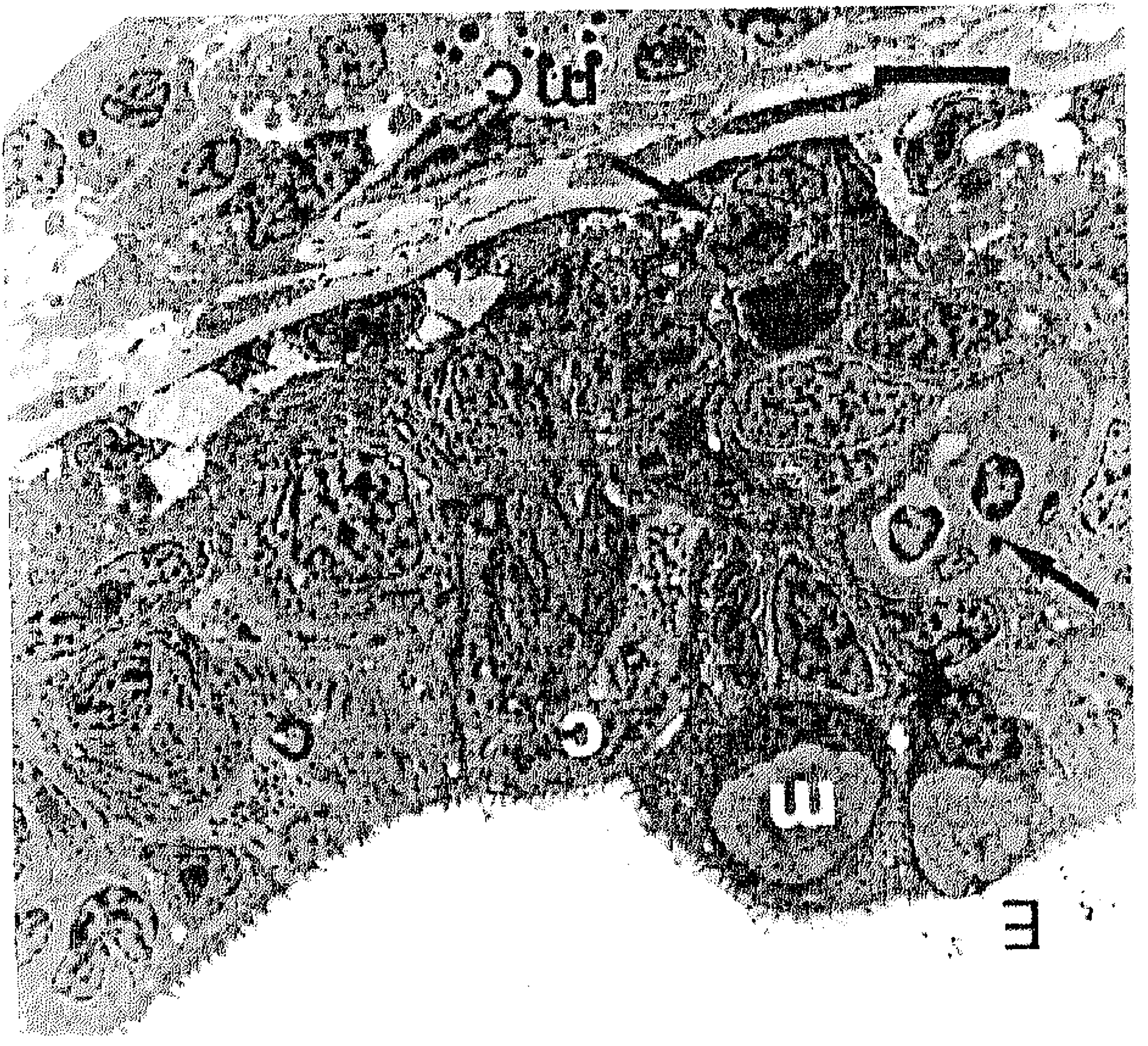
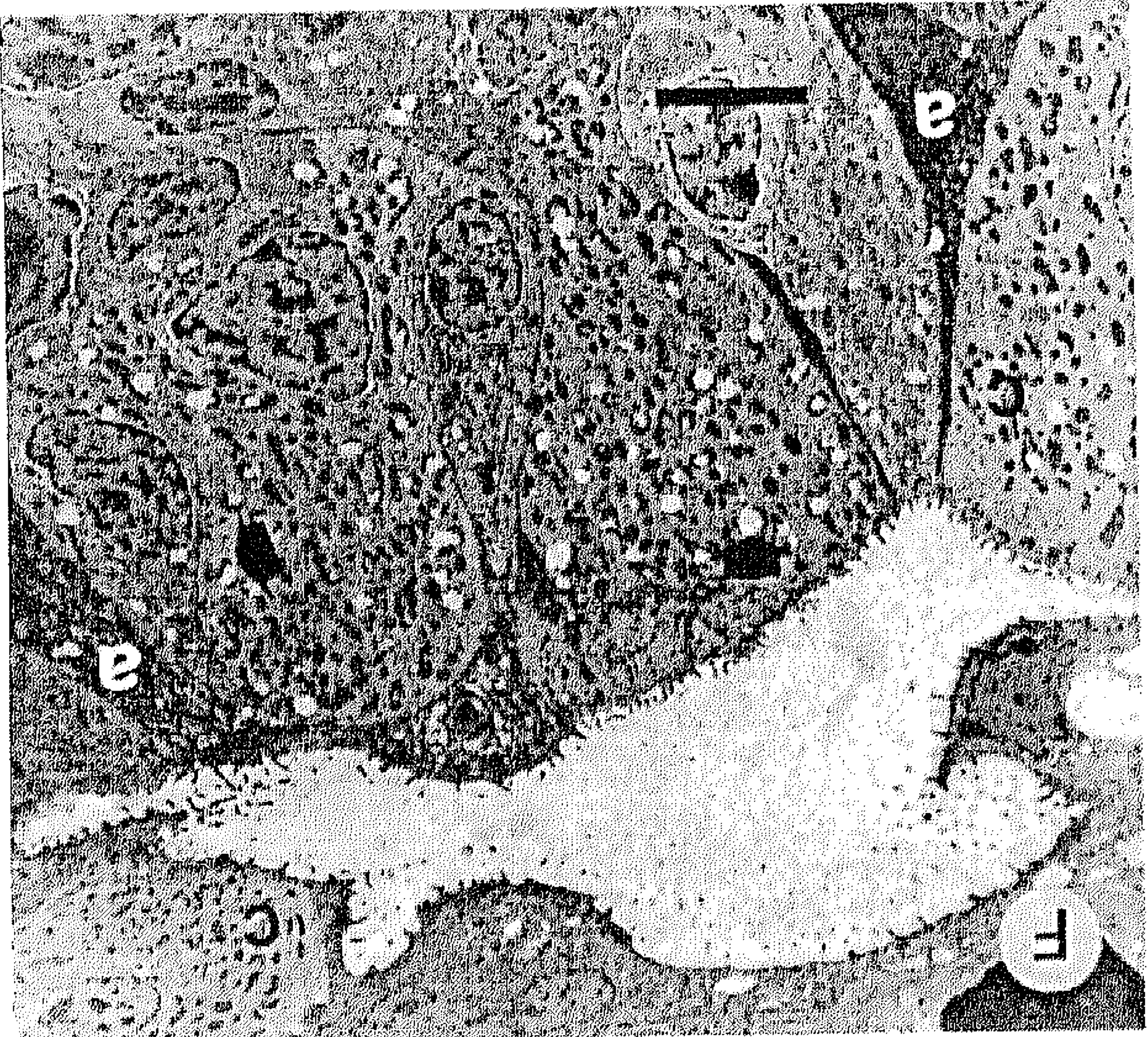
Infection with low numbers of preadult and adult *L. salmonis* had a marked effect on the integrity of the skin and gill epithelia of the Atlantic salmon. This is an indirect effect of the parasite, as it is reported from areas of epithelia where there was no obvious evidence of prior parasite attachment. The results of this study lead to the conclusion that many of the epithelial changes reported are similar to those described for stressors in general (Wendelaar Bonga 1997), including toxic agents (chemical stressors), handling, and a submissive position in hierarchy. These effects are likely hormone mediated, as a consequence of the infection causing an integrated stress response in the fish, resulting in increased levels of circulating cortisol and catecholamines. These results demonstrate that the effects of infection with *L. salmonis* can be divided into two distinct categories. The first is a direct effect of parasite attachment and feeding on the body surface, as reported by others (Jones et al. 1990; Johnson and Albright 1992a; Jonsdottir et al. 1992; MacKinnon 1993) and confirmed in this study, i.e., direct damage caused by parasite feeding, etc. The second is the indirect effect of the integrated stress response on the integrity of the skin and gill epithelia, including the osmoregulatory consequences in terms of gill  $\text{Na}^+/\text{K}^+$ -ATPase, as reported here for the first time.

