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Effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout, *Salmo gairdneri*

Pierre Laurent[1] **and Steve F. Perry**[2]

[1] Laboratoire de Morphologie Fonctionnelle et Ultrastructurale des Adaptations, CNRS, Strasbourg, France;
[2] Department of Biology, University of Ottawa, Ottawa Ontario, Canada

Summary. Daily intramuscular injection of cortisol (4 mg kg^{-1} body weight) in rainbow trout, *Salmo gairdneri*, for 10 days caused significant increases in the number and individual apical surface area of gill chloride cells per mm^2 of filament epithelium. Concomitantly, whole body influxes of sodium (Na^+) and chloride (Cl^-) increased. Acute (3 h) intra-arterial infusion of cortisol did not affect whole body Na^+ or Cl^- influx. A significant correlation was observed between both Na^+ and Cl^- influxes and the fractional apical surface area of filament chloride cells in control, sham (saline-injected) and experimental (cortisol-injected) fish. The chloride cells displayed similar ultrastructural modifications in trout undergoing cortisol treatment as in trout transferred to ion-deficient water. These findings suggest the existence of structure/function relationships in which branchial chloride cell morphology is an important determinant of Na^+ and Cl^- transport capacity. We conclude that chronic cortisol treatment enhances whole body Na^+ and Cl^- influxes by promoting proliferation of branchial chloride cells. The results of correlation analysis indicate that the chloride cell is an important site of NaCl uptake in freshwater rainbow trout.

Key words: Gill – Cortisol – Chloride cells – Ionic uptake – *Salmo gairdneri* (Teleostei)

In contrast to seawater, freshwater environments are characterized by extremely variable chemical properties including pH and ionic or gaseous composition (Dejours 1988). This variability has important consequences on the internal acid-base or ionic status of freshwater fish.

The variability of environmental conditions is most pronounced in euryhaline fish (e.g., salmonids) that are able to survive in seawater as well as in dilute freshwater approaching the osmolarity of distilled water (McDonald and Rogano 1986; Perry and Laurent 1989). In recent years, a variety of studies have focussed on the complex mechanisms whereby euryhaline fish adapt to diverse ionic environments (e.g., McDonald and Rogano 1986; Perry and Laurent 1989; see also review by Evans 1984).

In seawater or seawater-adapted fish, the chloride cell is accepted as being the principal effector of hypoosmoregulation (see review by Laurent 1989). The participation of the chloride cell, however, in hyperosmoregulation has been largely ignored in the past although branchial cell proliferation was observed in fish maintened in dilute media (Mattheij and Stroband 1971). The role of the chloride cell in adaptation of fish to dilute environments was not examined further until Laurent and Dunel (1980) reported a reversible proliferation of chloride cells after transfer of trout from freshwater to deionized water and vice-versa. Additional studies have confirmed that branchial chloride cell proliferation is a common response when fish are exposed to dilute NaCl environments (Laurent et al. 1985; Perry and Wood 1985; Avella et al. 1987). Most recently, Perry and Laurent (1989) demonstrated that chloride cell proliferation is an important adaptational response in rainbow trout adapted to NaCl-deficient water because it promotes an increase in gill Na^+ and Cl^- transporting capacity.

The aim of the present study was to quantify the relationship between the whole body influx of Na^+ or Cl^- and gill chloride cell morphology and thereby infer a role of the chloride cell in freshwater fish. Chronic cortisol treatment was used as a tool to induce chloride cell proliferation (Perry and Wood 1985). Measurements of whole body Na^+ and Cl^- influxes in control, sham-treated, and cortisol-treated fishes permitted correlation analysis over a wide range of chloride cell surface areas.

Materials and methods

Experimental animals

Rainbow trout (*Salmo gairdneri* Richardson) of either sex, weighing between 88 and 246 g (mean weight = 139.5 ± 3.7 (SE) g; experimental $n = 78$), were obtained from Thistle Springs Trout Farm (Ashton, Ontario) and transported to the University of Ottawa. Fish were held indoors in large fibreglass tanks (Living Stream; Toledo, Ohio) supplied with flowing, aerated and dechlorinated City of Ottawa water ($[Na^+] = 0.10$ mM; $[Cl^-] = 0.15$ mM; $[Ca^{2+}] = 0.35$–0.40 mM; $[K^+] = 0.03$ mM; pH = 7.5–8.0). Fish were acclimated to these conditions for at least 6 weeks prior to experimentation. Water temperature in both holding and experimental facilities varied between 10 and 16° C during the course of experiments. Photoperiod was kept constant at 12 h light:12 h dark. Fish were fed a diet of dried commercial trout pellets (Purina Trout Chow) daily, but were not fed 48 h before experiments commenced.

Send offprint requests to: Dr. Pierre Laurent, Laboratoire de Morphologie Fonctionnelle, CNRS, 23 Rue de Loess, BP 20 CR, F-67037 Strasbourg Cedex France

Protocol

(i) Chronic studies: Fish were separated into 3 groups and individual fish within these groups were transferred to opaque acrylic boxes (flux boxes) supplied with flowing, aerated water. The control group ($n=18$) were left untreated for 10 days; the sham group ($n=24$) received daily intramuscular injections of 0.1 ml physiological saline (Wolf 1963) for 10 days. The experimental group (cortisol-treated; $n=24$) received daily intramuscular injections of cortisol (hydrocortisone 21-hemisuccinate sodium salt; 4 mg kg^{-1} body weight) in 0.1 ml physiological saline (Perry and Wood 1985).

On day 11, whole body Na^+ or Cl^- influx ($JinNa^+$ or $JinCl^-$) was determined on separate groups of fish. Water flow to the boxes was terminated for a 3-h period during which the partial pressure of oxygen was maintained by vigorous aeration of the ambient water. Water temperature was stabilized by partially immersing the flux boxes in cooling baths. $JinNa^+$ or $JinCl^-$ was assessed by monitoring the disappearance of $^{22}Na^+$ (as NaCl; Amersham) or $^{36}Cl^-$ (as HCl; ICN) from the water after addition of 1.25 μCi (46.25 kBq) of isotope to each box and a 15 min mixing period. Water samples (10 ml) were removed following the mixing period and 3 h later to determine ^{22}Na or ^{36}Cl specific activity (DPM/μMol). Fish were sacrificed by overdose of anaesthetic (1 g l^{-1} of ethyl m-aminobenzoate (MS 222) added directly to the 3-l flux box), and a terminal blood sample (1 ml) was withdrawn via caudal puncture. The blood was centrifuged and the plasma stored (−20° C) for subsequent analyses of electrolytes. A portion of gill from the second arch (left side) was excised and processed for morphological examinations.

(ii) Acute studies: Fish were anaesthetized in a 1:10000 (W/V) solution of MS 222 adjusted to a pH of 7.0–7.5 with NaHCO. Indwelling dorsal aortic cannulae were implanted according to the method of Smith and Bell (1964) using flexible polyethylene tubing (Clay Adams PE 50; i.d.= 0.58 mm; o.d.=0.97 mm). After surgery, fish were placed into individual flux boxes (see above) in which they were allowed to recover for 48 h before experimentation. Dorsal aortic cannulae were flushed daily with saline.

Whole body Na^+ or Cl^- influxes were determined as described above. After this initial 3-h pre-infusion flux period, the boxes were rinsed with water for 15 min. Cortisol dissolved in saline (16.7 mg 100 ml^{-1} saline; pH=7.8–8.0), was administered to the fish by infusion into the dorsal aortic cannula (0.6 ml h^{-1}) using a syringe pump (Sage). $JinNa^+$ or $JinCl^-$ was determined during the first 3-h period of cortisol-infusion. Fish were sacrificed, and a terminal blood sample was taken (see above) and stored (−20° C) for later determination of plasma cortisol levels.

Analytical procedures

^{22}Na and ^{36}Cl activities were determined using liquid scintillation counting (LKB 1211 Rackbeta). Total Na^+ and Cl^- concentrations in water were determined by flame emission spectrophotometry (Varian Spectra 10) and amperometric titration (Buchler-Cotlove Chloridometer), respectively. From these measurements, whole body $JinNa^+$ and $JinCl^-$ were determined as described by Maetz (1956). Backflux correction was unnecessary.

Plasma [Na^+] was determined on diluted plasma (200×), while plasma [Cl^-] was measured on 100 μl of undiluted plasma, as described above. Plasma cortisol levels were determined by using a commercial cortisol (^{125}I) radioimmunoassay kit (Corning).

Morphological methods

A common fixation procedure was used to prepare gill tissue for scanning electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM). The procedure involved fixing small pieces of gill in 2.5% glutaraldehyde buffered with 0.15 M sodium cacodylate (pH=7.4) for 1 h at 4° C and subsequently in 2.5% osmium tetroxide in distilled water for 1 h at room temperature. Glutaraldehyde was used as it is known to cause less shrinkage in various tissues than formaldehyde (Mazzone et al. 1980).

Additional ultrastructural studies were made on animals during cortisol treatment and after transfer from Strasbourg tapwater ($Na^+=0.49$ mM, $Cl^-=0.82$ mM, $Ca^{1+}=2.44$ mM, pH=8.4) into ion-deficient water freshwater; $Na^+=0.14$ mM, $Cl^-=0.05$ mM, $Ca^{2+}=0.27$ mM, pH=7.5) and vice-versa.

Sampling of gill filaments: A central portion of arch II (left side) was excised from animals immediately after death. Each piece contained a few pairs of filaments. Most of the arch tissue was removed with a razor blade but anterior and posterior rows of filaments remained attached to the septum of the arch. These pieces were immersed in the fixative, subsequently rinsed in cacodylate buffer (0.15 M; pH=7.4; osmotic pressure=292 mOsm) and postfixed in 2.5% osmium tetroxide. Portions of anterior and posterior individual filaments were taken at the site where filaments separate from the septum. These pieces, which contained about 20 lamellae, were embedded in Araldite to allow cross-sectioning of the lamellae or the filament. Semithin sections (1 μm thick) and ultrathin sections were prepared with an automatic ultramicrotome (Ultracut, Reichert).

