Gill structure of a fish from an alkaline lake: effect of short-term exposure to neutral conditions

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Abstract: The morphology and morphometry of the gills of *Oreochromis alcalicus grahami*, a unique ureogenic teleost that lives in the alkaline environment of Lake Magadi, Kenya (pH 10, $Cco_2 = 180$ mmol $\cdot$ L^{-1}, temperature 30$-$40°C) were examined by transmission electron, scanning electron and light microscopy. Fish were examined in normal Lake Magadi water and 2$-$3 or 24 h after transfer to Lake Magadi water neutralized to pH 7 with HCl (i.e., CO_3^{2-} replaced with Cl$^-$), a treatment that caused severe reductions in urea excretion and O_2 uptake, internal acidosis, and ionoregulatory disturbance. In Lake Magadi water, the organization of the filament epithelium of the gill was similar to that of seawater teleosts. Indeed, chloride cells were located at the bottom of pits bordered by overlying pavement cells and flanked by typical accessory cells. Total numbers of chloride cells remained unchanged after transfer to pH 7, but after 2$-$3 h, many were covered by pavement cells, restricting their communication with the external milieu. At 24 h, this trend was reversed, an observation indicative of a reactivation of chloride cells. Mucous cells were located at maximum density on the trailing edge of the filament; most of them were empty after 24 h at pH 7. The harmonic mean thickness of the lamellar epithelium (blood-to-water diffusion pathway) was very small and not altered by acute or longer term exposure to pH 7. A model of alterations in ion and acid$-$base transport accompanying the morphological changes is presented.

Résumé : La microscopie électronique à transmission et à balayage, et la microscopie photonique ont servi à étudier la morphologie et la morphometrie des branchies chez *Oreochromis alcalicus grahami*, un téléostéen uréogène vivant dans les eaux extrêmement alcalines du lac Magadi, Kenya, (pH 10, $Cco_2 = 180$ mmol $\cdot$ L^{-1}, température 30$-$40°C. Les poissons ont été étudiés dans l'eau naturelle du lac Magadi et après 2$-$3 h et 24 h de séjour dans de l'eau du lac neutralisée par l'addition de HCl (pH 7, CO_3^{2-} remplacé par Cl$^-$), un traitement qui a entraîné une réduction considérable de l'excrétion d'urée et de la consommation de O_2, une acidose interne et un déséquilibre du contrôle des ions. Dans l'eau naturelle du lac, l'organisation de l'épithelium des filaments branchiaux s'est avérée semblable à celle qui prévaut chez les téléostéens marins: les cellules à chlorure sont situées au fond de cryptes bordées par des cellules pavimenteuses et flanquées de cellules accessoires typiques. Le nombre total des cellules à chlorure reste inchangé après transfert à pH 7 mais, au bout de 2$-$3 h, nombre de ces cellules sont recouvertes de cellules pavimenteuses qui bloquent leur contact avec la milieu externe. Après 24 h, cette tendance est inversée, ce qui semble indiquer une réactivation des cellules à chlorure. Les cellules à mucus sont présentes en densité maximale au bord de fuite des filaments; elles sont souvent vidées de leur contenu après 24 h d'exposition à pH 7. La moyenne harmonique de l'épaisseur de l'épithélium des lamelles (voie de diffusion du sang vers l'eau) est très faible et reste inchangée après une exposition prolongée à pH 7. Un modèle schématisant les modifications du transport des ions et de l'équilibre acide$-$base au cours des changements morphologiques est proposé.

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Introduction

The cichlid fish *Oreochromis alcalicus grahami*, formerly *Tilapia grahami* is the only species that lives in alkaline Lake Magadi, Kenya. The water of the springs feeding the lake has an osmolality of about 525 mosmol $\cdot$ kg^{-1}, a constant pH of 10, a constant P_{CO_2} of 0.3 Torr (1 Torr = 133.32 Pa), and a P_{O_2} that fluctuates diurnally from less than 20 to more than 400 Torr (Wood et al. 1989; unpublished observations). The "salinity" of Lake Magadi water is approximately equal to 50−65% seawater. The major difference is that anions are mainly represented by carbonates (Table 1).

The biology of this fish has been repeatedly explored since Coe (1966), and its particular resistance to extreme conditions has been studied by several investigators who have focused their attention on ion and acid−base regulation. More recently, it has been shown that the fish excretes no ammonia but rather is totally ureogenic, with a functional ornithine−urea cycle (Randall et al. 1989, Wood et al. 1989; Walsh et al. 1993). Most of the urea is excreted through the gills (Wood et al. 1994).

Recent studies on a variety of teleosts have revealed the importance of adaptive mechanisms at the morphological level in the gill epithelium of both freshwater and seawater fish. For instance, morphofunctional changes occur in the chloride cells of freshwater fish that are activated or deactivated according to acid−base status and (or) ionoregulatory status (Goss et al. 1992a, 1992b; Perry and Laurent 1989; Laurent and Perry 1990). In addition, the complex organization of the chloride cells, consisting of the association of chloride cells with accessory cells, in teleosts living in a hyperosmotic environment such as seawater (Dunel and Laurent 1973; Laurent and Dunel 1980) is seen as an adaptive mechanism for salt excretion (see review of Laurent 1989).

The first description of chloride cells on the filament epithelium of *O. a. grahami* was by Maina (1990, 1991). The first goal of the present study was to reinvestigate chloride cell organization in this hypo-osmoregulatory fish. Indeed, the presence of accessory cells beside the chloride cells was not mentioned by Maina.