At nonfeeding sites, infestation with *L. salmonis* caused necrosis in the pavement cells of the skin, increased apoptosis in the inner cell layers of the epidermis, and stimulated mucus discharge. Necrosis is a form of cellular death directly caused by toxic factors and other damaging agents. Pavement cell necrosis has been reported for a number of toxic chemicals in freshwater (Nolan et al. 1998) and also during seawater adaptation (Wendelaar Bonga and van der

Meij 1989; Uchida et al. 1996) and water acidification (Wendelaar Bonga et al. 1990). The necrotic cells were restricted to the pavement cell layer of parasitized salmon, suggesting that a change in the protective role of the mucus layer may have been a contributing factor, possibly by reduced protection as a result of a reduction in either total mucus coat or a change in mucus composition. The function of the mucus layer on the body surface of the fish and its components and composition have been reviewed, although there is still uncertainty about the involvement of mucus in ionoregulation in fishes (Shephard 1994). Increased mucus secretion in the present study was indicated by the numbers of discharging and predischARGE mucous cells seen in TEM and reduced total numbers of epidermal mucous cells in infested fish. Ectoparasites have been shown to reduce numbers of mucous cells in brown trout (*Salmo trutta*) epidermis (Pottinger et al. 1984), and stimulation of mucus discharge is a cortisol-mediated effect in rainbow trout (*Oncorhynchus mykiss*) (Iger et al. 1995).

The deletion of cells from a tissue is achieved by apoptosis, a physiologically controlled process that activates a genetically programmed mechanism that induces death and elimination of a cell. The stimulation of apoptosis in the skin and gill of parasitized fish is typical of the general epithelial response reported in many fishes (Wendelaar Bonga and van der Meij 1989; Nolan et al. 1998) and indicates increased aging, and therefore increased cell turnover, within these epithelia (Wendelaar Bonga 1997). The mechanisms controlling this process are not fully elucidated, but recently, it has been shown in vivo (Iger et al. 1995) and in vitro (Bury et al. 1997) that cortisol promotes apoptosis. The increased apoptosis in skin and gill epithelia of the sea lice infested fish in the present study may result from elevated circulating cortisol levels.

The overall structural integrity of the epithelia was disrupted, as indicated by the presence of widened intercellular spaces. These spaces are believed to be caused by hydrostatic pressure and osmotic forces and are prominent during periods of epithelial cell elimination through necrosis and apoptosis and increased mucus discharge. As numbers of cells are eliminated, the contacts between the remaining cells are reduced and the spaces become infiltrated with leukocytes. The stimulation of leukocyte infiltration and increased epithelial permeability are probably catecholamine-mediated effects (see review by Wendelaar Bonga 1997). The increase in electron density of the desmosomes connecting the epithelial cells of the skin was correlated with parasite infestation. These desmosomes may fortify the epidermis and reduce intercellular swelling. Heavily stained



**Fig. 5.** (A) Interlamellar region of gill from *S. salar* at 5 DPI with *L. salmonis* (3 lice/fish). Late-stage apoptotic chloride cells (a) are present as condensed apoptotic bodies located in a macrophage (MP), and electron-dense earlier stages in the chloride cell apoptotic pathway with dilated tubular systems can be seen (asterisks). Scale bar = 5  $\mu\text{m}$ . (B) Interlamellar region of gill from *S. salar* at 5 DPI with 3 lice/fish. Apoptotic chloride cells will be replaced at the original location by newly differentiated replacement chloride cells (asterisk). Scale bar = 5  $\mu\text{m}$ . (C) Primary filament epithelium of gill from *S. salar* at 5 DPI with 6 lice/fish. The normal epithelial structure is completely disrupted and the intercellular spaces (asterisks) are massively infiltrated by leukocytes (l); m, mucous cell. Scale bar = 10  $\mu\text{m}$ . (D) Chloride cells in the interlamellar region of gill from *S. salar* at 5 DPI with 6 lice/fish. Late-stage (la) and earlier-stage apoptotic chloride cells (ea) can be seen; asterisks, intercellular spaces. Scale bar = 5  $\mu\text{m}$ . (E) Gill lamellae from *S. salar* at 1 DPI with 10 lice/fish. The micrograph illustrates the disruption of the respiratory epithelium that underlies the wrinkling observed in the SEM views. The individual cells are shrunken and intercellular spaces are present (arrows). A shrunken chloride cell (c) is present on the lamella; e, erythrocyte; w, water. Scale bar = 5  $\mu\text{m}$ . (F) Chloride cells on the gill lamella of *S. salar* at 1 DPI with 10 lice/fish. Apoptotic chloride cells occur in the inner layers of the epithelium, while numbers of newly differentiating chloride cells are present also (asterisks). Leukocytes (l) are in the upper layer. p, pillar cell. Scale bar = 5  $\mu\text{m}$ .