Measurement of the apical surface area of chloride cells. This was accomplished using SEM. Pairs of filaments still attached to the septum were fixed with glutaraldehyde (see above) and postfixed for 1 h with osmium tetroxide in cacodylate buffer and dehydrated by the critical-point method. Alternatively, samples were dehydrated completely using an ethanol series, placed in two successive baths (2 min) of 1,1,1,3,3,3 hexamethyldisilazan (Aldrich) and then air-dried. The pairs of filaments were glued with silver paint on a specimen stub suitable for a Stereoscan 100 scanning electron microscope (Cambridge Ltd) in a way that maintained the lateral side of the filament in a plane parallel with the stub plate. The portion of the filament epithelium on the trailing edge of the filament (at the site of separation from the septum and close to the departure of the lamellae) was focussed on the screen and both anterior and posterior filaments were photographed at a magnification of 1000. At least 4 non-contiguous fields were photographed from each filament for morphometric measurements. A total of 48 fields was measured per fish. Chloride cell surface area was determined by underlining chloride cell perimeters on a digitizer tablet (Summagraphics) assisted by a microcomputer (CBM 8096SK). Data were computed by using a morphometric programme (written by von Giese, MTS Med. Techn. Apparate, GMBH, Tübingen, FRG, 1980) with a practical error of less than 1%. In addition to the mean

Table 1. The effects of chronic cortisol treatment on plasma Na^+ and Cl^- levels in rainbow trout (*Salmo gairdneri*).
\$ significantly different from corresponding control value ($P < 0.05$); *n* values are indicated in parentheses

	Controls (18)	Sham-treated (24)	Cortisol-treated (24)
$[Na^+]$ (mM)	139.4±2.4	140.5±2.3	136.8±1.9
$[Cl^-]$ (mM)	120.9±3.7	129.1±2.1 \$	124.9±2.1

Table 2. The effects of acute intra-arterial infusion of cortisol (over a 3-h period) on whole body influx of Na^+ ($JinNa^+$) or Cl^- ($JinCl^+$) in the rainbow trout (*Salmo gairdneri*); *n* values are indicated in parentheses

Condition	$JinNa^+$ ($\mu mol\ kg^{-1}\ h^{-1}$)	$JinCl^-$ ($\mu mol\ kg^{-1}\ h^{-1}$)
Pre-infusion	180.4±20.1 (6)	122.0±14.4 (6)
0–3 h Cortisol infusion	168.4±19.4	101.3±16.7

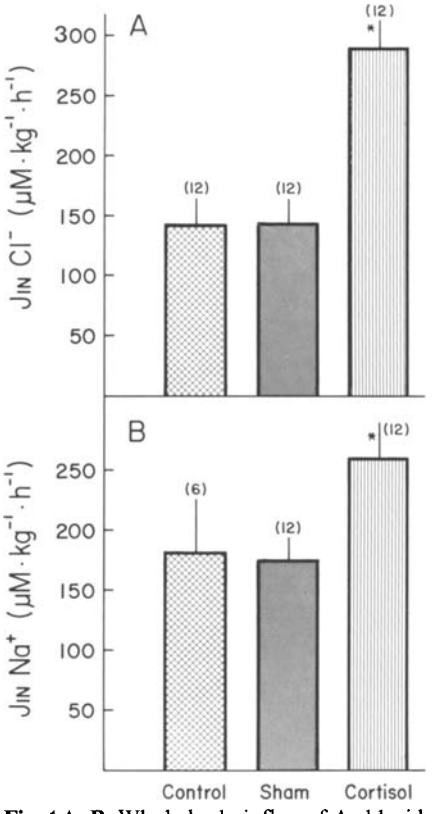


Fig. 1 A, B. Whole body influx of **A** chloride ($JinCl^-$) or **B** sodium ($JinNa^+$) in control, sham-, and cortisol-treated rainbow trout. Numbers of experimental animals are indicated in parentheses; * significantly different from control value ($P < 0.05$)

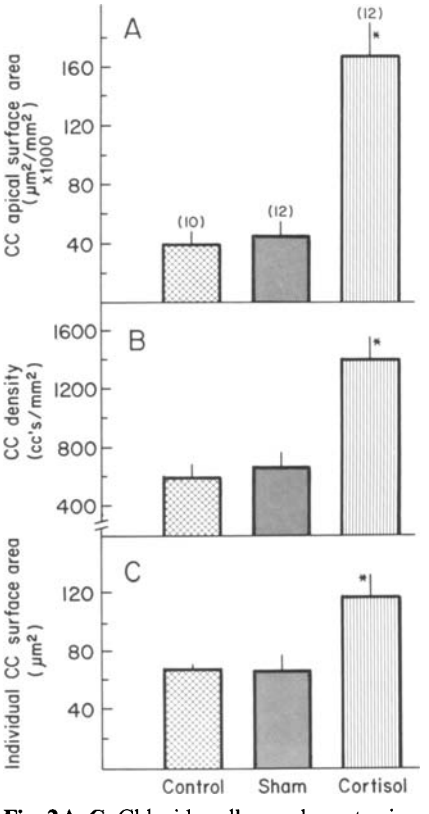


Fig. 2 A–C. Chloride cell morphometry in control, sham-, and cortisol-treated rainbow trout including **A** fractional chloride-cell (*CC*) apical surface area (per unit of filament epithelium area) (× 1000), **B** CC density, and **C** surface area of individual CC's. Numbers of experimented animals are indicated in parentheses; * significantly different from control value. All other details as in Fig. 1

apical surface of individual cells, the fractional surface area of chloride cells per unit of filament epithelium area was also calculated.

Statistical analysis

In Figures and Tables, variability of the data is indicated by ±1 SE. Results have been statistically analysed using paired or unpaired Student's *t*-test between appropriate sample means; 5% was taken as the fiducial limit of confidence.

Results

The effects of chronic (10 days) cortisol treatment on plasma Na^+ and Cl^- concentrations are shown in Table 1. No significant differences were observed between control and cortisol-treated fish, whereas a small rise in plasma $[Cl^-]$ was observed in sham-treated fish.

Whole body influxes of Na^+ ($JinNa^+$) and Cl^- ($JinCl^-$) were significantly increased after cortisol treatment (Fig. 1), but were unaffected by acute (3 h) intra-arterial infusion of cortisol (Table 2).

Several morphometric characteristics of the chloride cell population of the filament epithelium were assessed in parallel with Na^+ and Cl^- influx measurements. Cortisol treatment caused an increase in (i) surface area of individual chloride cells, (ii) chloride cell density, and (iii) fractional chloride cell area (Fig. 2). The mean area occupied by chloride cell apical surfaces represented about 2.5% of the trailing edge of the filament surface in control or sham-treated trout and nearly 10% in cortisol-treated trout.

To quantify the relationships between Na^+ or Cl^- influx and chloride cell morphometry, correlations were calculated between Na^+ or Cl^- influx and fractional chloride

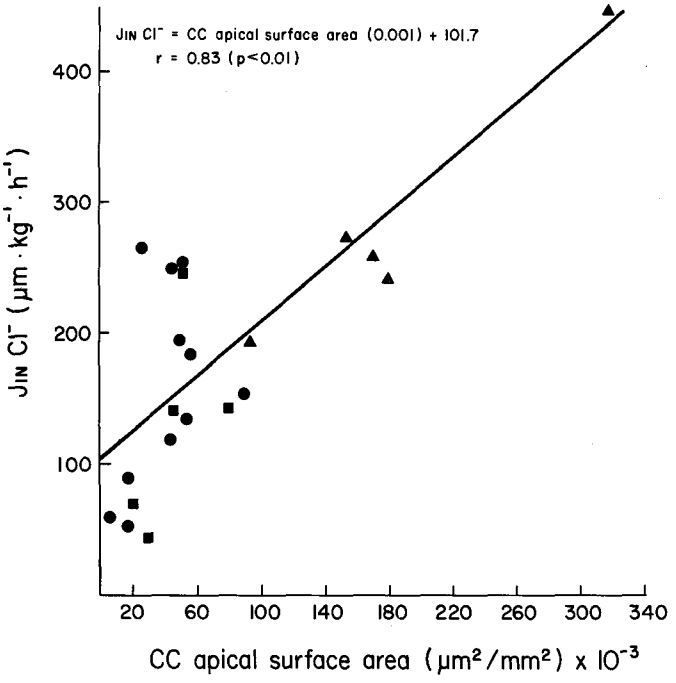


Fig. 3. Correlation of whole body chloride influx ($JinCl^-$) and fractional filament chloride-cell apical surface area in control (*filled squares*), sham- (*filled circles*), and cortisol-treated (*filled triangles*) rainbow trout. The regression equation was calculated from all data (controls, sham-, and cortisol-treated)

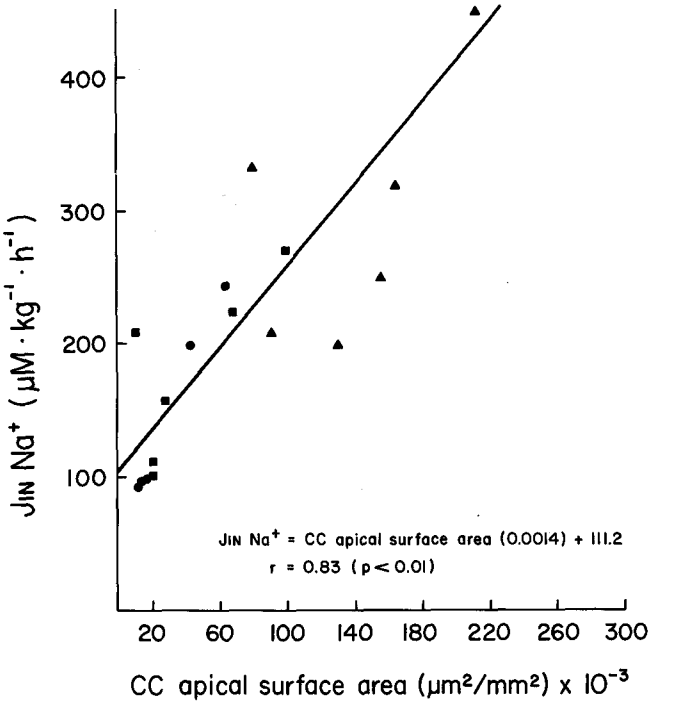


Fig. 4. Correlation of whole body sodium influx ($JinNa^+$) and fractional filament chloride-cell apical surface area. All other details as in Fig. 3

cell apical surface area. All available data were incorporated from the control, sham- and cortisol-treated animals. Both $JinNa^+$ and Jin Cl^- correlated significantly with the total surface area of chloride cell apical membranes per unit of filament epithelium (fractional surface area) (Figs. 3, 4).

Considering the results from individual fish, it was occasionally observed that control and sham-treated fish displayed unusually high values of $JinNa^+$ or $JinCl^-$. These fish also displayed unusually large numbers of chloride cells with enlarged apical surfaces both on the filament and lamellar epithelia. Individuals with low (well below average) Na^+ or Cl^- influx values were never observed in cortisol-treated trout. $JinNa^+$ and $JinCl^-$ always were high (well above average) and the filament epithelium was encumbered with chloride cells. Representative SEM micrographs from individual control, sham- and cortisol-treated fish displaying low and high Na^+ or Cl^- influxes are shown in Figs. 5–10.