A second goal was to evaluate possible changes in morphology of the chloride cells or any other epithelial components and general gill organization following acute transfer of the fish to Lake Magadi water adjusted to pH 7 with HCl (replacing CO_3^{2-} and HCO_3^- with Cl^-). It has been shown that exposure of the alkaline-adapted Lake Magadi tilapia to this neutral-pH water causes severe internal acid−base disturbance (metabolic acidosis), severe ionoregulatory disturbance (elevated plasma Cl^- and depressed plasma Na^+), a severe reduction in urea excretion (80−100%), a progressive reduction in O_2 uptake, and increased mortality (Wood et al. 1989, 1994; Wright et al. 1990).

Materials and methods

Oreochromis alcalicus grahami weighing 1−6 g were collected by net in late February 1992 from Fish Springs Lagoon at the edge of Lake Magadi (cf. Coe 1966). The fish were held in plastic buckets (20 individuals in 20 L of lagoon water). The water was vigorously aerated and changed twice

Table 1. Mean measured composition of normal (pH 10) and neutralized (pH 7) Lake Magadi water in these experiments.

	pH 10	pH 7
pH	9.94	7.05
CO_3^{2-} (mequiv. $\cdot$ L^{-1})	265	0
HCO_3^- (mequiv. $\cdot$ L^{-1})	40	0.4
Cl^- (mequiv. $\cdot$ L^{-1})	108	420
Na^+ (mequiv. $\cdot$ L^{-1})	342	358
Osmolality (mosmol $\cdot$ kg^{-1})	525	572

a day; the ammonia level was less than 1 μmol $\cdot$ L^{-1}. The water composition is given in Table 1.

Titration of Lake Magadi water to pH 7

The effect of environmental pH on gill organization was studied by transferring *O. a. grahami* from Lake Magadi water at pH 10 to lake water titrated to pH 7 with HCl (Table 1). The responses observed cannot be attributed solely to the change in pH from 10 to 7, but to a combination of pH change, basic equivalent removal, and Cl^- addition.

Experimental protocols

Fish were sacrificed after 2−3 and 24 h exposure to pH 7, with appropriate control fish held at pH 10 (N = 5−10 per group). Four treatment groups were examined: (*i*) freshly collected, held at pH 10, and examined within 2−3 h of capture; (*ii*) acute (2−3 h) exposure to pH 7; (*iii*) 24 h captivity, held at pH 10; and (*iv*) 24 h exposure to pH 7. Parallel physiological studies of these four groups have been published (Wright et al. 1990; Wood et al. 1994).

Morphological techniques

Gill samples

Scanning electron microscopy (SEM), light microscopy (LM), and transmission electron microscopy (TEM) techniques have been described in detail elsewhere (Laurent et al. 1985).

Opercular epithelium

The opercular epithelium was sampled from some individuals and examined with SEM after the same fixation procedure as for the gills. The epithelium was kept in place on the opercular bone. The whole surface of the operculum was examined and the numerical density of externally open chloride cells measured by simply counting the cells present on a given area of epithelium.

Morphometry

Determination of cell densities, chloride cell apical surface area, and harmonic mean thickness of the water-to-blood diffusion barrier were performed according to the methods described by Laurent and Hebibi (1989) and Laurent and Perry (1990).

Statistical methods have been described previously (Laurent and Hebibi 1989; Laurent and Perry 1990).

Fig. 1. (*a*) Representative SEM of the filament epithelium in a control *O. a. grahami* (pH 10). The arrows point to chloride cells. Stars indicate mucous cells. Scale bar = 5 μm. (*b*) Close-up of the apical crypt of a chloride cell (*cc*). The arrow points to one of the accessory cell processes encrusting the apical surface of a chloride cell. *pvc*, pavement cell. Scale bar = 1 μm.
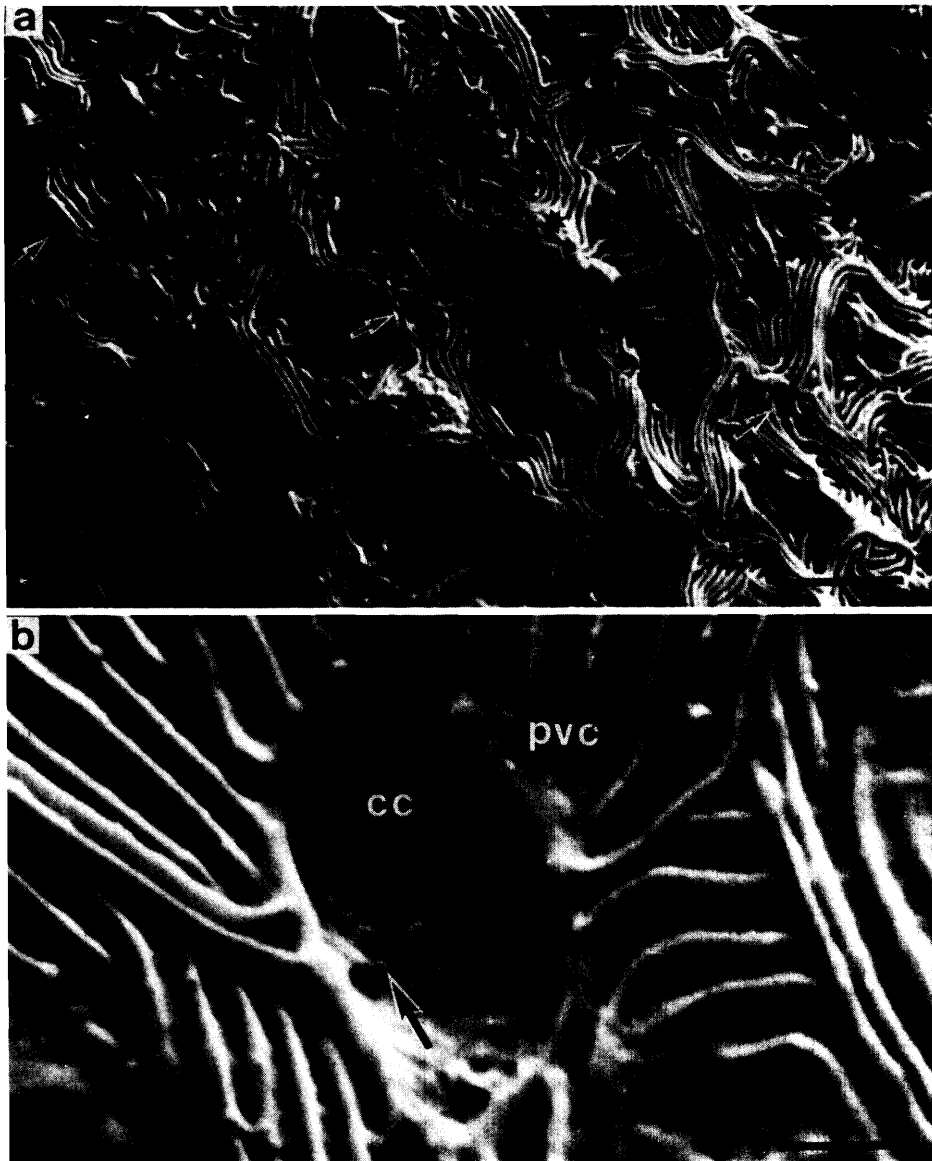
Results