**Table 3.** Parameters investigated in *S. salar* infected with numbers of preadult and adult *L. salmonis*.

| Parameter                                    | Control         | 3 lice/fish        | 6 lice/fish        | 10 lice/fish       |
|--|-----------------|--------------------|--------------------|--------------------|
| 1 DPI  |                 |                    |                    |                    |
| Sodium                                       | 170 $\pm$ 2.5   | 164 $\pm$ 3.1      | 167 $\pm$ 2.6      | 159 $\pm$ 2.2*     |
| Chloride                                     | 141 $\pm$ 4.5   | 136 $\pm$ 2.6      | 142 $\pm$ 4.2      | 134 $\pm$ 2.2      |
| Na:Cl  | 1.20 $\pm$ 0.02 | 1.20 $\pm$ 0.02    | 1.18 $\pm$ 0.02    | 1.19 $\pm$ 0.01    |
| Gill Na <sup>+</sup> /K <sup>+</sup> -ATPase | 8.2 $\pm$ 0.97  | 8.3 $\pm$ 0.41     | 8.7 $\pm$ 0.38     | 9.7 $\pm$ 0.41     |
| Epidermal mucous cells                       | 91.9 $\pm$ 6.2  | 63.5 $\pm$ 16.9**  | 66.3 $\pm$ 6.1**   | 65.1 $\pm$ 8.1**   |
| 5 DPI  |                 |                    |                    |                    |
| Sodium                                       | 166 $\pm$ 1.1   | 169 $\pm$ 3.0      | 168 $\pm$ 1.4      | 162 $\pm$ 2.4      |
| Chloride                                     | 132 $\pm$ 1.5   | 143 $\pm$ 3.7*     | 142 $\pm$ 1.9*     | 137 $\pm$ 2.4      |
| Na:Cl  | 1.26 $\pm$ 0.01 | 1.19 $\pm$ 0.01*** | 1.18 $\pm$ 0.01*** | 1.20 $\pm$ 0.02**  |
| Gill Na <sup>+</sup> /K <sup>+</sup> -ATPase | 8.9 $\pm$ 0.82  | 14.43 $\pm$ 1.04** | 15.88 $\pm$ 1.30** | 15.0 $\pm$ 0.67*** |
| Epidermal mucous cells                       | 91.3 $\pm$ 4.6  | 62.3 $\pm$ 3.7**   | 65.4 $\pm$ 7.4**   | 64.4 $\pm$ 4.5**   |
| 10 DPI                                       |                 |                    |                    |                    |
| Sodium                                       | 169 $\pm$ 1.1   | 171 $\pm$ 2.4      | 170 $\pm$ 1.7      | 171 $\pm$ 1.3      |
| Chloride                                     | 137 $\pm$ 1.6   | 136 $\pm$ 1.9      | 142 $\pm$ 1.8      | 135 $\pm$ 1.0      |
| Na:Cl  | 1.24 $\pm$ 0.02 | 1.27 $\pm$ 0.01    | 1.20 $\pm$ 0.01    | 1.26 $\pm$ 0.01    |
| Gill Na <sup>+</sup> /K <sup>+</sup> -ATPase | 7.0 $\pm$ 0.27  | 7.9 $\pm$ 1.42     | 7.8 $\pm$ 1.04     | 13.3 $\pm$ 0.70**  |
| Epidermal mucous cells                       | 90.8 $\pm$ 6.2  | 54.2 $\pm$ 8.2**   | 57.6 $\pm$ 11.6**  | 50.0 $\pm$ 6.5***  |

Note: Data for serum sodium and chloride values are mmol/L, gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity is mmol ATP·mg protein<sup>-1</sup>·h<sup>-1</sup>, and total numbers of mucous cells are per millimetre of skin epidermis. Data are expressed as means  $\pm$  SE for  $n = 7$ . \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  compared with control values.

**Table 4.** Serum protein, urea, and total calcium of *S. salar* at 1 DPI with numbers of preadult and adult *L. salmonis*.

| Serum parameter        | Control       | 3 lice/fish   | 6 lice/fish   | 10 lice/fish    |
|------------------------|---------------|---------------|---------------|-----------------|
| Protein (mg/dL)        | 28.7 $\pm$ 6  | 42.3 $\pm$ 5  | 42.0 $\pm$ 5  | 50.7 $\pm$ 2*   |
| Urea (mmol/L)          | 2.3 $\pm$ 0.2 | 2.2 $\pm$ 0.1 | 2.3 $\pm$ 0.2 | 3.0 $\pm$ 0.1*  |
| Total calcium (mmol/L) | 2.7 $\pm$ 0.2 | 3.1 $\pm$ 0.2 | 3.3 $\pm$ 0.2 | 3.6 $\pm$ 0.1** |