For practical reasons (primarily because the filament epithelium generally is flat and parallel with the specimen support of the microscope, allowing precise measurements of chloride cell morphometry), the quantification of chloride cells was restricted to the filament epithelia. Nevertheless, the lamellar chloride cell population was examined qualitatively to allow comparison with those present on the neighbouring filament surface. Two representative examples are shown: Figs. 11 and 12 illustrate portions of lamellar and filament epithelia, respectively, for an individual control fish displaying low ionic influx. On both epithelia, chloride cells were rare and displayed small apical surfaces. Conversely, it is clear that chloride cells with large apical surfaces were abundant on both lamellar (Fig. 13) and filament (Fig. 14) epithelia in a fish displaying high ionic influx. These observations indicate that chloride cell distribution and morphology change in parallel on the lamella and filament. Thus, the chloride cell invasion of the lamella appears to be closely related to proliferation of these cells on the filament. In cortisol-treated trout, invasion of the lamella is considerable (Fig. 15). Concomitantly, chloride cells are extremely dense on the filament (Fig. 16).

Careful examination of the apical surface of chloride cells revealed several types of villous organisation. In trout displaying low flux rates (where chloride cells presumably were not very active), villi were scarce, small and punctiform (Fig. 12). In trout displaying high flux rates, villi were densely packed, long and spine-like (Fig. 16) or formed continuous ridges (Fig. 7). Often, these 2 latter types of organisation were observed on neighboring cells (Fig. 16). Finally, it was common to observe chloride cells with flat,

Fig. 5. SEM of filament epithelium of a control trout displaying low Na^+ influx ($JinNa^+ = 93.4\ \mu M \cdot kg^{-1} \cdot h^{-1}$); *asterisk* chloride cell apical surface; *mc* mucus cell

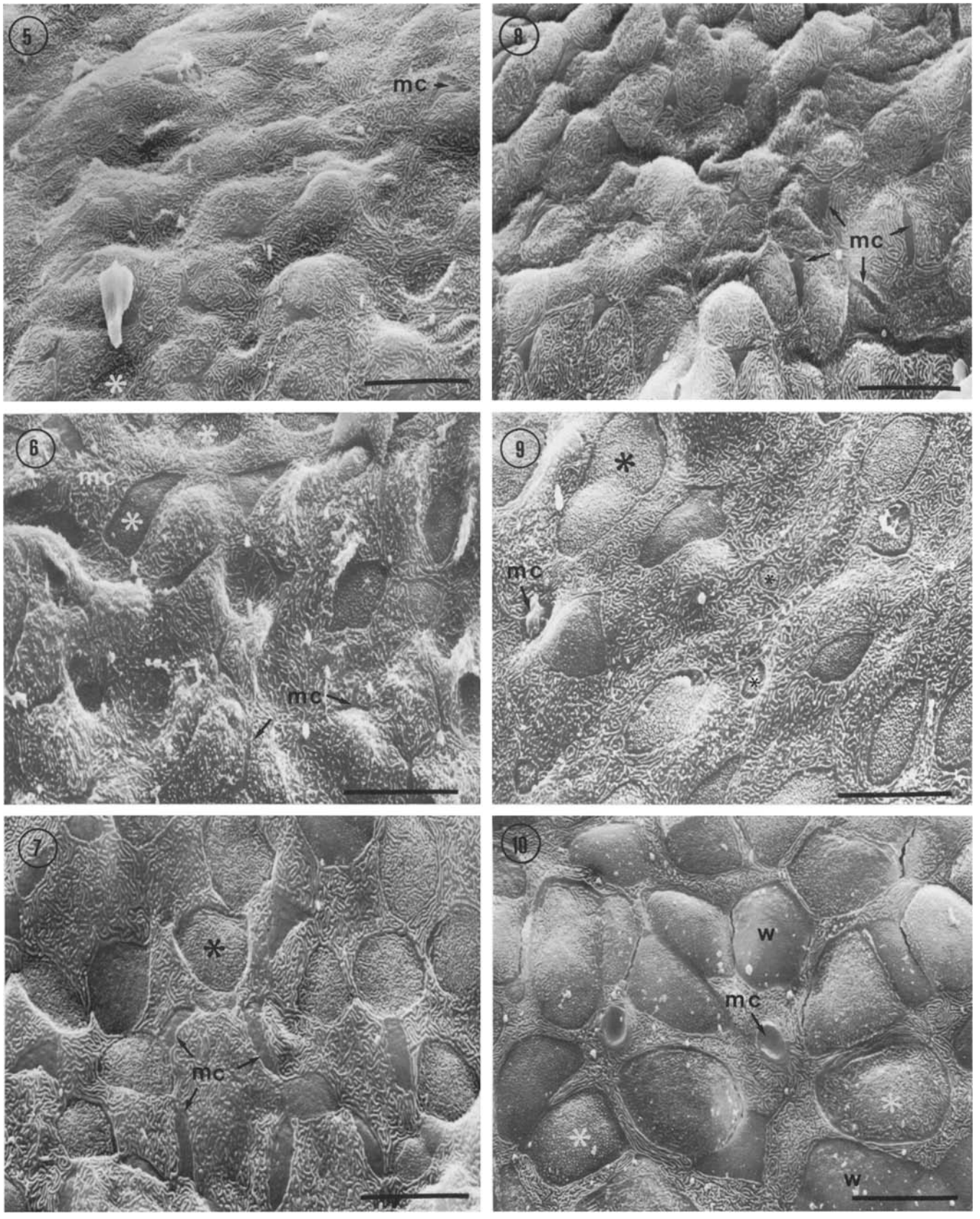
Fig. 6. SEM of filament epithelium of a control trout displaying high Na^+ influx ($JinNa^+ = 203.9\ \mu M \cdot kg^{-1} \cdot h^{-1}$)

Fig. 7. SEM of filament epithelium of a cortisol-treated trout displaying high Na^+ influx ($JinNa^+ = 198.8\ \mu M \cdot kg^{-1} \cdot h^{-1}$)

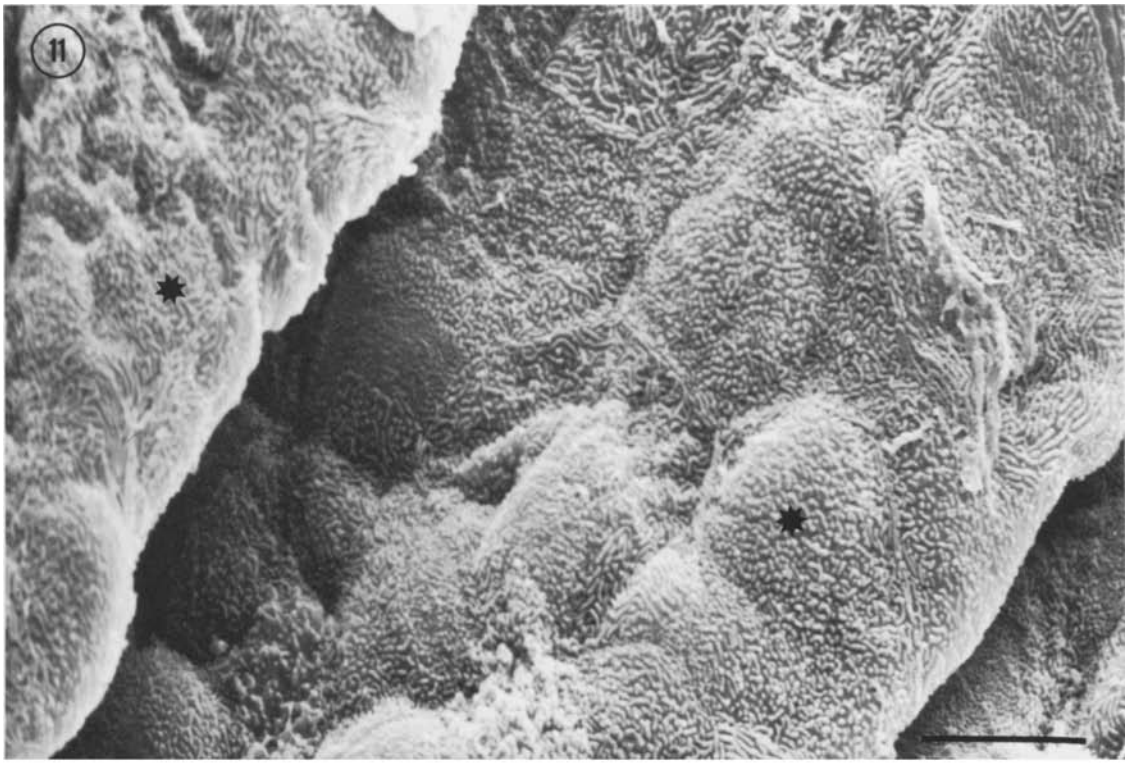
Fig. 8. SEM of filament epithelium of a control trout displaying low Cl^- influx ($JinCl^- = 55.3\ \mu M \cdot kg^{-1} \cdot h^{-1}$)

Fig. 9. SEM of filament epithelium of a control trout displaying high Cl^- influx ($JinCl^- = 154.4\ \mu M \cdot kg^{-1} \cdot h^{-1}$)

Fig. 10. SEM of filament epithelium of a cortisol-treated trout displaying high Cl^- influx ($JinCl^- = 533\ \mu M \cdot kg^{-1} \cdot h^{-1}$); *w* worn-out chloride cell



Compare Figs. 5 and 8 with Figs. 6 and 9, respectively, and note that trout displaying high influx rates have substantially greater numbers of chloride cells than trout with low influx rates (there were no trout with low influx rates in the cortisol-treated groups) (Figs. 7, 10). *Asterisk* chloride cell; *mc* mucus cell; *w* worn-out chloride cell. *Bar*: 20 μm