The gill epithelia of *O. a. grahami* at pH 10

Two types of epithelia must be distinguished: the epithelium of the filament and the epithelium of the lamella.

The filament epithelium

This epithelium, which envelopes the core of the filament, is thick and multistratified. It comprises three well-differentiated types of cell: chloride, pavement, and mucous cells (Fig. 1*a*). In addition, a large population of less differentiated cells is present.

The chloride cells: In contrast to typical freshwater teleosts, *O. a. grahami* has chloride cells located in the bottom of a crypt, the rim of which is constituted by the flanges of the surrounding pavement cells. The depth of this crypt and the size of the aperture are variable (see below). Chloride cells of *O. a. grahami* are strictly confined to the filament epithelium, where they extend from the interlamellar space to the whole trailing edge of the filament. Chloride cells of *O. a. grahami* are flanked by accessory cells. The accessory cell body lies beside the chloride cell, in contact with it, sending cytoplasmic processes to the apex of the chloride cell. Thus, the apical membrane at the bottom of the pit is a mosaic of stripes of both cells (Fig. 1*b*). TEM (Fig. 2) clearly shows that the occluding zonule, the tight part of the junctional apparatus between the main and the accessory cell membranes, is shallow, less than 0.05 μm compared with 0.5 μm between chloride cell and pavement cell.

Not all chloride cells are in contact with the external

Fig. 2. Representative TEM of the apical region of a chloride cell in *O. a. grahami*. Beside the chloride cell (*cc*), note the accessory cell (*ac*) sending its cytoplasmic processes (asterisks) encrusting the chloride cell (compare with Fig. 1*b*). Note that the junctions between *ac* and *cc* are shallow (arrows) compared with the junctions with pavement cells (pvc) (arrow head). *c*, crypt. Scale bar = 1 μm.
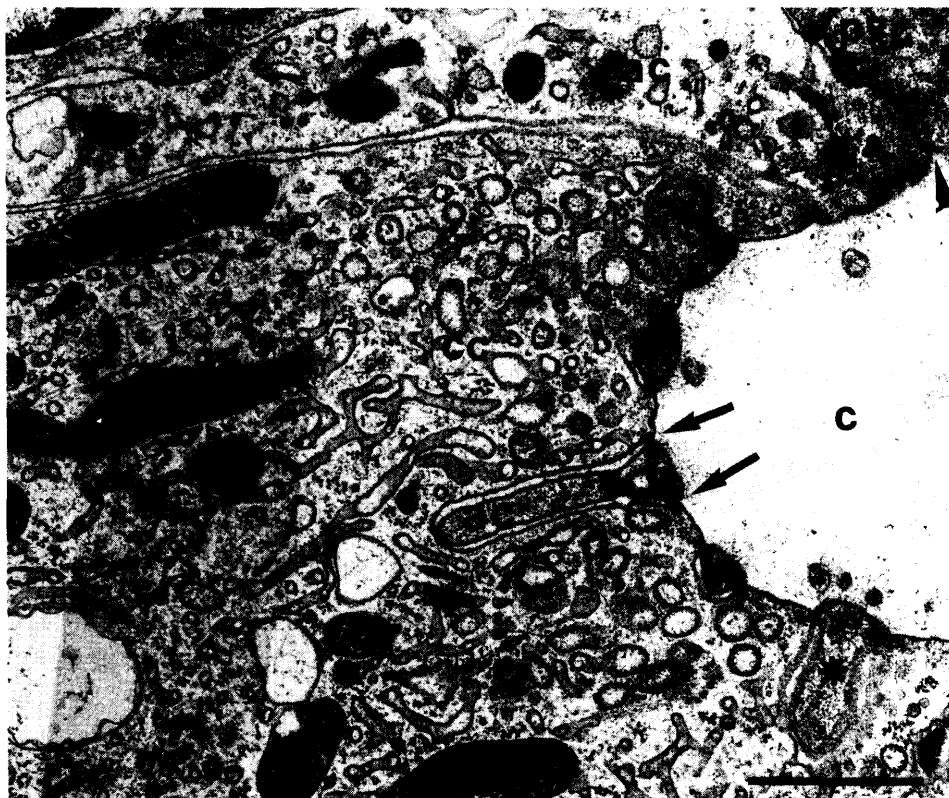
milieu. TEM shows that the buried chloride cells may be either fully developed, still in the process of development, or even undergoing a process of degeneration. The percentage of "open" chloride cells, as assessed with LM, was about 20% in freshly caught fish but fell to about 10% after 24 h holding at pH 10 in the laboratory (Figs. 3 and 4).