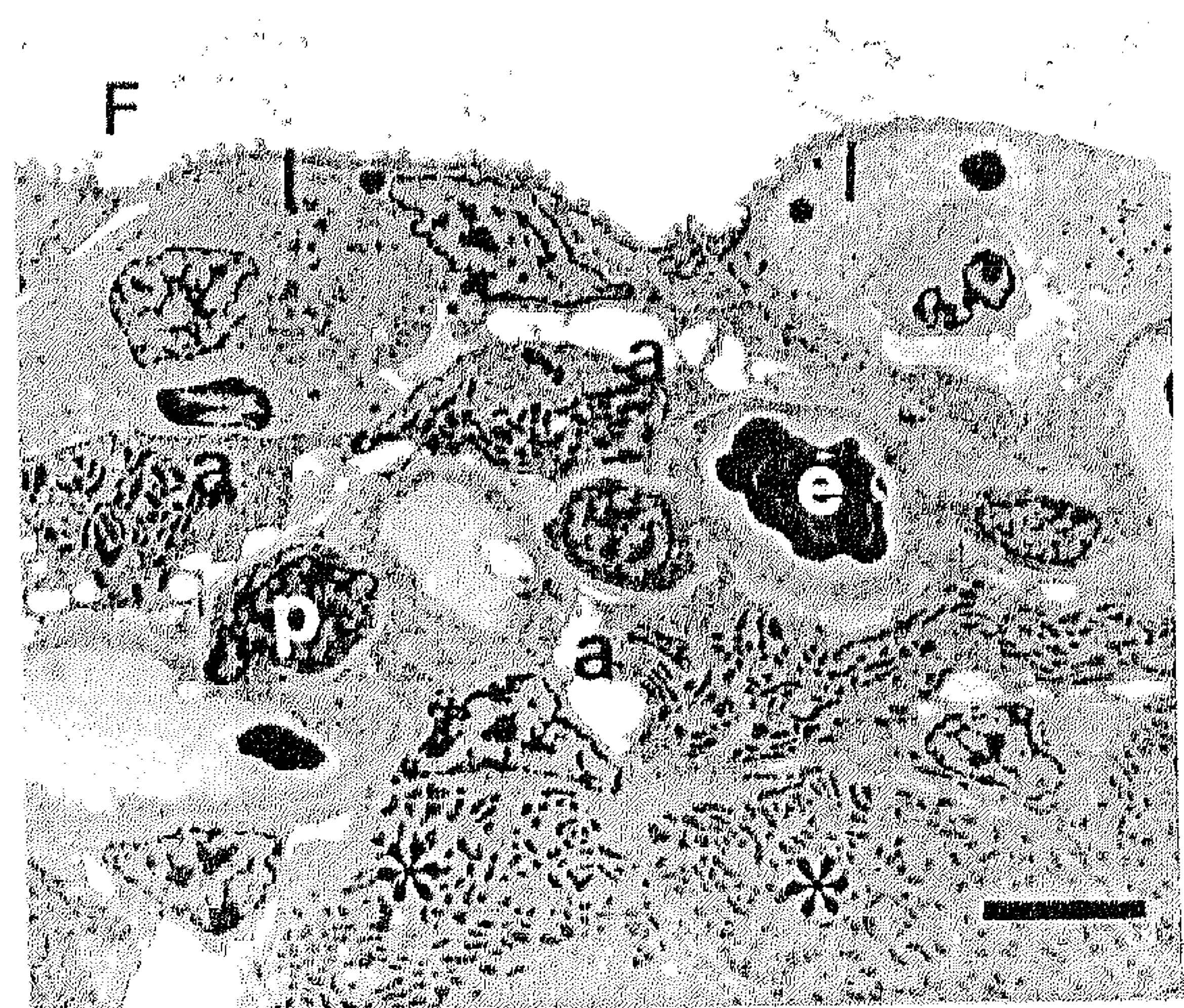
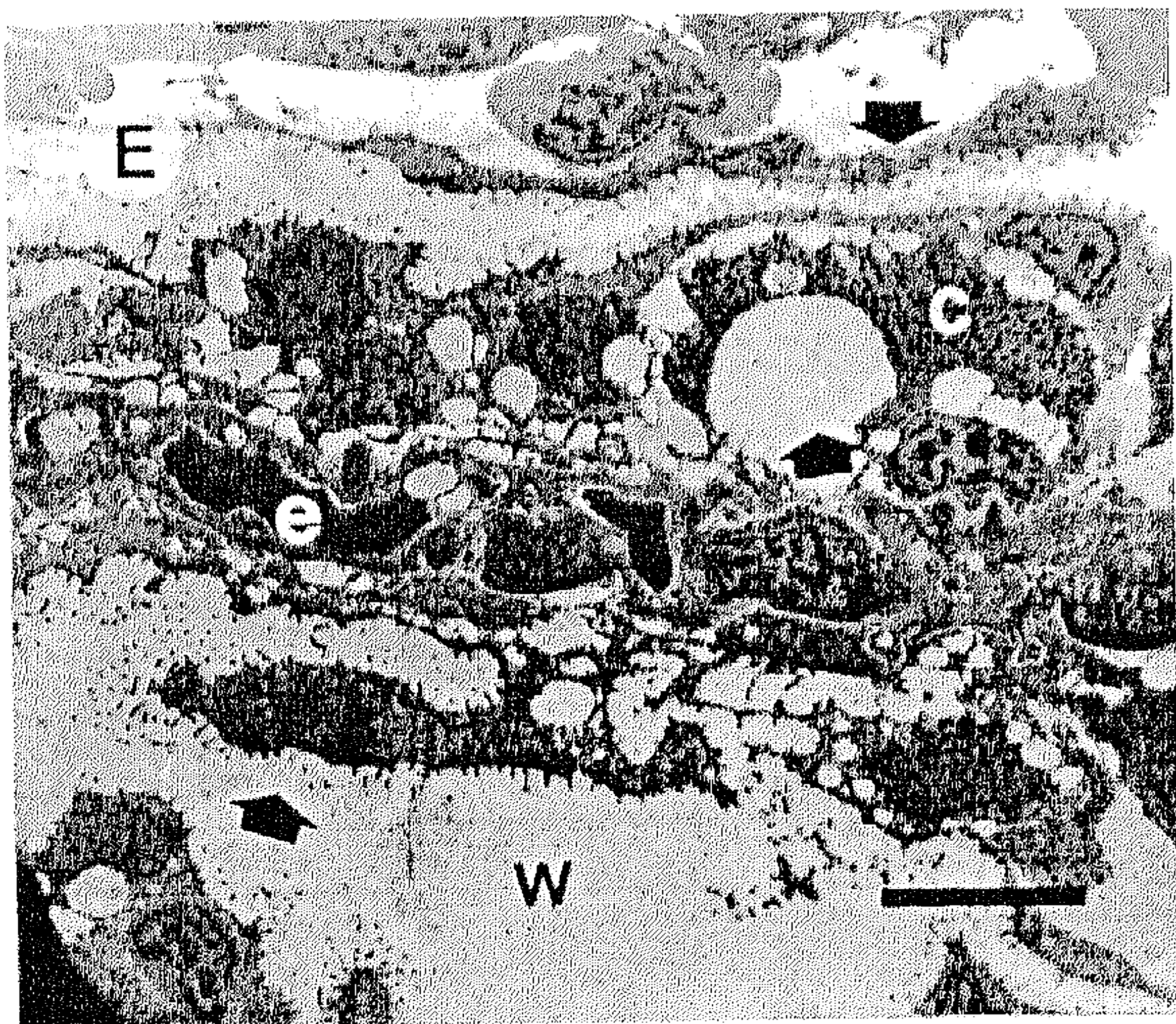
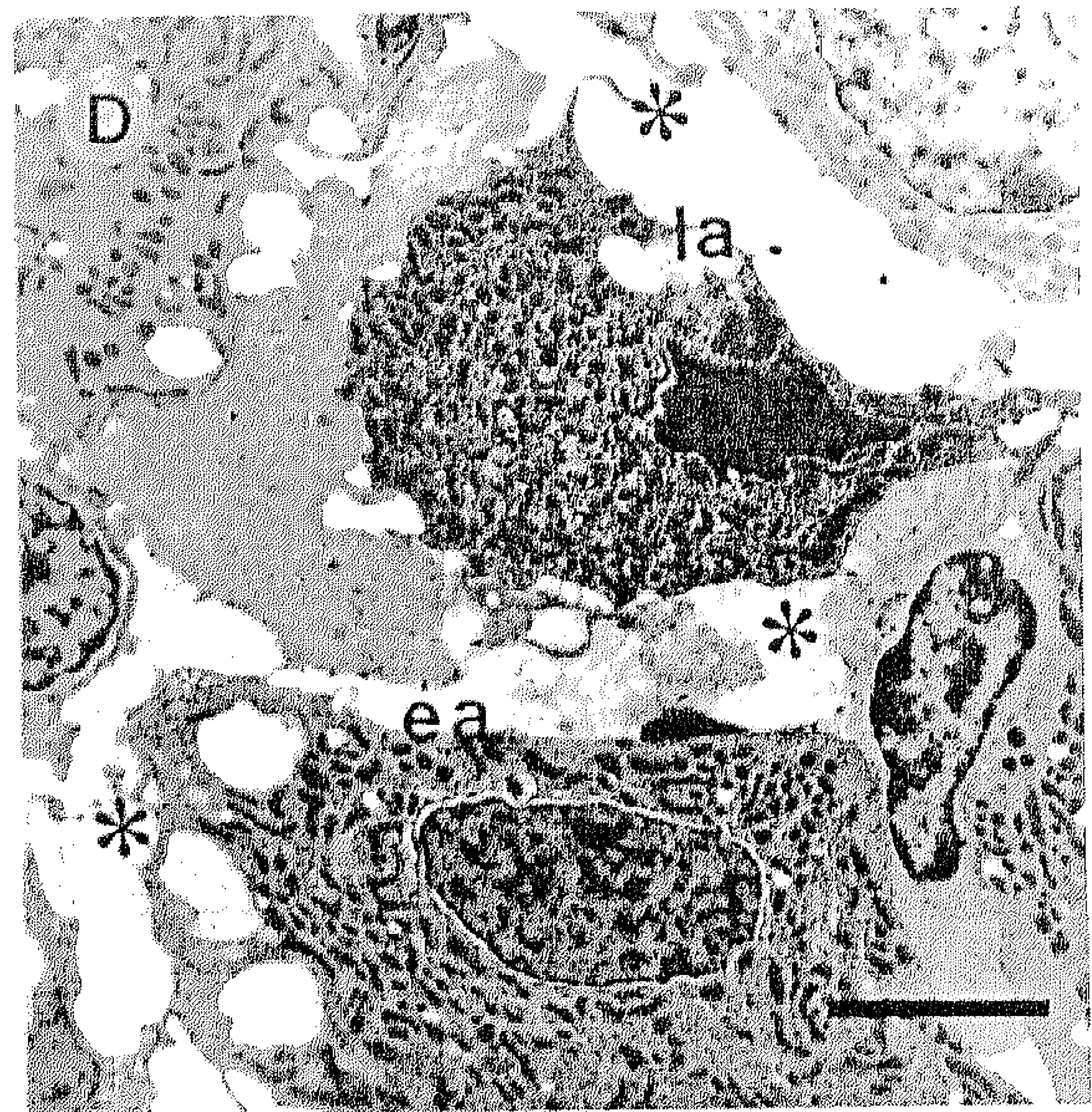