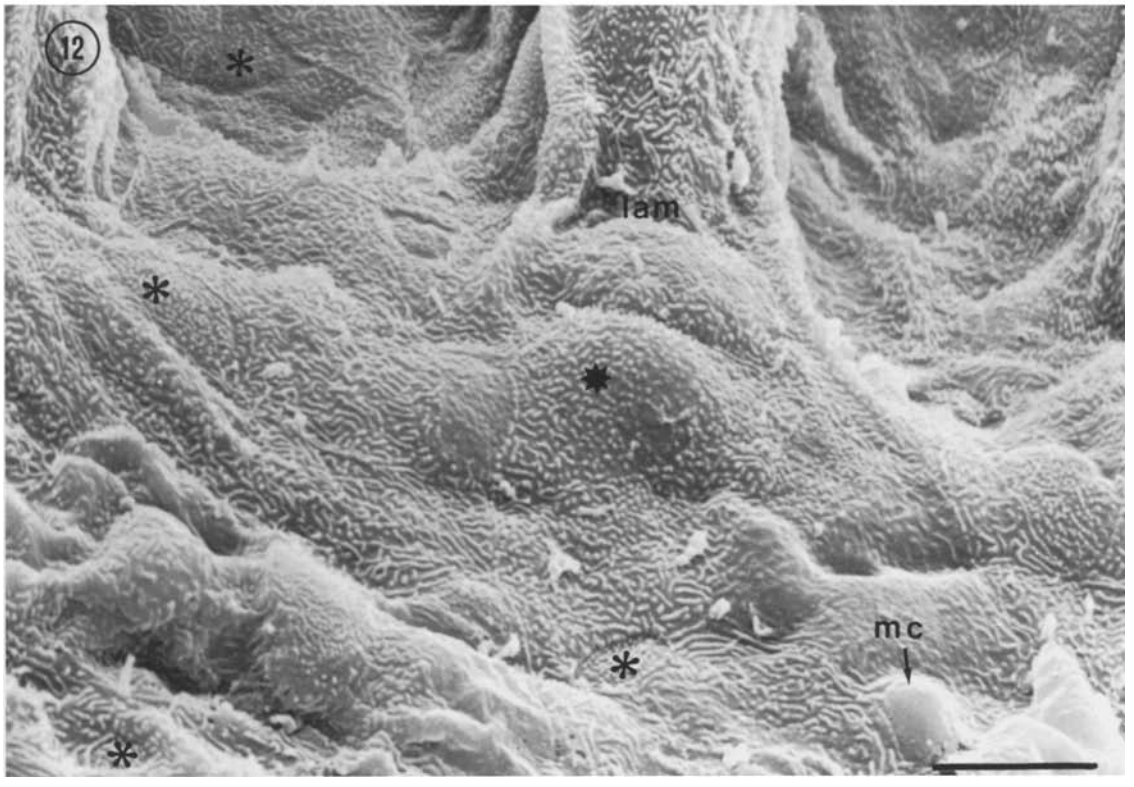
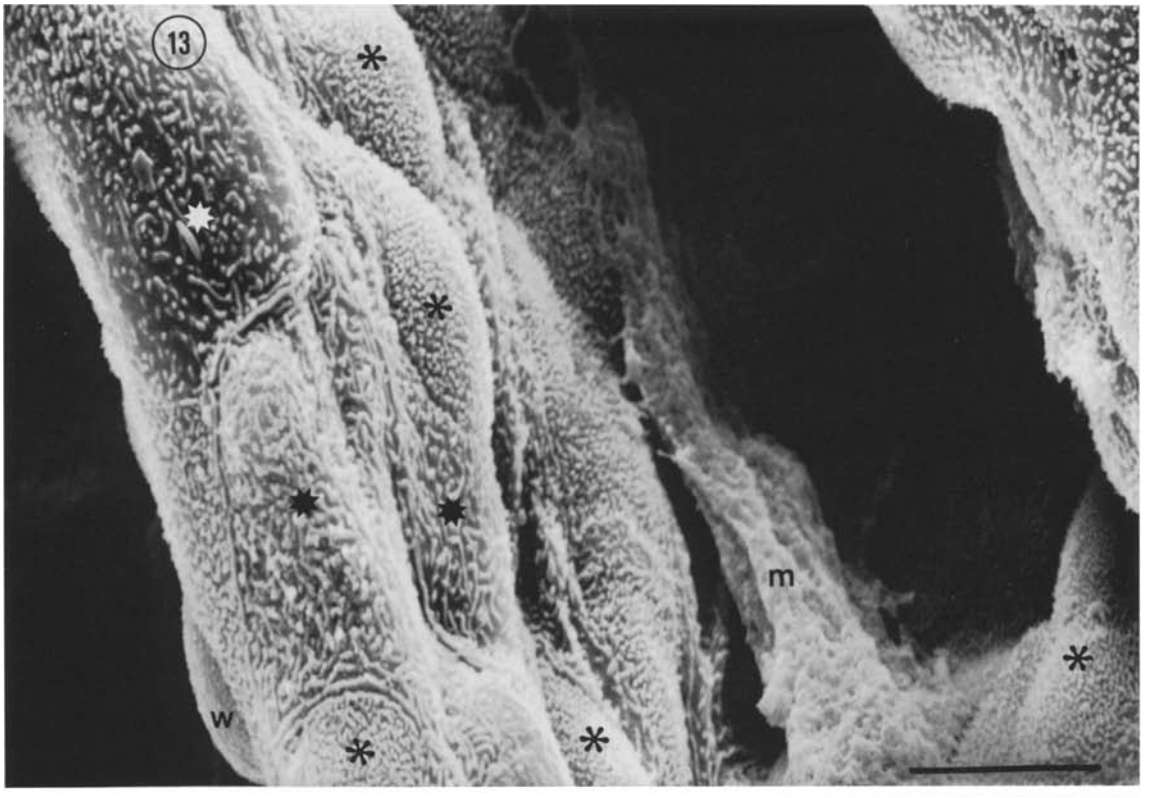


Fig. 11. SEM of lamellar epithelium of a control trout displaying low Na^+ influx ($JinNa^+ = 97.4\ \mu M \cdot kg^{-1} \cdot h^{-1}$). Note the absence of chloride cells. *Bar*: 5 μm

Fig. 12. SEM of filament epithelium from the same trout as in Fig. 11. The base of the lamella (*lam*) shown in this figure is that of the lamella shown in Fig. 11. Note the scarce and small chloride cells (*asterisks*) on the filament and absence of this type of cell on the lamella (Fig. 11). Examples of pavement cells (*star*) are marked both on lamella and filament. *Bar*: 5 μm

smooth, non-ornamented surfaces. This type of cell was noted most frequently in cortisol-treated trout (Fig. 10), but also was occasionally present in control trout displaying high ionic influx rates (Fig. 14). We also found intermediate stages between smooth and villous apical surfaces (Fig. 14). It is important to note that mucus cells were easily (e.g., Fig. 16) distinguished from smooth-surfaced chloride cells (Fig. 14).

The ultrastructure of the chloride cell and particularly of its apical region was studied comparatively under condi-



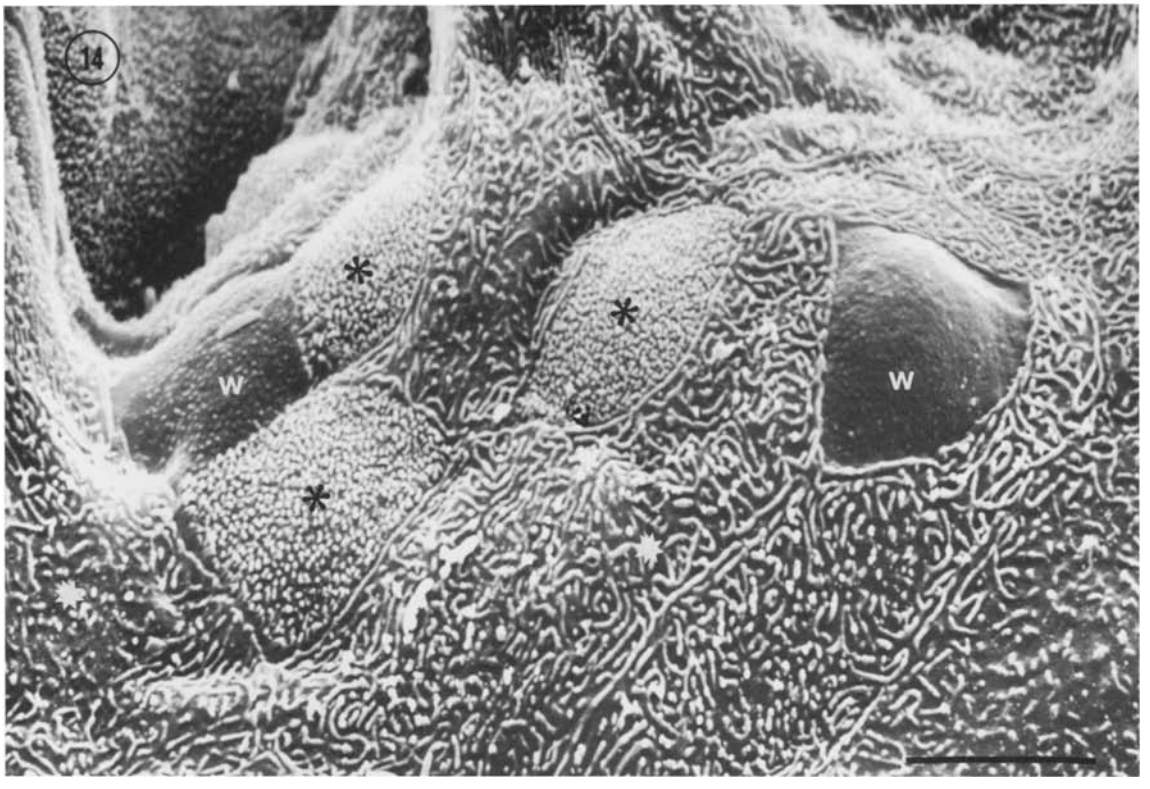
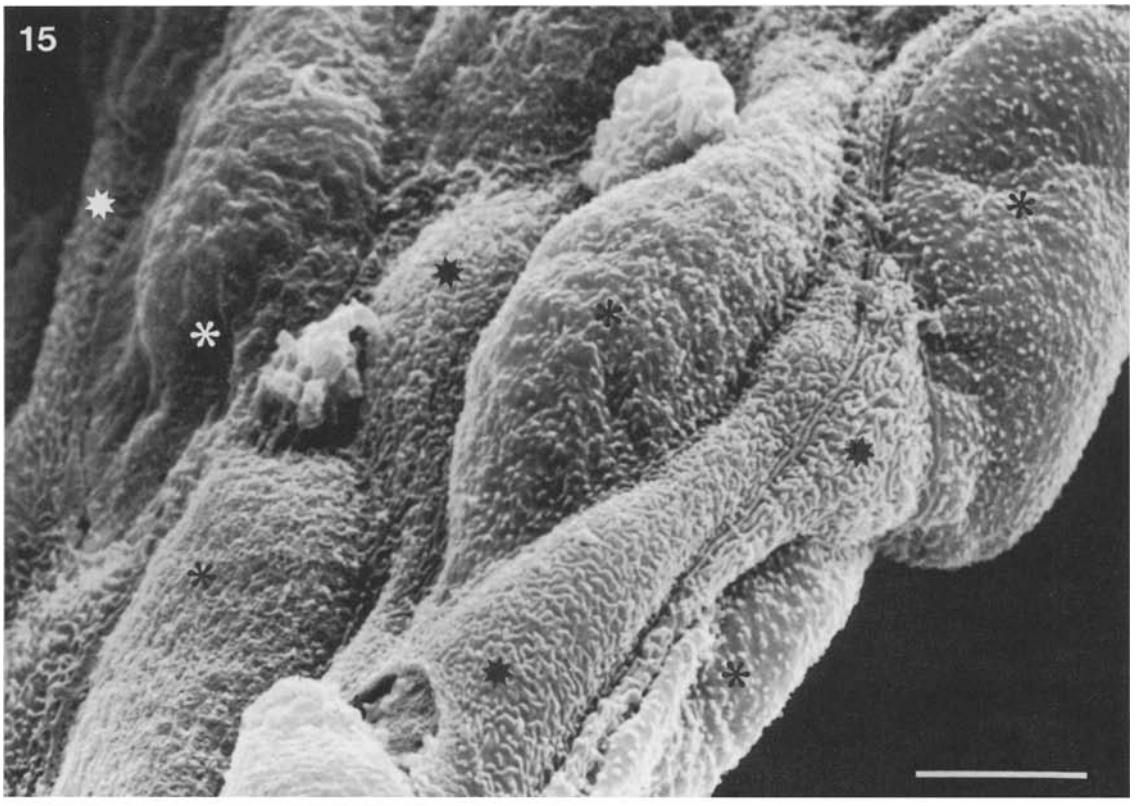