The pavement cells of the filament: Pavement cells occupy more than 95% of the filament surface. Measurement with the digitizer gave a mean apical surface of 79.5 ± 4.9 μm^2 per pavement cell. Observed with SEM (Fig. 5*a*), the apical surface shows two regions: (*i*) a series of concentric ridges parallel to the hexagonal limit of the cell, and (*ii*) a smooth central area with occasionally occurring microvilli. The total surface area is inversely correlated with the number of ridges.

With TEM, pavement cells have different shapes: some are very flat, others are more cuboidal. The internal organization of the cuboidal cells suggests a high degree of activity, with a large number of mitochondria, numerous Golgi systems, a large population of vesicles, and a well-developed subapical cytoskeleton. Coated vesicles emanating from the Golgi system were seen moving toward the apical or the basolateral membrane.

The mucous cells: The mucous cells are present in the filament epithelium but scarce on the lamellae. Their maximum

density occurs on the trailing edge, some are present in the interlamellar region, but they are scarce on the leading edge. As shown in Fig. 6, unmyelinated nerve fibers (less than 0.2 μm in diameter) were often seen close to the mucous cells. Counting demonstrated that the density of mucous cells decreased significantly after 24 h of captivity in the laboratory at both pH levels (Fig. 3).

The epithelium of the lamella

The respiratory gases move across this epithelium. At both pH levels, the harmonic mean diffusion distance decreased significantly by about 30% from the control values of 1.45 ± 0.07 μm after 2−3 h captivity, but there was no significant effect of exposure to pH 7 (Fig. 7A).

Lamellar pavement cells are rather different in form from those of the filament. In particular, they are very flat and are delineated by a double rim marking their border with neighbouring cells (Fig. 5*b*). Thus, the surface of the cell is relatively smooth and bears only a few microvilli. Another characteristic is its large area. Measurements were made in a single fish to compare pavement cell surface areas: 90 ± 4 μm^2 on the filament and 341 ± 29 μm^2 on the lamella.

With TEM, the lamellar epithelium is generally bilayered. The external layer consists of very flat cells, the pavement cells observed with SEM (see above). Some of them exhibit abundant organelles, suggesting a high level of activity. A variable extracellular space is interposed between the two

Fig. 3. (a) LM measurements of epithelial cell densities on cross sections of filaments in control fish and after 2−3 h exposure to pH 7 water. An asterisk indicates a significant difference ($p < 0.05$) from the pH 10 group at the same time. (b) LM measurements of epithelial cell densities on cross sections of filaments in control fish held for 24 h at pH 10 and in experimental fish after 24 h exposure to pH 7. *, significant difference ($p < 0.05$) from pH 10 group at the same time. CC, chloride cells; MC, mucous cells.
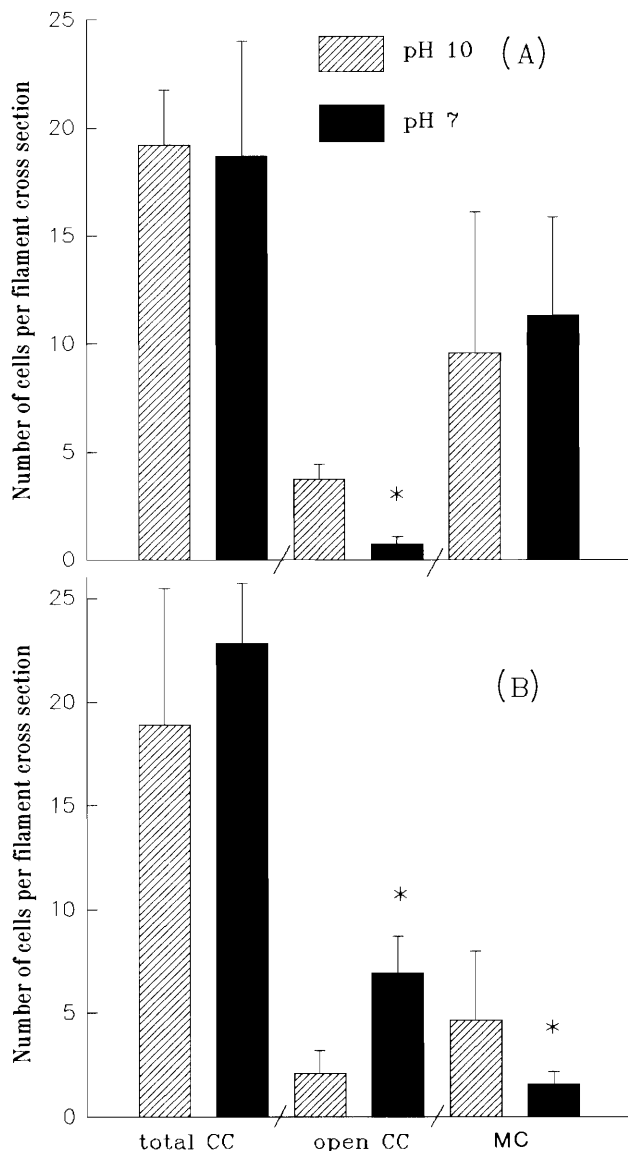
layers. The inner layer comprises both large cells characterized by a high nucleus-to-cytoplasm ratio and small cells with small nuclei.

The gill epithelia of *O. a. grahami* after transfer to pH 7
The individual apical surface area of chloride cells, as measured by SEM from the size of the aperture of the chloride cell pits, increased significantly by about 3-fold after 24 h at pH 7 (Fig. 7B). During the first 2−3 h at pH 7, the density of cells open to the outside decreased significantly, but

returned to close to the control value at 24 h (Fig. 7C). In consequence, the fractional surface area displayed a biphasic evolution: a significant, rapid removal of chloride cells from the surface at pH 7 followed by a subsequent reappearance at 24 h to over twice the original fractional surface area (Fig. 7D). The individual surface area, density, and fractional surface area of the chloride cells remained unchanged in control fish held for the same period at pH 10 (Figs. 7B−7D).