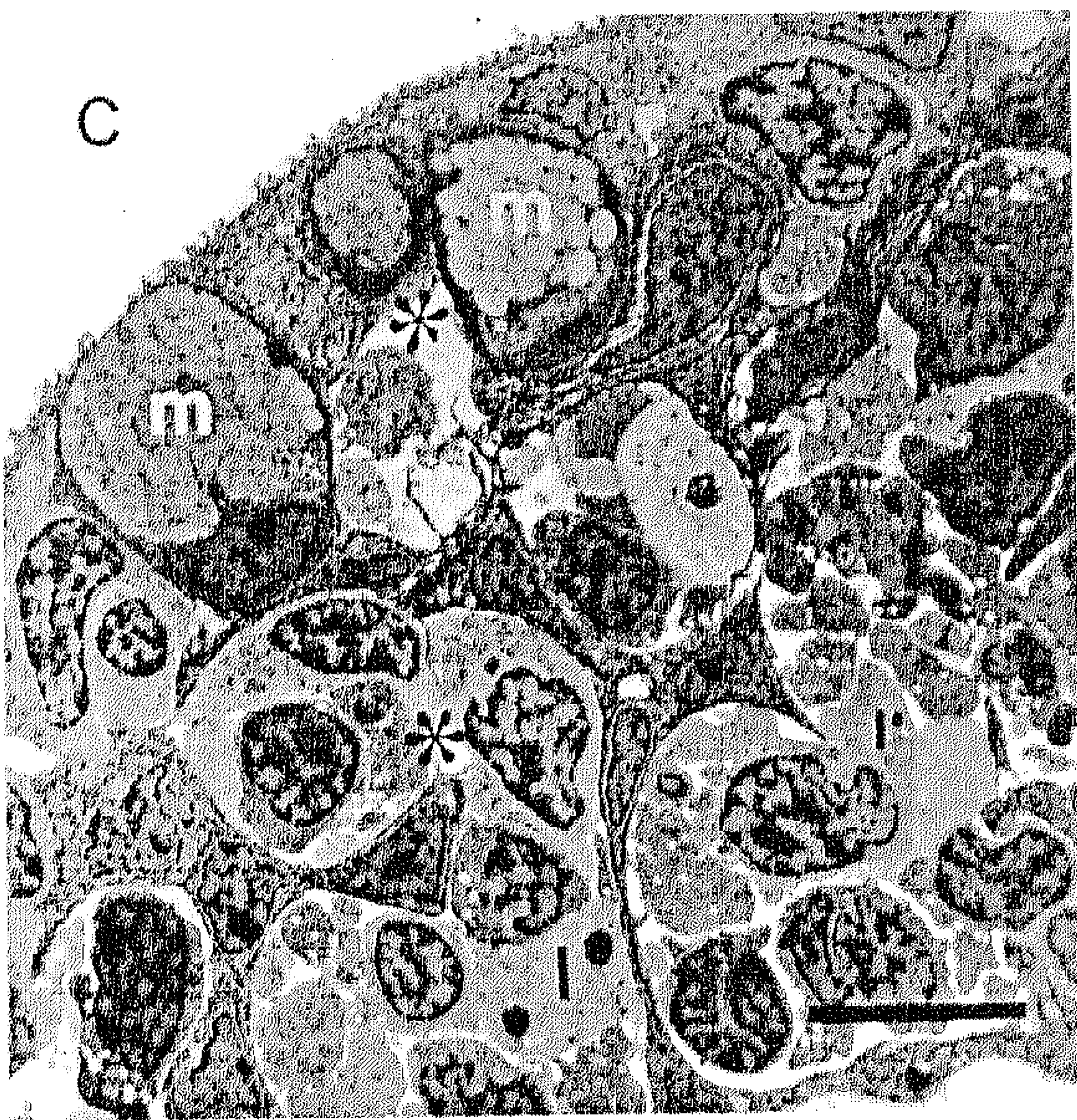
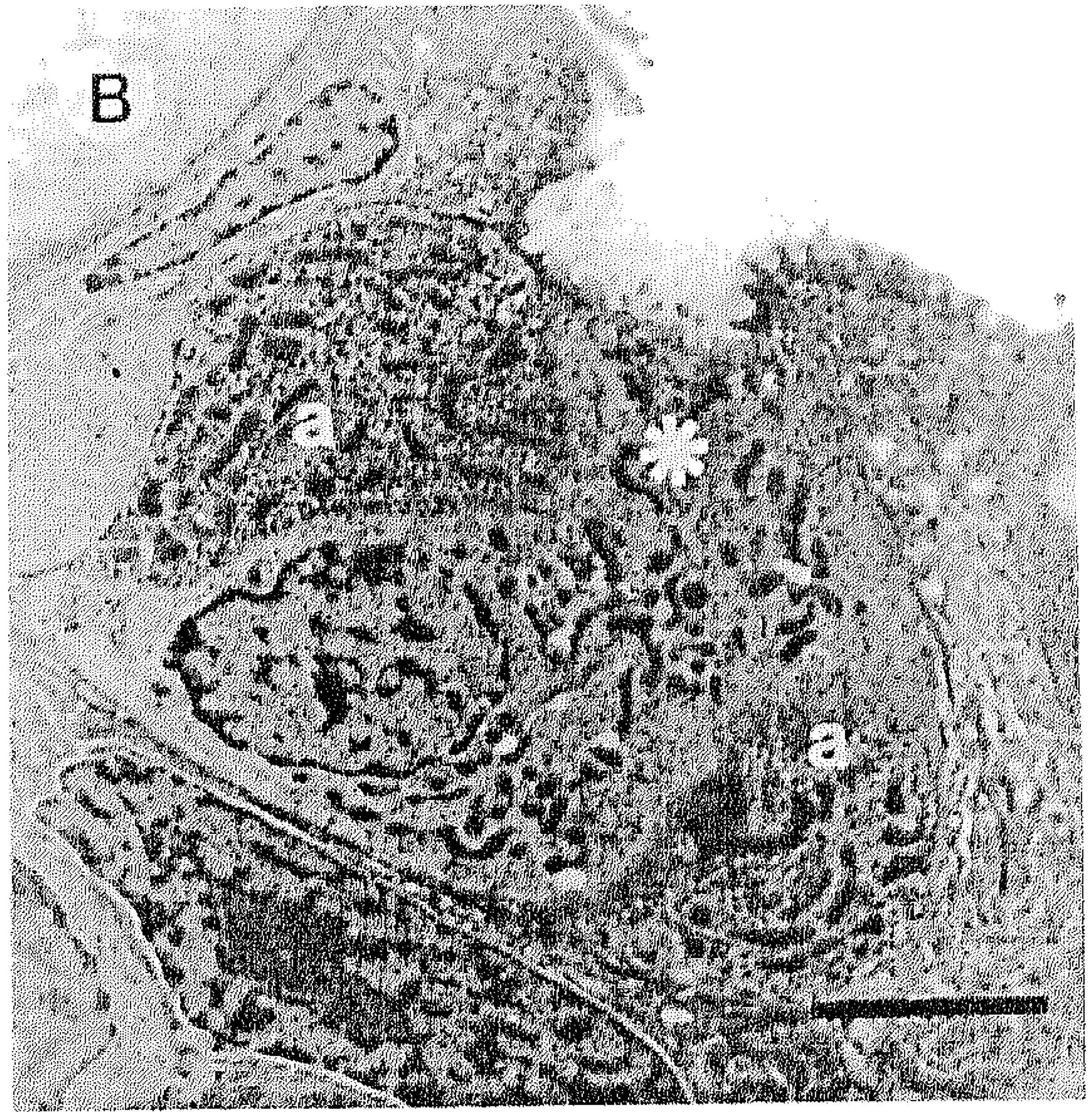
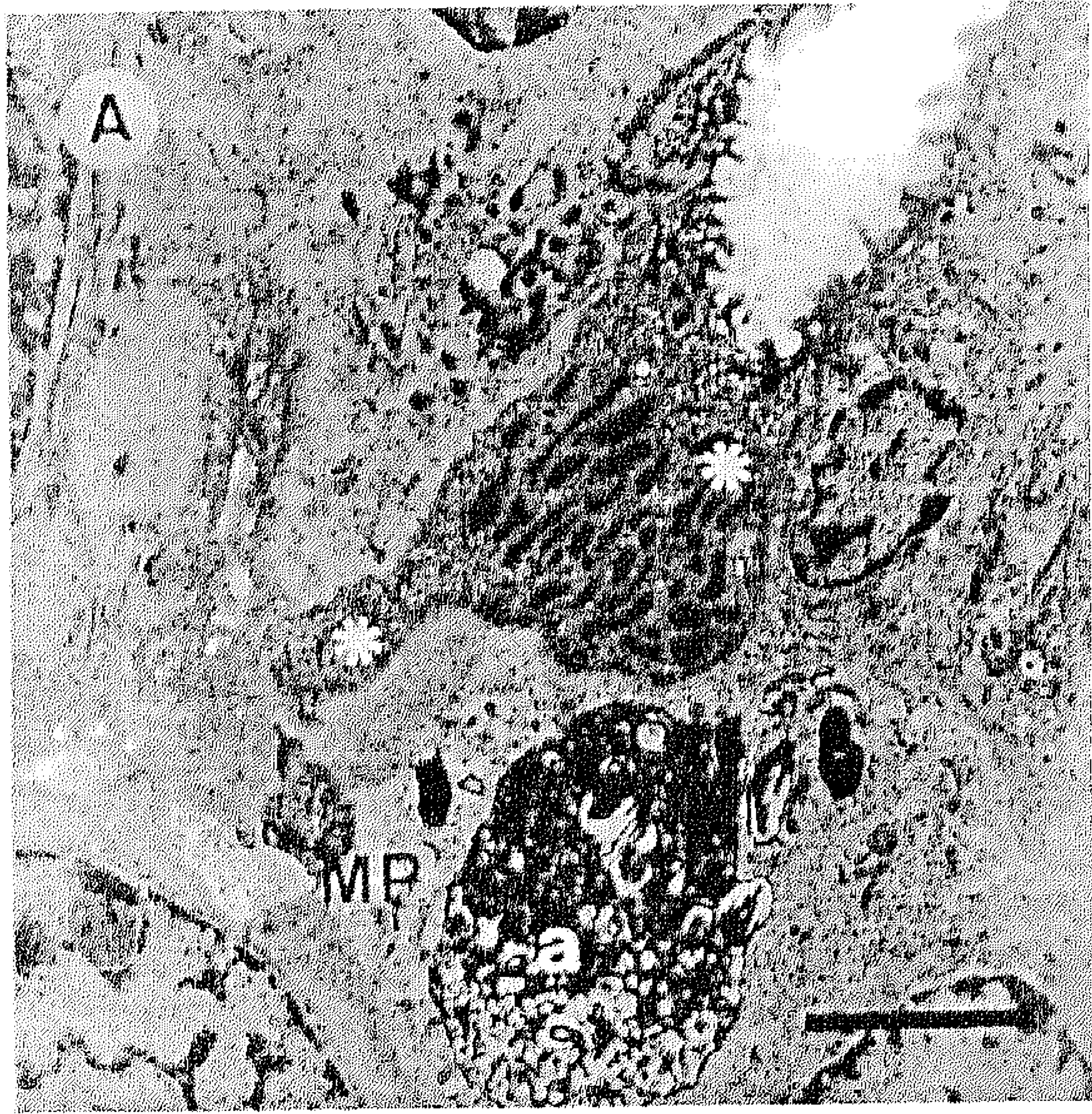
Note: Data are expressed as means  $\pm$  SE for  $n = 7$ . \* $P < 0.05$  and \*\* $P < 0.01$  compared with control values.