Fig. 13. SEM of lamellar epithelium of a control trout displaying high Cl^- influx ($JinCl^- = 194.3\ \mu M \cdot kg^{-1} \cdot h^{-1}$). *Bar*: 5 μm

Fig. 14. SEM of filament epithelium from the same trout as in Fig. 13. The base of lamella shown is that of the lamella shown in Fig. 13. Note the presence of large and numerous chloride cells of similar size and displaying the same apical morphology on both epithelia. A fragment of mucus (*m*) is still present within the interlamellar space (Fig. 13). Same symbols as in Fig. 13. *Bar*: 5 μm

tions of low or high NaCl influx. In natural tapwater (Fig. 17), chloride cells contacted the external milieu only via a small portion of the apical membrane. Numerous coated vesicles (0.10–0.25 μm in diameter) were concentrated beneath the apical membrane; these vesicles were much less numerous in the remainder of the cell. Chloride cells were often separated from the gill surface by a thin lining of pavement cells, especially in trout displaying low influx rates. Chloride cells were, therefore, not visible with



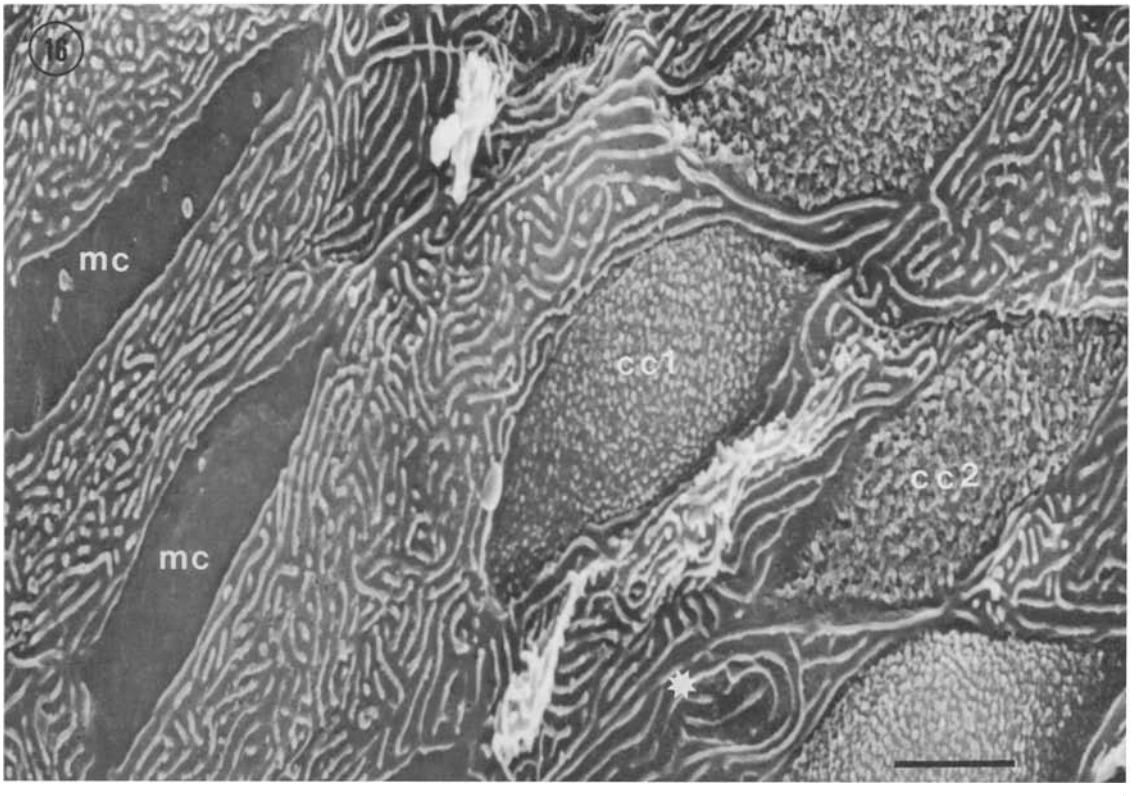
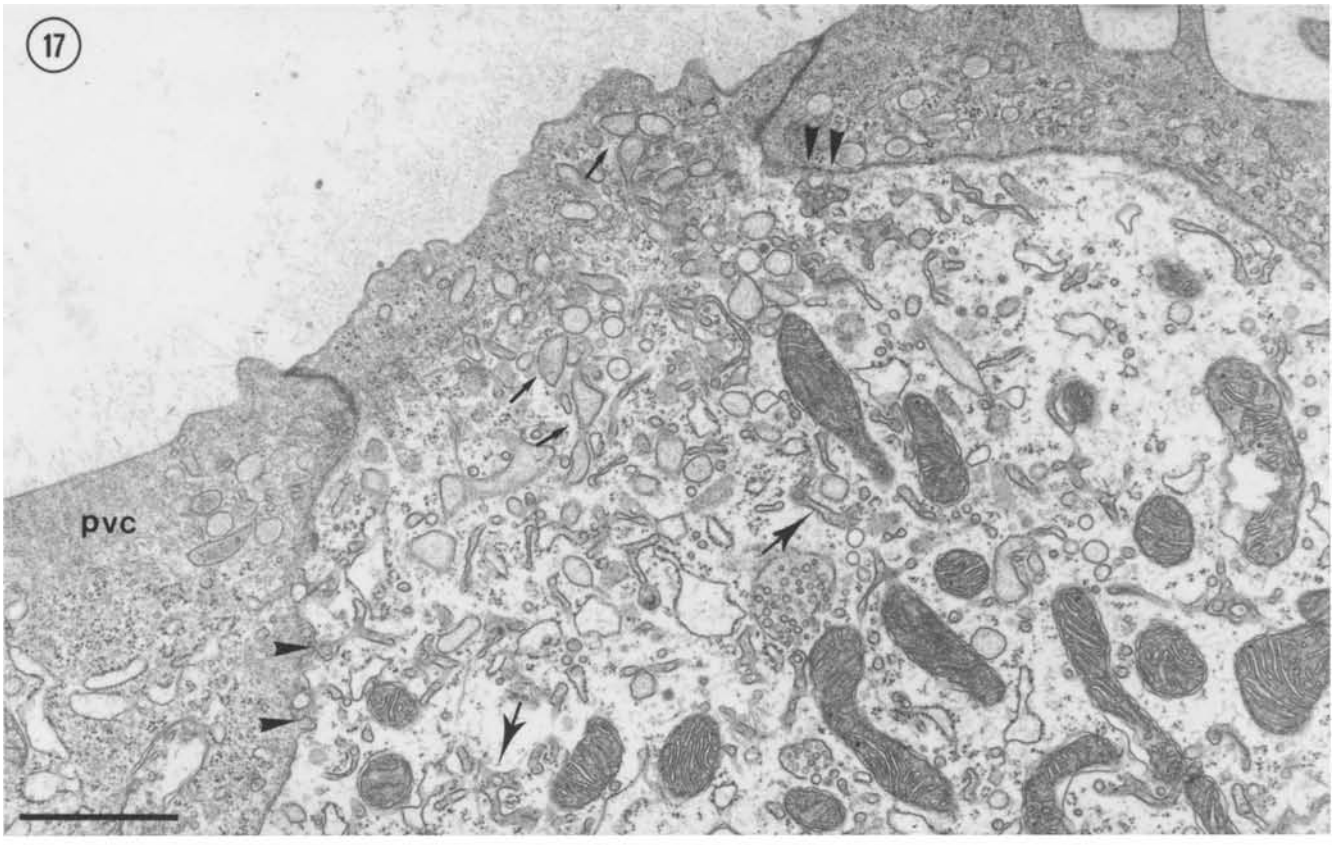


Fig. 15. SEM of lamellar epithelium of a cortisol-treated trout displaying high Na^+ influx ($JinNa^+ = 206\ \mu M \cdot kg^{-1} \cdot h^{-1}$). Note the drastic reduction of the lamellar surface lined up with pavement cells. *Bar*: 5 μm

Fig. 16. SEM of filament epithelium of the same cortisol-treated trout as in Fig. 15. Note some polymorphism of the apical surface morphology of chloride cells (*cc1*, *cc2*) and a clear difference between the apical surface of chloride and mucus cells (*mc*). *Bar*: 5 μm

Fig. 17. TEM of the apical portion of a filament chloride cell of rainbow trout adapted to Strasbourg tapwater for 2 weeks (ion-rich freshwater, see Materials and methods for ionic composition). Note the small apical region of the chloride cell in contact with the external milieu, the lining of pavement cell (*pvc*), the vesiculo-tubular system located at the apex of the cell (*small arrows*), the scarceness of the tubular system (this system actually is constituted by long and branched narrow invaginations of the chloride cell basolateral plasma membrane, *arrowheads*). *Bar*: 1 μm