With LM, additional data were obtained concerning these changes. During a short, 2- to 3-h exposure to pH 7, the populations of chloride cells in the filament epithelium remained constant but a significant removal of chloride cells from the surface was observed (Fig. 3A). This confirms the SEM study. After 24 h of exposure to pH 7, the total number of chloride cells again remained unaffected, but the number of chloride cells in contact with the external milieu increased significantly (Fig. 3B). Again, these data confirm the SEM study.

The density of mucous cells was assessed in parallel with the chloride cell study. Short-term exposure to pH 7 did not modify the number of mucous cells present on the trailing or the leading edge of the filament (which were shown together in Fig. 3). After 24 h, the number of mucous cells decreased dramatically (Fig. 3B). A careful examination with TEM showed that some cells were still present but empty of their mucous droplets. In addition, the fish kept in control pH 10 water in the laboratory for 24 h also exhibited a reduction of their mucus cell populations, though the effect was not as great as in the pH 7 group.

The length of the diffusion pathway, expressed as the harmonic mean of the distance between the blood and the external milieu, was not affected by short-term exposure (2−3 h) to pH 7 (Fig. 7A). After 24 h, in both control conditions and pH 7, the length of the diffusion pathway decreased significantly, by about 30%.

The opercular epithelium
The epithelium covering the inner part of the operculum has a structure similar to that of the gill filament, including a high density of chloride cells. The present study showed that chloride cells are present in the operculum of *O. a. grahami* at a density of slightly less than 1000/mm^2 (Fig. 5c). While there was no statistical analysis, this density is less than in the filament epithelium, where the mean density is about 2300 cells/mm^2. As in the gill, the opercular chloride cells were located at the bottom of a pit with an aperture of 2−3 μm^2. Interestingly, the pavement cells exhibited a size and surface ornamentation (apical ridges) similar to those of the filament epithelium. As yet no ultrastructural studies have been done on these cells.

Discussion

Oreochromis alcalicus grahami must overcome two major physiological problems that are not faced by other fish: acid−base regulation and nitrogenous waste excretion in a severely alkaline environment. How does morphological study help us to understand how these problems are solved?

Morphofunctional adaptation of the gill to life at pH 10
The present study has demonstrated that the chloride cells of

Fig. 4. Selected aspects of the link between chloride cells (*cc*) and the external environment in *O. a. grahami*, which explain the mechanism of closure. (*a*) The chloride cell lying at the bottom of its crypt is exposed to the environmental water (*p*). Note the rim constituted by several pavement cell processes (★) (generally 3). (*b* and *c*) The same chloride cell (*cc*) a distance of several ultrathin sections away showing the process of closure by means of the interdigitation of pavement cell processes, from an individual held at pH 7 for 24 h. *p*, environmental water. (*d*) Complete closure of the crypt (arrowhead). The chloride cell (*cc*) has no further link with the outside. The internal lacuna will be progressively resorbed.
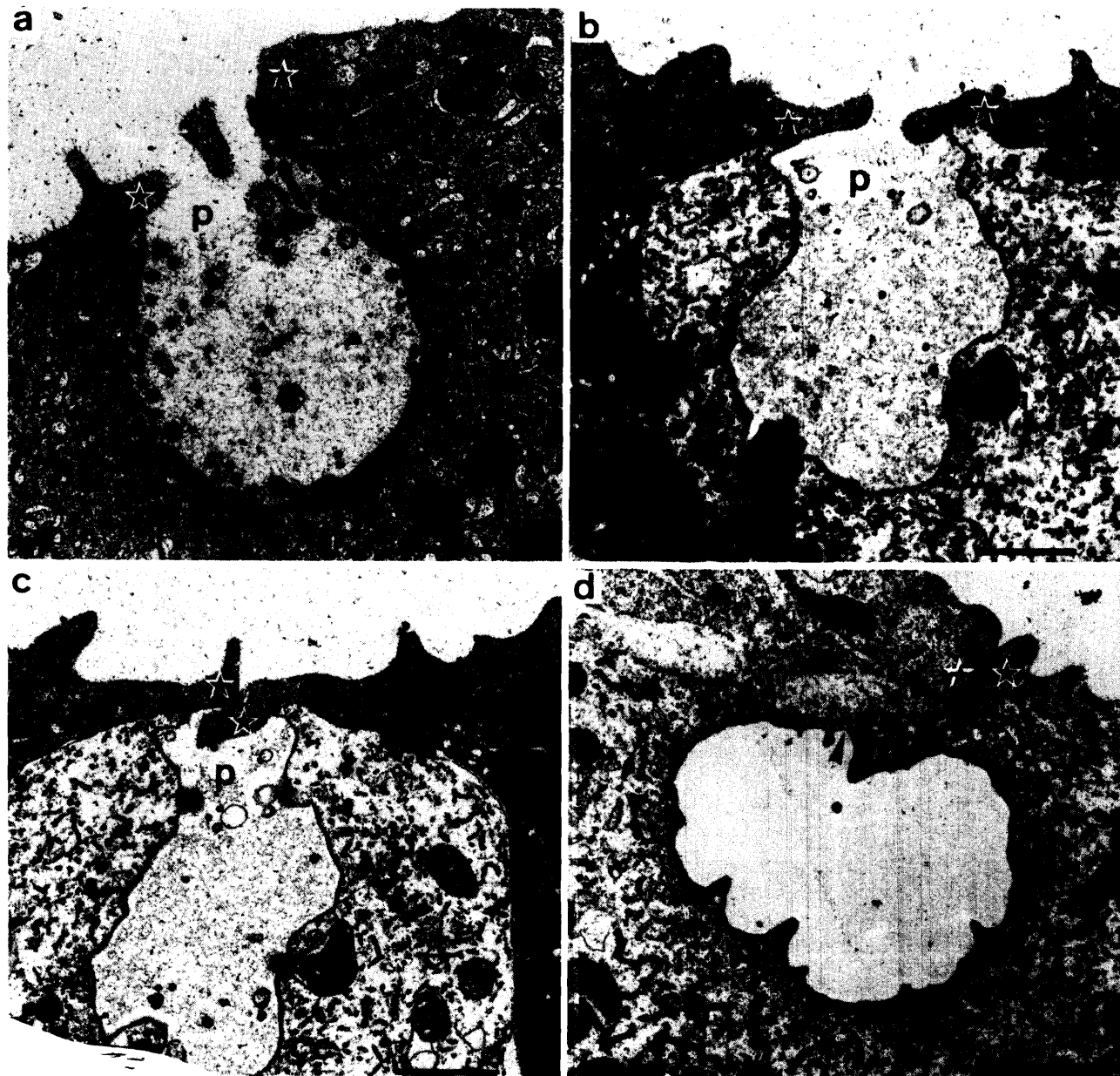
O. a. grahami are similar in morphology to those of typical marine teleosts but rather different from those of freshwater teleosts. The intimate association of the chloride cells with accessory cells is particularly diagnostic (see the review by Pisam and Rambourg 1991). Without doubt, in *O. a. grahami* these companion cells of the chloride cell are morphologically identical with the accessory cells described by Dunel and Laurent (1973) and Dunel (1975) in seawater teleosts. Therefore, it seems likely that the gills carry out the excretion of "salt" according to the standard, well-documented seawater-type model (see reviews by Laurent 1989; Foskett et al. 1983; Wood and Marshall 1994). From the concentrations of the relevant ions and the acid–base equivalents in the environment (Table 1) and the blood plasma of *O. a. grahami* (Wright et al. 1990; Wood et al. 1994), the electrochemical gradients involved have recently been calculated (Wood et al. 1994). There are strong inwardly directed gradients for Na^+ and basic equivalents $(CO_3^{2-}-HCO_3^{-}-OH^{-})$ but an outwardly directed gradient for Cl^- with respect to Lake Magadi water at pH 10. In