desmosomes in the present study were observed predominantly in the epidermis of fish that had relatively high levels of damage. In a study of sea lice effects on skin of Atlantic salmon, electron-dense desmosomes between cells have also been shown in the skin of control fish. However, a different tissue fixative for TEM analysis was used (MacKinnon 1993).

In the gill, catecholamine action also increases branchial blood flow, resulting in increased gill perfusion and blood pressure (Satchell 1991; Wendelaar Bonga 1997). The

lamellar swelling reported in the present study in parasitized fish may reflect the action of high levels of catecholamines released as a result of parasite-induced stress. We are confident that the effects we report in the gills do not result from any direct action of the parasite, as we infected our fish with preadult and adult parasites, while only the copepodid and chalimus stages have been reported to attach to the gills (Johnson and Albright 1992a, 1992b). Inspection of the gills from our infected fish by light microscopy and SEM revealed no parasites or parasite damage to the gills. In addition, at the water temperature in our experiment (15°C), the generation time from egg to infective stage is estimated at 7.4 days (Johnson and Albright 1991a), so if successful reproduction had occurred, the infective stages would have been active shortly before the 10 DPI sample point. This was when the 3 and 6 lice/fish groups (but not 10 lice/fish group) showed recovery.

Infestation with sea lice increased gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and induced chloride cell apoptosis without greatly disrupting hydromineral balance. Elevated cortisol levels in fish stimulate chloride cell differentiation in vitro (McCormick 1995) and improve hypoosmoregulation in vivo



(Madsen 1990; Cornell et al. 1994), as well as inducing chloride cell apoptosis *in vitro* (Bury et al. 1997). Cortisol is a pluripotent hormone whose effects can be advantageous at moderately elevated levels and deleterious at highly elevated levels (Wendelaar Bonga 1997). In parasitized fish in the present study, the infection increased gill  $\text{Na}^+/\text{K}^+$ -ATPase activity at 5 DPI in all groups, increased aging of the mature chloride cells, and stimulated differentiation of a new chloride cell population, and these responses may have contributed to the maintenance or restoration of hydromineral balance. At 10 DPI, gill  $\text{Na}^+/\text{K}^+$ -ATPase activity and chloride cell apoptosis had returned to control levels in the 3 and 6 lice/fish groups. However,  $\text{Na}^+/\text{K}^+$ -ATPase activity and chloride cell apoptosis in the 10 lice/fish group remained above control levels, indicating that recovery was incomplete and that the stress was greater at this infestation level.

The effects on the chloride cells of the 10 lice/fish group were the strongest. These were correlated functionally at 1 DPI with decreased serum sodium and increased urea, total calcium, and protein in this group. Teleosts are ammoniotelic and excrete most nitrogenous waste through the gill as ammonium ( $\text{NH}_4^+$ ). This occurs through the chloride cells, and elevated levels of urea in the blood are indicative of a rise in the production of nitrogenous waste products, as is typical for stressed fish (Wendelaar Bonga 1997), and may reflect impaired excretion of these products as a result of disrupted gill function. This is further supported by the decreased sodium and increased calcium levels. The former reflects the activity of increased gill  $\text{Na}^+/\text{K}^+$ -ATPase activity to eliminate excess sodium influx and (or) effects of reduced drinking rate, while the latter cannot be explained. Another study has reported increased plasma chloride and decreased serum total protein in Atlantic salmon infested with adult *L. salmonis* (Grimnes and Jakobsen 1996). This contrasts with the present study where we report an increase in serum protein, no increase in serum sodium, and a small increase in serum chloride at 5 DPI in the 3 and 6 lice/fish groups. However, the study of Grimnes and Jakobsen (1996) involved much higher infestation levels on considerably smaller fish (median infestation intensity 64 lice/fish at 11 days and 42 lice/fish at 31 days following infection with 86–176 infective copepodids/40-g fish) and these numbers of lice caused osmoregulatory failure.

The results of this study are based on single groups of fish for each experimental treatment. Although several replicate tanks per treatment are preferable, this was not possible. However, we are confident that the effects we report here are representative, as we have carried out later experiments infecting groups of salmonids with the crustacean ectoparasite *Argulus foliaceus*. Here, using a similar methodology and replicate treatment tanks, no differences were noted between replicate tanks for any treatment group at any time (Ruane et al. 1999).

We report here the presence of mucous cell lined ducts in the head region of Atlantic salmon. These ducts have not been reported previously, to the best of our knowledge, and we have confirmed the presence of these ducts in other salmonids (*S. trutta* and *O. mykiss*). We propose that, under normal circumstances, these ducts provide a rich supply of mucus to flow back over the body surface. Sea lice infestation of the scaleless head region can be severe (Pike 1989).

The parasites may find this area attractive because the mucus provides a rich food source and preferentially gather there during heavy infestation, when competition between parasites for feeding resources may be high.

In conclusion, infection of post-smolt Atlantic salmon with low numbers of the sea louse *L. salmonis* has marked effects on the epithelia of the skin and gills, both locally in the skin as a result of parasite attachment and feeding (a direct effect) and overall as a result of the stress response of the fish to infection (an indirect effect). Hydromineral balance in the blood is not greatly affected, but increased gill  $\text{Na}^+/\text{K}^+$ -ATPase activity indicates that while the epithelial integrity is compromised, a high rate of sodium turnover occurs. The fish could apparently adapt to an initial infection level of 3–6 lice/fish within 10 days, but not to 10 lice/fish. Thus, infestation is stressful and at higher infection levels creates a prolonged period during which the overall compromised integrity of the skin and gill epithelia, as well as stress-related immunosuppression, may render the fish susceptible to secondary infections.

### Acknowledgments

The excellent technical assistance of J.C.A. van der Meij (transmission electron microscopy) and H.P.M. Geurts (scanning electron microscopy) is gratefully acknowledged. This work was supported by a grant from the Graduate School M&T to D.T.N.

### References

- Barton, B.A., and Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish Dis.* 1: 3–26.
- Berland, B. 1993. Salmon lice on wild salmon (*Salmo salar* L.) in western Norway. In *Pathogens of wild and farmed fish: sea lice*. Edited by G.A. Boxshall and D. Defaye. Ellis Horwood Limited, London, U.K. pp. 179–187.
- Blackstock, N., and Pickering, A.D. 1982. Acidophilic granular cells in the epidermis of the brown trout, *Salmo trutta* L. *Cell Tissue Res.* 210: 259–269.
- Bury, N.R., Li, J., Lock, R.A.C., and Wendelaar Bonga, S.E. 1997. Cortisol protects against copper induced necrosis and promotes apoptosis in fish gill chloride cells *in vitro*. *Aquat. Toxicol.* 40: 193–202.
- Cornell, S.C., Portesi, D.M., Veillette, P.A., Sundell, K., and Speckler, J.L. 1994. Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo salar*) in the post-smolt stage. *Fish Physiol. Biochem.* 13: 183–190.
- Flik, G., Wendelaar Bonga, S.E., and Fenwick, J.C. 1984.  $\text{Ca}^{2+}$ -dependent phosphatase and  $\text{Ca}^{2+}$ -dependent ATPase activities in plasma membranes of eel gill epithelium. III. Stimulation of branchial high-affinity  $\text{Ca}^{2+}$ -ATPase activity during prolactin-induced hypercalcemia in American eels. *Comp. Biochem. Physiol. B. Comp. Biochem.* 79: 521–524.
- Grimnes, A., and Jakobsen, P.J. 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. *J. Fish Biol.* 48: 1179–1194.
- Heuch, P.A., Parsons, A., and Boxaspen, K. 1995. Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? *Can. J. Fish. Aquat. Sci.* 52: 681–689.