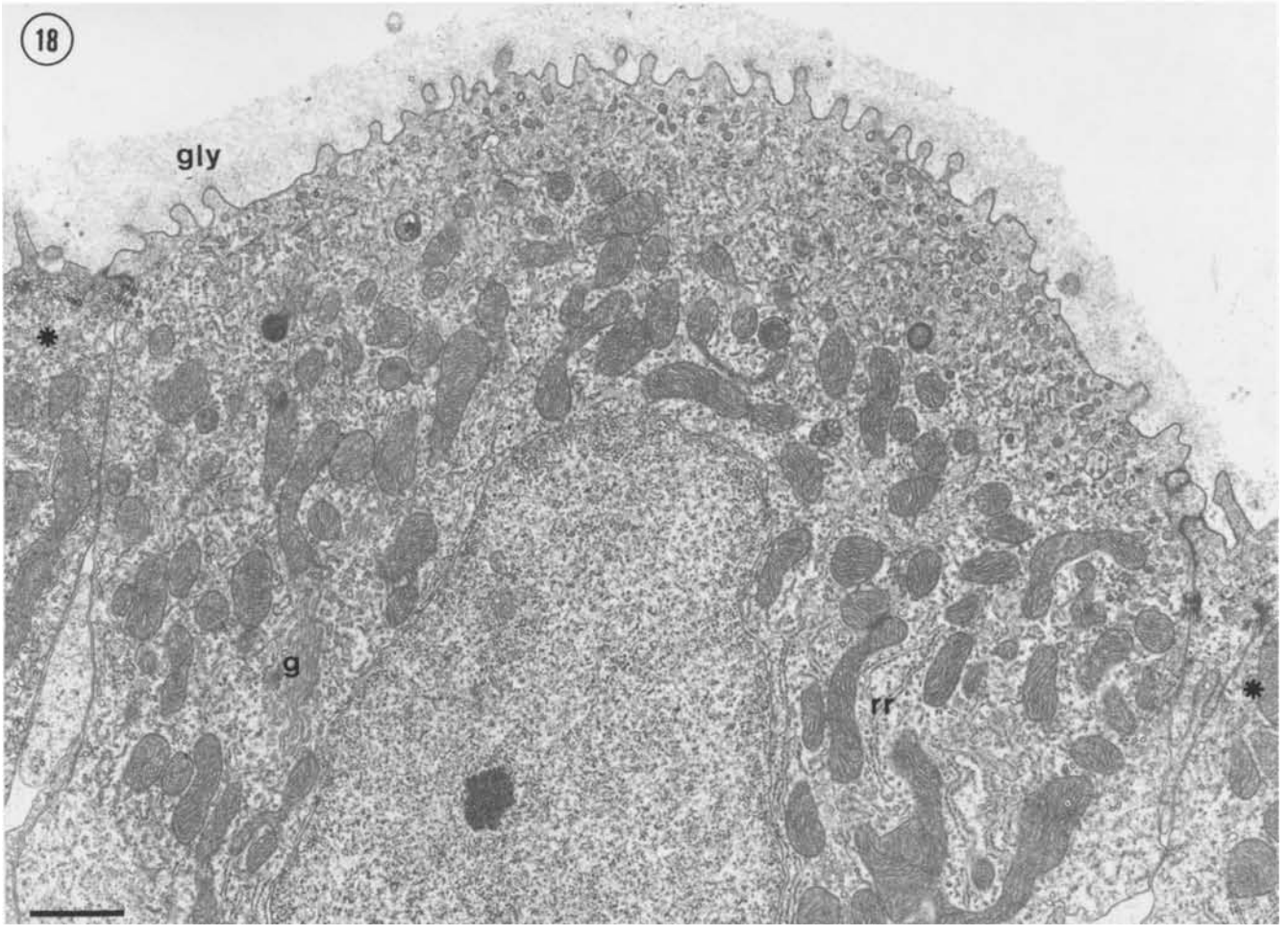
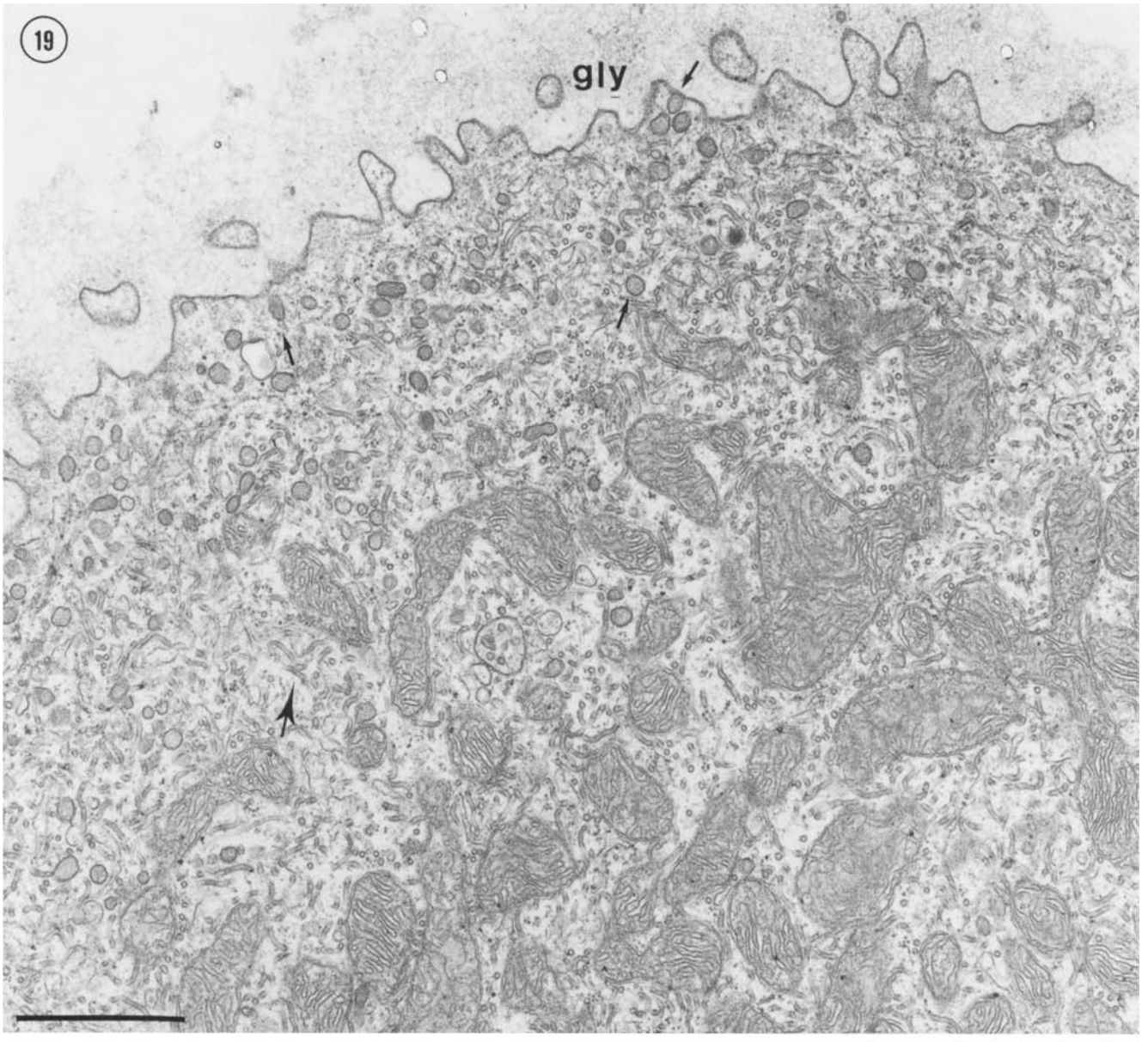


Fig. 18. Low-magnification TEM of a chloride cell from a rainbow trout maintained in Strasbourg tapwater, after 3 daily injections of cortisol. Three adjacent chloride cells are pictured. Note the expansion of the apical surface and its increased ornamentation, the tight distribution of mitochondria associated with a dense network of tubules, the abundance of Golgi complexes (*g*) and the rough endoplasmic reticulum (*rr*); *gly* glycocalix. *Bar*: 1 μm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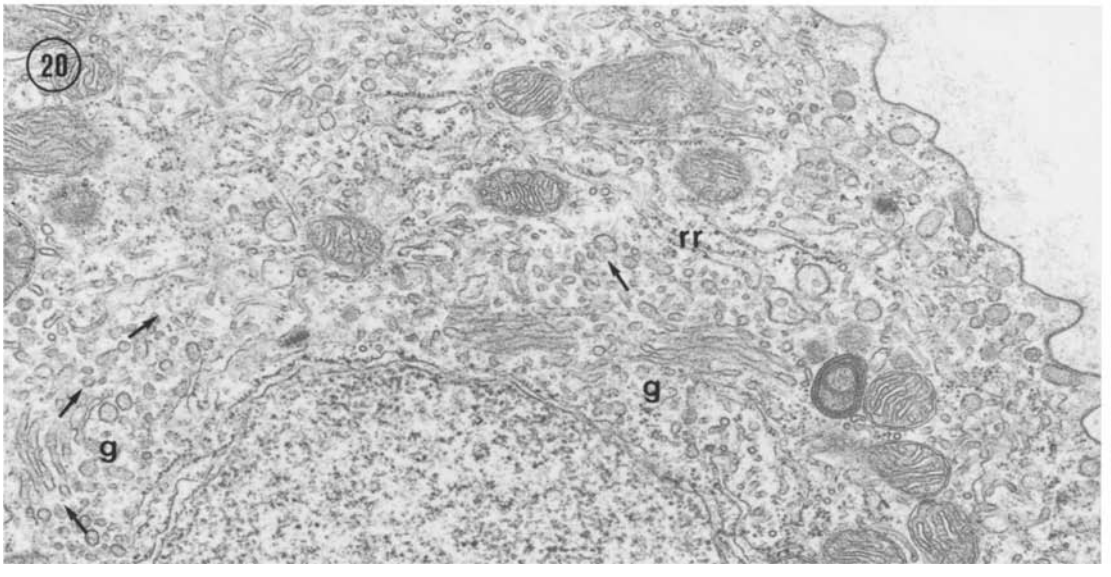
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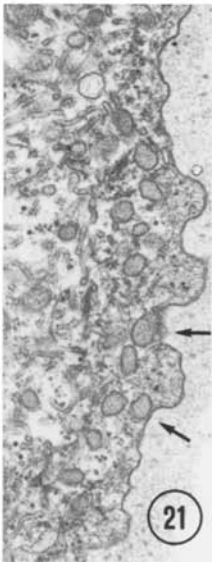
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the scanning electron microscope but were revealed by TEM. In trout with low influxes, the tubular system (baso-lateral infoldings) of the chloride cell was loosely distributed; the mitochondria were irregular in size and shape and appeared to be dispersed sparsely within the cytoplasm.

In trout displaying high ionic influxes (cortisol-treated), the chloride cell apical membrane area was increased. Fig. 18 shows a chloride cell from a trout on the third day of daily cortisol injections. The villous arrangement now formed a pattern of microridges (microplicae). The number of coated vesicles was increased (Fig. 19), some of them approaching the apical membrane with which they were fused (Fig. 21). A careful examination of high-magnification electron micrographs suggests that these vesicles originate from the Golgi complex (Fig. 20). The tubular system was dense and in close association with numerous mitochondria (compare Figs. 17 and 20).