Fig. 5. SEM of gill and opercular epithelia in *O. a. grahami* at pH 10. (*a*) Surface ornamentation of the filament pavement cells (*pvc*). The white arrows delimit two neighbouring cells. The black arrow points to the pit of a chloride cell. Scale bar = 10 μm. (*b*) The surface of the lamella, showing a large pavement cell (*pvc*) delineated by a ridge (arrows) parallel to the ridge of the neighbouring cells. Note the absence of the concentric ridges seen on the filament. The surface is uneven. Scale bar = 10 μm. (*c*) Opercular
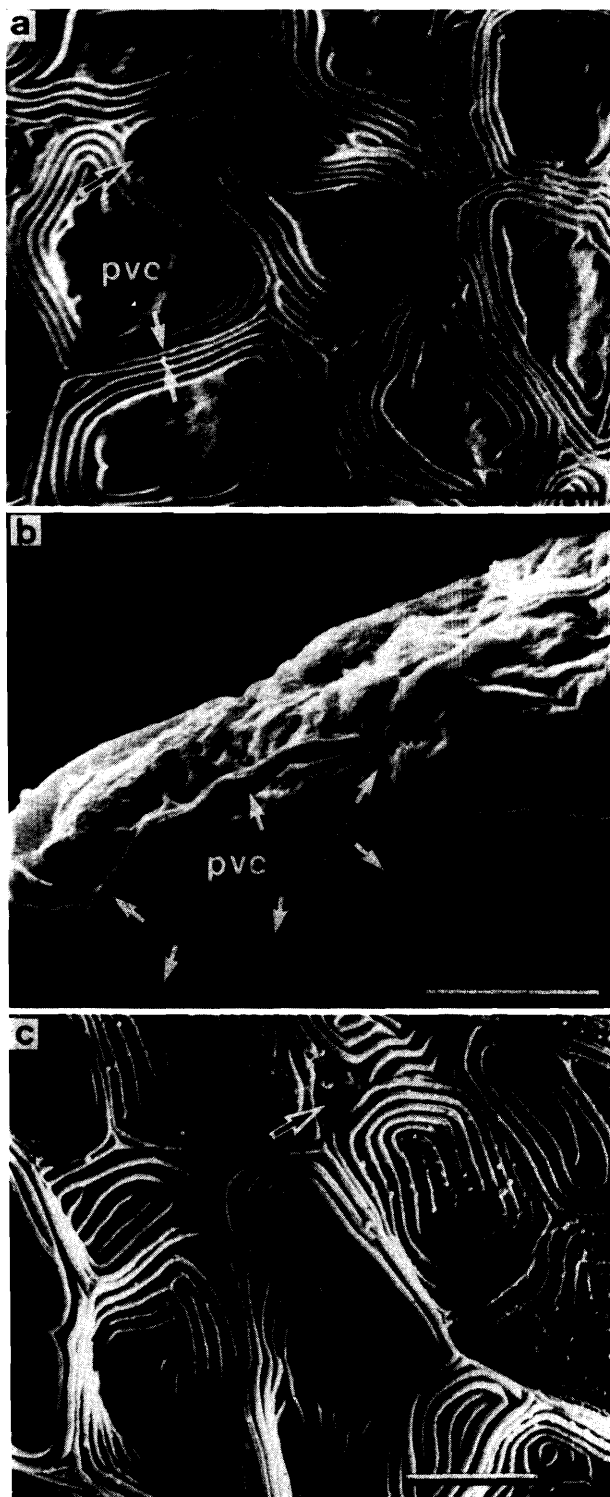
epithelium. Note the same type of ornamentation as on the filament. The arrow points to a chloride cell pit. Scale bar = 5 μm.