- Holst, J.C., Nilsen, F., Hodneland, K., and Nylund, A. 1993. Observations of the biology and parasites of postsmolt Atlantic salmon, *Salmo salar*, from the Norwegian Sea. *J. Fish Biol.* **42**: 962-966.
- Iger, Y., Balm, P.H.M., Jenner, H.A., and Wendelaar Bonga, S.E. 1995. Cortisol induced stress-related changes in the skin of rainbow trout (*Oncorhynchus mykiss*). *Gen. Comp. Endocrinol.* **97**: 188-198.
- Johnson, S.C., and Albright, L.J. 1991a. Development, growth, and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. *J. Mar. Biol. Assoc. U.K.* **71**: 425-436.
- Johnson, S.C., and Albright, L.J. 1991b. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *Can. J. Zool.* **69**: 929-950.
- Johnson, S.C., and Albright, L.J. 1992a. Comparative susceptibility and histopathology of the response of naive Atlantic, chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Dis. Aquat. Org.* **14**: 179-193.
- Johnson, S.C., and Albright L.J. 1992b. Effects of cortisol implants on the susceptibility and the histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). *Dis. Aquat. Org.* **14**: 195-205.
- Johnson, S.C., Blaylock, R.B., Elphick, J., and Hyatt, K.D. 1996. Disease induced by the sea louse (*Lepeophtheirus salmonis*) (Copepoda: Caligidae) in wild sockeye salmon (*Oncorhynchus nerka*) stocks of Alberni Inlet, British Columbia. *Can. J. Fish. Aquat. Sci.* **53**: 2888-2897.
- Jones, M.W., Sommerville, C., and Bron, J. 1990. The histopathology associated with the juvenile stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* **13**: 303-310.
- Jonsdottir, H., Bron, J.E., Wootten, R., and Turnbull, J.F. 1992. The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. *J. Fish Dis.* **15**: 521-527.
- MacKinnon, B.M. 1993. Host response of Atlantic salmon (*Salmo salar*) to infection by sea lice (*Caligus elongatus*). *Can. J. Fish. Aquat. Sci.* **50**: 789-792.
- Madsen, S. 1990. The role of cortisol and growth hormone in seawater adaptation and development of hypoosmoregulatory mechanisms in sea trout parr (*Salmo trutta trutta*). *Gen. Comp. Endocrinol.* **79**: 1-11.
- McCormick, S.D. 1995. Hormonal control of gill Na<sup>+</sup>,K<sup>+</sup>-ATPase and chloride cell function. In *Cellular and molecular approaches to fish ionic regulation*. Edited by C.M. Wood and T.J. Shuttleworth. Academic Press Inc., San Diego, Calif. pp. 285-315.
- Nolan, D.T., Haddingh, R.H., Jenner, H.A., and Wendelaar Bonga, S.E. 1998. The effects of exposure to Rhine water on the sea trout smolt (*Salmo trutta trutta* L.): an ultrastructural and physiological study. In *New concepts for sustainable management of river basins*. Edited by P.H. Nienhuis, R.S.E.W. Leuven and A.M.J. Ragas. Backhuys Publishers, Leiden. pp. 261-271.
- Pickering, A.D., Pottinger, T.G., and Carragher, J.F. 1989. Differences in the sensitivity of brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson, to physiological doses of cortisol. *J. Fish Biol.* **34**: 757-768.
- Pike, A.W. 1989. Sea lice — major pathogens of farmed Atlantic salmon. *Parasitol. Today*, **5**: 291-297.
- Pottinger, T.G., and Pickering, A.D. 1985. The effects of 11-keto-testosterone and testosterone on the skin structure of brown trout, *Salmo trutta* L. *Gen. Comp. Endocrinol.* **59**: 335-342.
- Pottinger, T.G., Pickering, A.D., and Blackstock, N. 1984. Ectoparasite induced changes in epidermal mucification of the brown trout, *Salmo trutta* L. *J. Fish Biol.* **25**: 123-128.
- Ritchie, G., Mordue, A.J., Pike, A.W., and Rae, G.H. 1993. The reproductive output of *Lepeophtheirus salmonis* adult females in relation to temperature and photoperiod. In *Pathogens of wild and farmed fish: sea lice*. Edited by G.A. Boxshall and D. Defaye. Ellis Horwood Limited, London, U.K. pp. 153-165.
- Ruane, N.M., Nolan, D.T., Rotllant, J., Tort, L., Balm, P.H.M., and Wendelaar Bonga, S.E. 1999. Modulation of the response of rainbow trout (*Oncorhynchus mykiss* Walbaum) to confinement by an ectoparasitic (*Argulus foliaceus* L.) infestation and cortisol feeding. *Fish Physiol. Biochem.* **20**: 43-51.
- Satchell, G.H. 1991. *Physiology and form of fish circulation*. Cambridge University Press, Cambridge, U.K.
- Shephard, K.L. 1994. Functions for fish mucus. *Rev. Fish Biol. Fish.* **4**: 401-429.
- Tully, O., Poole, W.R., and Whelan, K.F. 1993. Infestation parameters for *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae) parasitic on sea trout, *Salmo trutta* L., off the west coast of Ireland during 1990 and 1991. *Aquacult. Fish. Manage.* **24**: 545-555.
- Uchida, K., Kaneko, T., Yamauchi, K., and Hirano, T. 1996. Morphometrical analysis of chloride cell activity in the gill filaments and lamellae and changes in Na<sup>+</sup>,K<sup>+</sup>-ATPase activity during seawater adaptation in chum salmon fry. *J. Exp. Zool.* **276**: 193-200.
- Wedemeyer, G.A. 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In *Fish stress and health in aquaculture*. Edited by G.K. Iwama, A.D. Pickering, J.P. Sumpter, and C.B. Schreck. Cambridge University Press, Cambridge, U.K. pp. 35-71.
- Wendelaar Bonga, S.E. 1997. The stress response in fish. *Physiol. Rev.* **77**: 591-625.
- Wendelaar Bonga, S.E., and van der Meij, J.C.A. 1989. Degeneration and death, by apoptosis and necrosis, of the pavement and chloride cells in the gills of the teleost *Oreochromis mossambicus*. *Cell Tissue Res.* **255**: 235-243.
- Wendelaar Bonga, S.E., Flik, G., Balm, P.H.M., and van der Meij, J.C.A. 1990. The ultrastructure of chloride cells in the gills of the teleost *Oreochromis mossambicus* during exposure to acidified water. *Cell Tissue Res.* **259**: 575-585.
- Wootten, R., Smith, J.W., and Needham, E.A. 1982. Aspects of the biology of the parasitic copepods *Lepeophtheirus salmonis* and *Caligus elongatus* on farmed salmonids, and their treatment. *Proc. R. Soc. Edinb. Sect. B (Biol. Sci.)*, **81**: 185-197.