The morphometric changes in the branchial chloride cell population induced by cortisol treatment can be mimicked when trout are transferred to ion-deficient water. After 3 weeks in ion-deficient water, the apical membrane of chloride cells was extended greatly by formation of microplicae due to the fusion of coated vesicles (Fig. 22). The tubulo-vesicular system (baso-lateral infoldings) also was increased. Upon return to normal Strasbourg tapwater for 1 week, the surface area of apical membranes was decreased. In addition, the complex system of microplicae was replaced by irregular and short villi. Membrane was retrieved from the plasma as suggested by Fig. 23 and presumably recycled within the chloride cell.

Discussion

It has long been recognized that cortisol, the major corticosteroid of fish, plays an important role in hypoosmoregulating fish (Chan et al. 1967; Mayer et al. 1967; Henderson and Chester Jones 1967; Hirano and Utida 1968; Epstein et al. 1971). In addition, cellular receptors for this hormone exist in teleost gill (Sandor et al. 1984; Chakraborti et al. 1985). There is accruing evidence that cortisol increases Na^+ secretion in marine teleosts (Lahlou 1980).

In hyperosmoregulating (freshwater) fish, the role of cortisol is less certain. Both directions of acclimation (from freshwater to seawater and vice-versa) cause an elevation of plama cortisol levels. In superfused interrenal tissue, deletion of perfusate [Na^+] has no effect, but its reintroduction in the perfusate stimulates the release of cortisol. In contrast, a decrease of Cl^- concentration causes a pronounced increase in cortisol release (Decourt and Lahlou 1986). Recently, it was shown that transfer of rainbow trout from natural freshwater into [NaCl]-deficient water induces a significant increase in plasma cortisol levels (Perry and Laurent 1989). In the freshwater eel, interrenalectomy reduces Na^+ uptake, a situation which is restored by administration of cortisol (Mayer et al. 1967). No similar data are yet available for trout but the present findings demonstrate that cortisol treatment increases influx of both Na^+ and Cl^-. Thus, cortisol appears to promote salt uptake in freshwater fish, a conclusion strengthened by the impact of cortisol on the chloride cell population in rainbow trout noted in this study.

The concurrent increases of chloride cell surface area and $JinNa^+$ or $JinCl^-$ suggest that these cells are an important site of NaCl uptake in freshwater. It is worth mentioning that significant positive correlations were found not only in cortisol-treated trout but in control fish as well. In other words, this correlation concerns a wide range of influx values, from the smallest to the largest, measured in this study regardless of treatment. The significant linear increase of Na^+ or Cl^- influx as a function of chloride cell apical surface area suggests that NaCl influx is limited by the availability of apical membrane "transport sites". Incidentally, the parallel changes observed in chloride cell abundance and their morphology on both lamellar and filament epithelia support the concept of a common role of chloride cells regardless of their location. There is no reason, so far, to consider the function of chloride cells to be different on filaments and on lamellae.

There are no data yet available concerning the relationship between the morphology of the chloride cell apical membrane and the rate of branchial ion transport in freshwater fish. A limited number of attempts have been made concerning several other types of epithelia such as turtle bladder (see review by Steinmetz 1986), nephron segments (Kaisling 1985) and their cellular adaptation to different physiological conditions. For example, morphometric studies (Stetson and Steinmetz 1983) show that stimulation of H^+ secretion in the turtle urinary bladder is associated with an increase in the luminal membrane surface area of the cells containing carbonic anhydrase. Simultaneously, there is a decrease in the volume density of the tubulo-vesicular membrane structures in the apical region of these cells. On this material, SEM has revealed that physiological conditions provoking a stimulation of H^+ secretion are associated with conversion of apical microvilli to microplicae (Stetson and Steinmetz 1983).

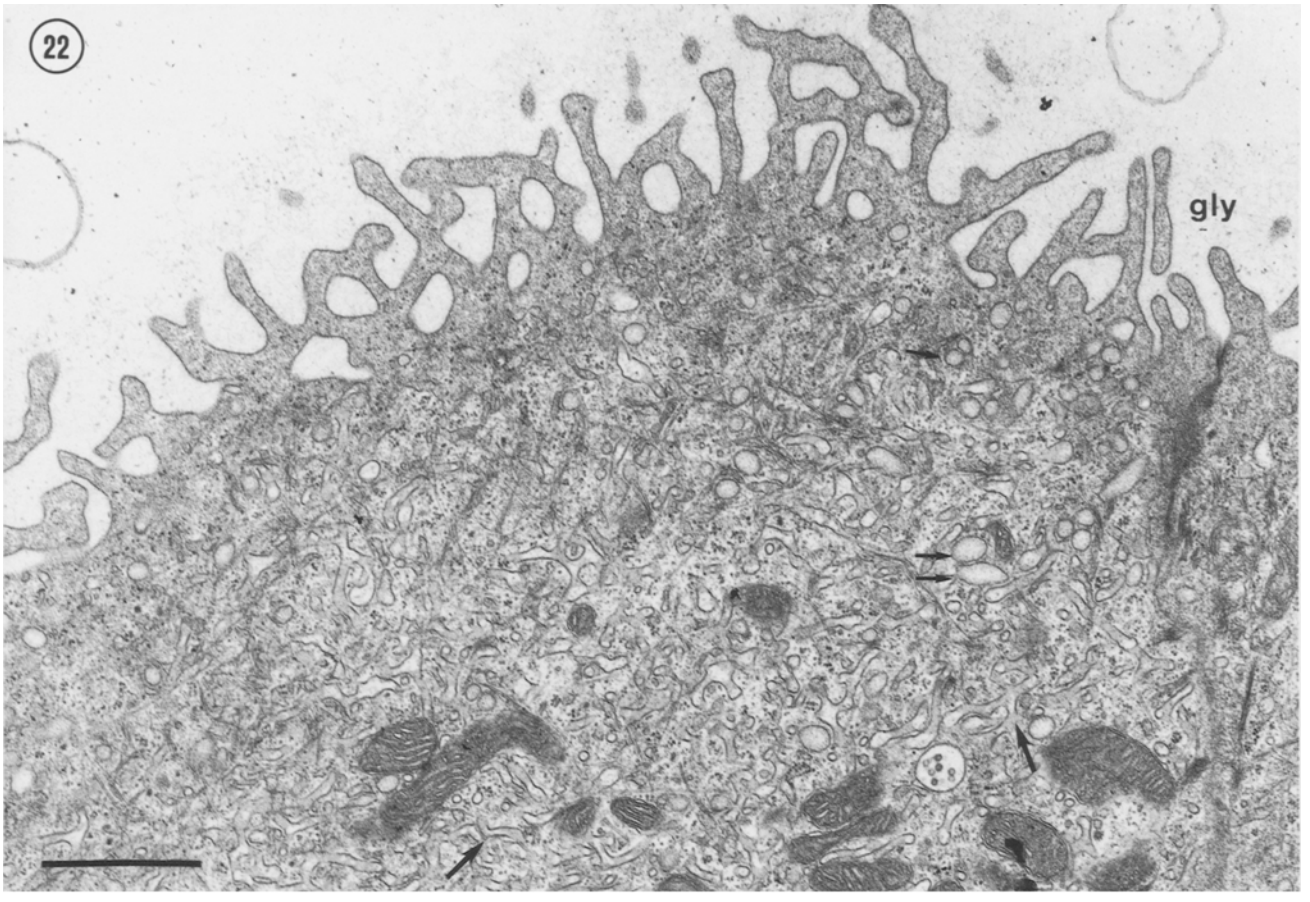
In the collecting ducts of rats with acute respiratory or chronic metabolic acidosis, the luminal surface area of the so-called I (intercalated) cells is increased with a concomitant decrease of the tubulo-vesicular membrane structures in the apical region of the cell. This observation suggests that membrane material is being transferred from the tubulo-vesicular system into the apical plasma membrane (Madsen and Tisher 1983, 1984).

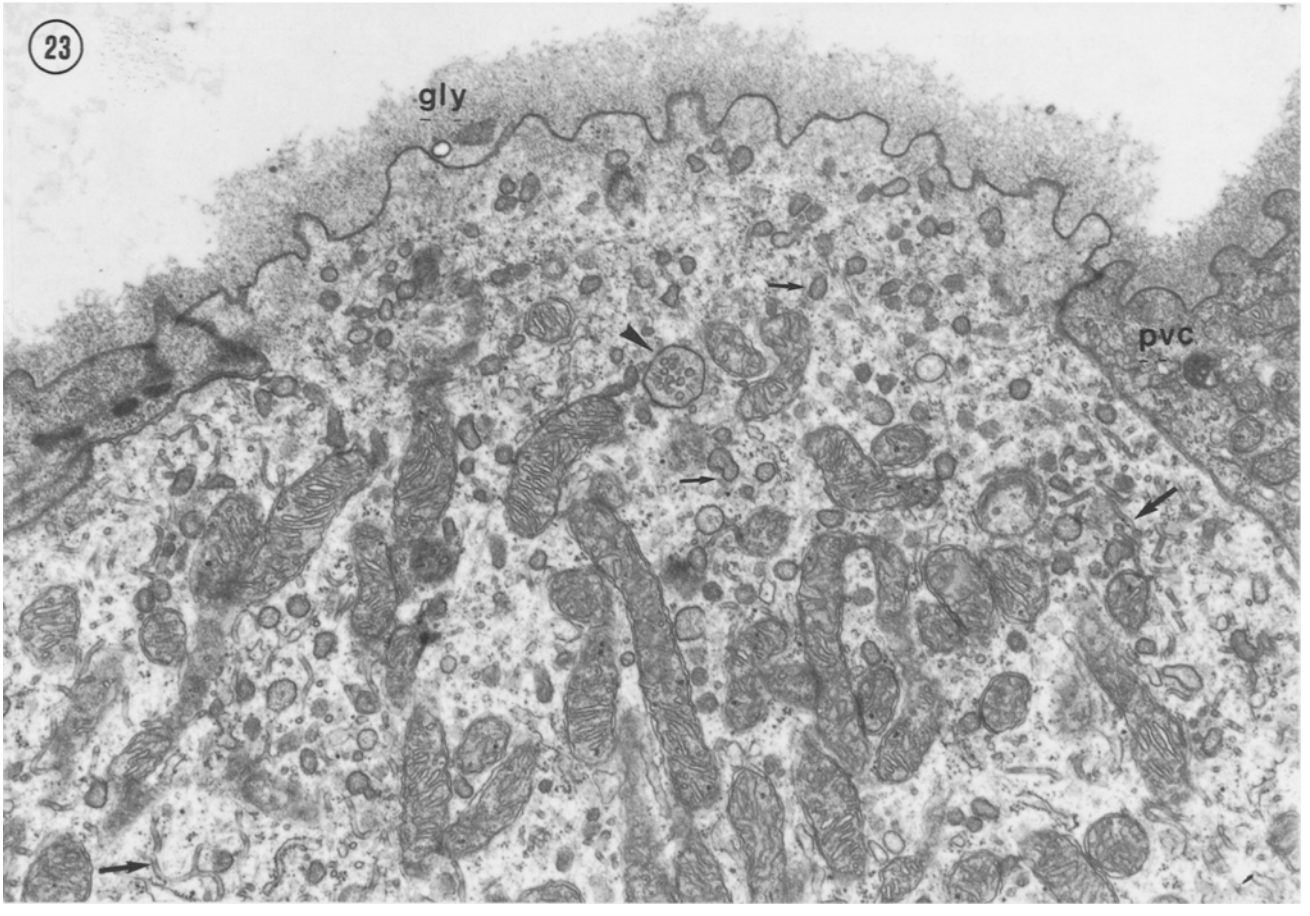
The freshwater chloride cell has a morphology similar to the above mentioned cells (see review by Laurent 1984). It has a tubular system (infoldings of the baso-lateral mem-