addition, since the environment is hyperosmotic (525 versus 351 mosmol · kg^{-1} internally), the fish must continually drink the surrounding water in order to achieve water balance (Maloiy et al. 1978; Skadhauge et al. 1980). *Oreochromis alcalicus grahami* is therefore faced with a continual net entry of both Na$^+$ and basic equivalents, yet a loss of Cl$^-$. Presumably, the gills therefore excrete this excess of Na$^+$ and of CO$_3^{2-}$ − HCO$_3^-$ −OH$^-$ while at the same time taking up Cl$^-$ from the environment. Thus, the standard seawater model should be modified to the extent that CO$_3^{2-}$ − HCO$_3^-$ −OH$^-$ replaces Cl$^-$ in the outwardly directed transport system (compare Figs. 8*a* and 8*c*). In particular, 1 CO$_3^{2-}$ or 2 HCO$_3^-$ or 2 OH$^-$, together with Na$^+$ and K$^+$, would be transported into the cell on the neutral cotransporter along the Na$^+$ electrochemical gradient, and the basic equivalents would leave through apical channels and (or) via apical Cl$^-$/HCO$_3^-$ exchange (Goss et al. 1992*a*, 1992*b*, 1994), a process assisting in Cl$^-$ uptake and basic equivalent excretion (Fig. 8*a*). As in the seawater model, excess Na$^+$ probably exits through the "leaky" paracellular junctions (Sardet et al. 1979).

The second problem encountered by *O. a. grahami* concerns the clearance of its nitrogenous waste, urea. Movement of urea across many epithelia depends on facilitated transport (Marsh and Knepper 1992), but the cellular sites and mechanisms by which it is excreted through the gills of *O. a. grahami* are presently unknown. We did not confirm the presence of the particular cell type that Maina (1991) suggests is involved in urea secretion. The chloride cell is a possible candidate, for it is a site of accumulation of urea and various other experimentally injected substances in the seawater-adapted eel (Masoni and Garcia-Romeu 1972). Another possibility is paracellular diffusion of this small noncharged molecule via the leaky junctions.

The blood-to-water diffusion distance for *O. a. grahami* (harmonic mean) previously reported by Maina (1990, 1991) and Maina et al.[2] is unusually short relative to that in other freshwater and seawater fish. Indeed, 1.45 μm is less than the harmonic mean thickness measured in the highly aerobic *Oncorhynchus mykiss* adapted to seawater (1.65 μm; Laurent and Hebibi 1989) and close to that of some fast-swimming pelagic teleosts (for a review see Hughes 1984). Clearly, the severity of the environment has not resulted in protective thickening. Rather, this thin diffusion barrier may be seen either as an adaptation to facilitate urea excretion or as an adaptation to the severely hypoxic conditions that are routinely encountered and well tolerated by *O. a. grahami* (unpublished observations).

The density of the mucous cell population on the gills of *O. a. grahami* is quite comparable to that of the seawater-

Fig. 6. TEM of a mature mucous cell (*mc*) in the gill filament epithelium of *O. a. grahami* at pH 10 (the arrow indicates the apical aperture). The filament epithelium is multilayered, with two basal lamina: 1 is located underneath the epithelium itself, and 2 supports the wall of the central venous sinus filled with blood (*bl*). Between them is a loose parenchyma containing smooth muscle and nerve fibers (*nf*, arrowheads). Note the nerve fiber profiles ($\star$) close to the mucous cell. *w*, water. Scale bar = 1 μm.


adapted rainbow trout (Laurent and Hebibi 1989), other marine teleosts (Laurent 1984), and the related euryhaline species *Oreochromis mossambicus* (P. Laurent, unpublished observation). Thus, the severity of the alkaline environment does not seem to quantitatively affect the protective role of the mucous coat. So far, the chemistry of the mucous in this species has not been studied.

Morphofunctional changes in the gill at pH 7

When *O. a. grahami* was transferred to Lake Magadi water neutralized to pH 7 with HCl, no significant change was observed in chloride cell organization. However, the relationship of the chloride cells with the external medium was dramatically altered. The results of the present study are strong evidence for control of chloride cell function after transfer to pH 7 by the action of pavement cells in reducing chloride cell access to the external environment. Indeed, there was a biphasic response, the pavement cells initially covering (at 2−3 h) and then uncovering (at 24 h) the chloride cells.

This observation suggests the existence of an adaptive mechanism to pH 7. The inwardly directed gradients for basic equivalents ($CO_3^{2-} - HCO_3^- - OH^-$) were reversed, and the Cl^- gradient became inwardly directed as a result of the elevation of external Cl^- (Table 1) (Wood et al. 1994). Nevertheless, the external medium remained hyperosmotic and blood osmolality did not change (Wright et al. 1990). Therefore, the ion and acid−base regulatory problem changed from one of Na^+ and basic equivalent gain to one of Na^+ and Cl^- loading and basic equivalent loss, resulting in elevated plasma Cl^- and metabolic acidosis (Wright et al. 1990). As basic equivalent loading was immediately lowered after transfer to pH 7, Cl^- loading started and internal metabolic acidosis quickly developed (Wood et al. 1994). During acute exposure to pH 7, there was a need to quickly shut down basic equivalent excretion, including apical Cl^-/HCO_3^- efflux exchange. The reduction of chloride cell exposure due to covering by pavement cells (Fig. 8b) can therefore be viewed as a mechanism to inhibit these processes. Indeed, modulation of chloride cell function in