Fig. 19. High-magnification TEM of a chloride cell from cortisol-treated rainbow trout. Note the structure of the vesicles (*arrows*) approaching the apical membrane with which they appear to fuse (*large arrow*) and the 1-μm thick cell coat (glycocalix: *gly*). This micrograph suggests that vesicles carry the glycocalix for insertion into the cell membrane. *Bar*: 1 μm

Fig. 20. High-magnification TEM of a chloride cell from cortisol-treated trout as in Fig. 19. The Golgi complex appears to participate in the elaboration of apical vesicles containing a fibrous material (*arrows*). Same magnification as in Fig. 19

Fig. 21. High-magnification TEM of the apical portion of a chloride cell from the same trout as in Fig. 20 showing vesicles containing a fibrous material. Some vesicles appear to contact the apical membrane of the chloride cell (*arrows*) suggesting a process of fusion. Same magnification as in Fig. 19


22
gly


23
gly
pvc

brane), a vesiculo-tubular system (related to the apical membrane), a villous apical (luminal) membrane. It is rich in carbonic anhydrase (Rahim et al. 1988). Presumably, it participates in Na^+/H^+ exchange whereby Na^+ influx is coupled to excretion of H^+ either via an electroneutral Na^+/H^+ antiporter or indirectly via a Na^+ conductive pathway linked to a primary electrogenic proton pump (Avella and Bornancin 1988). The results of our study suggest that a structural/functional relationship exists in the chloride cell similar to that described for other epithelia. This is evident when comparing chloride cell ultrastructure in trout displaying low (e.g., Fig. 12) or high (e.g., Fig. 14) ionic influx rates regardless of the cause of the influx rise (e.g., cortisol, this study; acclimation to ion-deficient water, Perry and Laurent 1989) or as yet undetermined (e.g., some control trout, this study). The density of the apical membrane villi is dramatically increased under high influx conditions. A comparison of chloride cells from rainbow trout transferred from City of Strasbourg tapwater into natural ion-deficient water of the Soultzeren mountain basin (Fig. 22) and vice-versa (Fig. 23) reveals a similar type of morphological transformation that has been shown to occur during acidosis in cells of the rat collecting duct (see above). Similar observations were obtained from cortisol-treated trout (this study). Further studies should be devoted to the mechanism of apical membrane expansion when chloride cells are stimulated. The fusion of vesicles to the apical membrane which is present in fish displaying high influx of Cl^- or Na^+, suggests that some material, functionally implicated in the transport process, might be incorporated within the apical membrane. The use of specific immunocytochemical or autoradiographic markers may help to solve this question.

In summary, the plasticity of the branchial chloride cell population observed in response to altered environmental conditions and the significant correlation of chloride cell apical surface area and Na^+ or Cl^- influx rates suggest that these cells play a vital role in maintaining NaCl homeostasis in freshwater salmonids. The results of the present study strongly suggest that the chloride cell is an important site of NaCl uptake in freshwater trout. Comparison of their ultrastructure with that of other epithelial cells involved in H^+ secretion suggests that similar events might occur in the chloride cell concomitantly with Na^+ absorption when this process is stimulated.

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Fig. 22. High-magnification TEM of a chloride cell from rainbow trout transferred into ion-deficient water and adapted for 3 weeks (see Materials and methods for ionic composition). Note the sponge-like organization of apical microplicae, the well developed network of baso-lateral tubules (*large arrows*) and the numerous vesicles (*small arrows*) beneath the apical membrane. *gly* glycocalix. Same magnification as in Fig. 19

Fig. 23. High-magnification TEM of a chloride cell from rainbow trout transferred to Strasbourg (ion-rich) freshwater for one week after being previously adapted to ion-deficient freshwater for three weeks (see Materials and methods for ionic composition). Compare with Fig. 22. After returning to ion-rich feshwater the chloride cell apical surface is partly covered with pavement cells (*pvc*). Microplicae shown in Fig. 22 (ion-deficient freshwater) are replaced by small villi. Note, also, the scarceness of the tubular system (*large arrows*) and the presence of vesicles containing an electron-dense material (*small arrows*). The Golgi system does not appear well developed. This micrograph suggests the retrieval of membrane material by vesicular trafic. *Bar*: 1 μm

References

Avella M, Masoni A, Bornancin M, Mayer-Gostan N (1987) Gill morphology and sodium influx in the rainbow trout (*Salmo gairdneri*) acclimated to artificial freshwater environments. J Exp Zool 241:159–161

Avella M, Bornancin M (1988) A new analysis of ammonia and sodium transport through the gills of the freshwater rainbow trout (*Salmo gairdneri*). J Exp Biol 142:155–175

Chakraborti PK, Chakraborti A, Weisbart M (1985) A high affinity cortisol receptor in the gill of the brook trout, *Salvenillus fontinalis*. Am Zool [Abstr] 25:82

Chan DKO, Chester Jones I, Henderson IW, Rankin JC (1967) Studies on the experimental alteration of water and electrolyte composition in the eel (*Anguilla anguilla* L.). J Endocrinol 37:297–317

Decourt C, Lahlou B (1986) In vitro studies on the release of cortisol from interrenal tissue in trout (*Salmo gairdneri*) II Action of changes in extracellular electrolytes. Comp Biochem Physiol 85A:747–753

Dejours P (1988) Respiration in water and air: Adaptation- regulation- Evolution. Elsevier, Amsterdam

Epstein FH, Cynamon M, McKay W (1971) Endocrine control of Na-K-ATPase and seawater adaptation in *Anguilla rostrata*. Gen Comp Endocrinol 48:425–431

Evans DH (1984) The roles of gill permeability and transport mechanisms in euryhalinity. In: Hoar WS, Randall DJ (eds) Fish Physiology. vol XB, New York, Academic Press, pp 239–283

Henderson IW, Chester Jones I (1967) Endocrine influences on the net extrarenal fluxes of sodium and potassium in the European eel (*Anguilla anguilla* L.). J Endocrinol 37:319–325

Hirano T, Utida S (1968) Effects of ACTH and cortisol on water movement in isolated intestine of the eel, (*Anguilla japonica*). Gen Comp Endocrinol 11:373–380

Kaisling B (1985) Structural adaptation to altered electrolyte metabolism by cortical distal segments. Fed Proc 44:2710–2716

Lahlou B (1980) Les hormones dans l'osmoregulation des Poissons. In: Ali MA (ed) Environmental Physiology of fishes. Nato Series, New York London Plenum, pp 201–240

Laurent P (1984) Gill internal morphology. In: Hoar WS, Randall DJ (eds) Fish Physiology. vol XA, New York, Academic Press, pp 73–183

Laurent P (1989) Gill structure and function: fish. In: Wood SC (ed) Comparative pulmonary physiology, New York, Marcel Dekker, pp 69–120

Laurent P, Dunel S (1980) Morphology of gill epithelia in fish. Am J Physiol 238:R147–R159

Laurent P, Hobe H, Dunel-Erb S (1985) The role of environmental sodium chloride relative to calcium in gill morphology of freshwater salmonid fish. Cell Tissue Res 240:675–692

McDonald DG, Rogano MS (1986) Ion regulation by the rainbow trout, *Salmo gairdneri*, in ion poor water. Physiol Zool 59:318–331

Madsen KM, Tisher CC (1983) Cellular response to acute respiratory acidosis in rat medullary collecting duct. Am J Physiol 245:F670–F679

Madsen KM, Tisher CC (1984) Response of intercalated cells of rat outer medullary collecting duct to chronic metabolic acidosis. Lab Invest 51:268–276

Maetz J (1956) Les échanges de sodium chez le poisson *Carassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. J Phys Paris 48:1085–1099

Mattheij JAM, Stroband HWJ (1971) The effects of osmotic experiments and prolactin on the mucus cells in the skin and the ionocytes in the gills of the teleost *Cichlasoma biocellatum*. Z Zellforsch Mikrosk Anat 121:93–101

Mayer N, Maetz J, Chan DKO, Forster ME, Chester Jones I (1967) Cortisol, a sodium excreting factor in the eel (*Anguilla anguilla* L.) adapted to seawater. Nature 214:1118–1120

Mazzone RW, Kornblau S, Durand CM (1980) Shrinkage of lung after chemical fixation for analysis of pulmonary structure-function relations. J Appl Physiol: Resp Environ Exerc Physiol 48(2):382–385

Perry SF, Laurent P (1989) Adaptational responses of rainbow trout to lowered external NaCl concentration: contribution of the branchial chloride cell. J Exp Biol (in press)

Perry SF, Wood CM (1985) Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. J Exp Biol 116:411–433

Sandor T, DiBattista JA, Medhi AZ (1984) Glucocorticoid receptors in the gill tissue of fish. Gen Comp Endocrinol 53:353–364

Smith LS, Bell GR (1964) A technique for prolonged blood sampling in free swimming salmon. J Fish Res Board Canada 21:711–717

Steinmetz PR (1986) Cellular organization of urinary acidification. Am J Physiol 251:F173–F187

Stetson DL, Steinmetz PR (1983) Role of membrane fusion in CO_2 stimulation of proton secretion by turtle bladder. Am J Physiol 245:C113–C120

Rahim SM, Delaunoy JP, Laurent P (1988) Identification and immunocytochemical localization of two different isoenzymes in teleostean fish erythrocytes and gill epithelia. Histochemistry 8:451–459

Wolf K (1963) Physiological salines for freshwater teleosts. Prog Fish Cult 25:135–140

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