Fig. 7. (A) TEM measurement of the harmonic mean thickness (i.e., diffusion pathway) of the lamellar epithelium of
O. a. grahami. †, significant difference ($p < 0.05$) from the corresponding group at 2−3 h. (B) SEM measurement of the
individual chloride cell apical surface area (i.e., apical crypt area) in *O. a. grahami*. †, significant difference ($p < 0.05$) from the
corresponding group at 2−3 h; *, significant difference ($p < 0.05$) from the pH 10 group at the same time. (C) SEM measurement
of the density of chloride cells in contact with the external milieu in the filament epithelium of *O. a. grahami*. †, significant
difference ($p < 0.05$) from the corresponding group at 2−3 h; *, significant difference ($p < 0.05$) from the pH 10 group at the
same time. (D) Computation of the fractional surface area of open chloride cells per unit of filament surface area in *O. a. grahami*.
†, significant difference ($p < 0.05$) from the corresponding group at 2−3 h; *, significant difference ($p < 0.05$) from the pH 10
group at the same time.
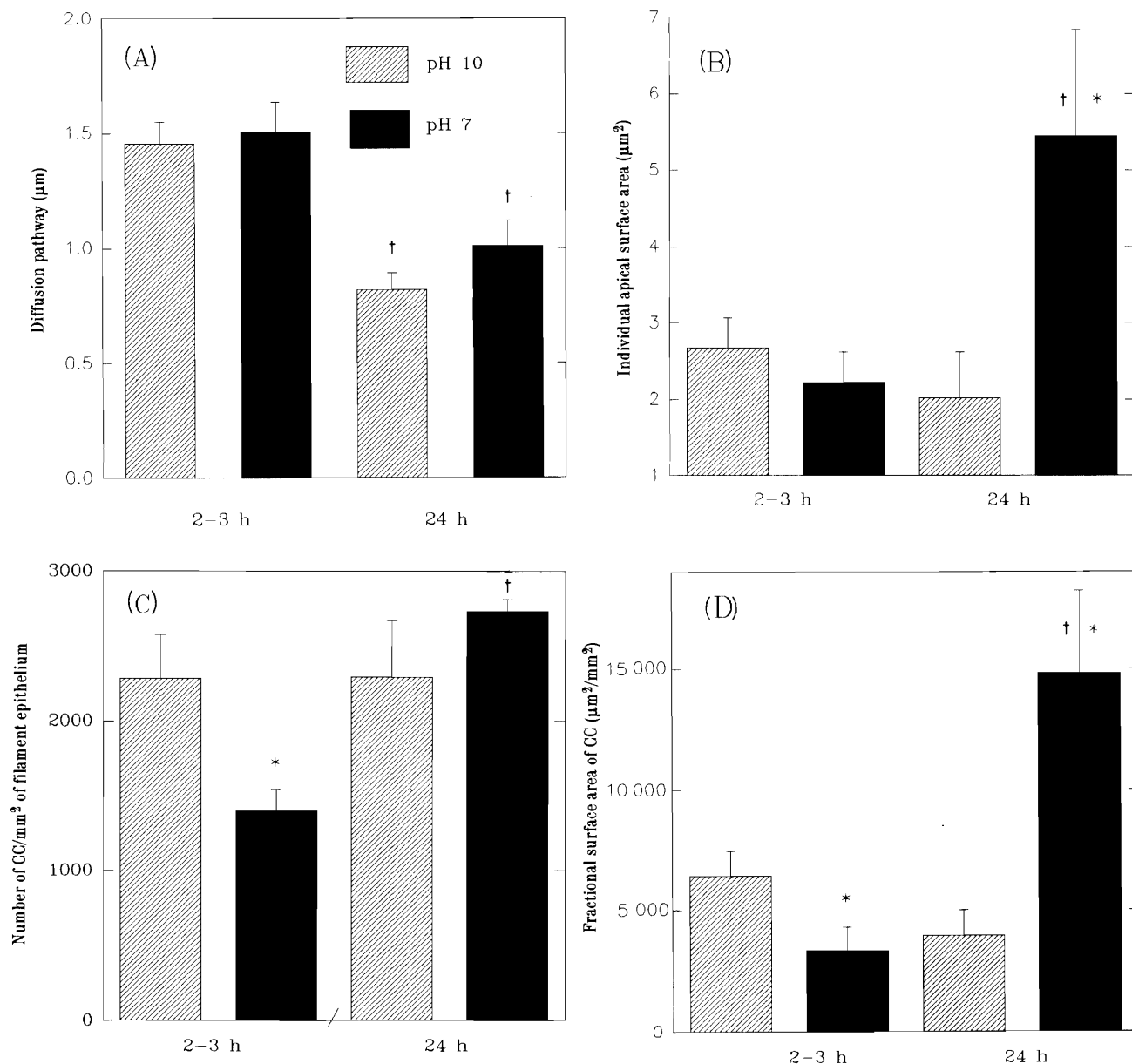
response to acid−base disturbance or hormonal stimulation
by means of pavement cell covering or uncovering has been
well documented in many other teleosts, including *O. mos-
sambicus* (Goss et al. 1992*a*, 1992*b*; Perry et al. 1992). The
subsequent reopening of chloride cell communication with
the external milieu after 24 h at pH 7 would allow the excre-
tion of excess Cl^-, because Cl^- loading would now be
detrimental to the fish. Na^+ excretion would follow pas-
sively through the paracellular leaky junctions (Fig. 8*c*).
This would now represent the standard model for chloride

cell function in seawater (see Laurent 1989; Wood and
Marshall 1994), with Cl^- instead of $CO_3^{2-}-HCO_3^--OH^-$
entering the neutral cotransporter and exiting via apical chan-
nels. It is also possible that the apical Cl^-/HCO_3^- exchange
process would be reversed so as to excrete Cl^- in exchange
for HCO_3^- uptake (Fig. 8*c*).

At pH 7, production of urea is greatly impaired and oxy-
gen consumption is decreased (Wood et al. 1994). This study
proved that these effects were not the consequence of change
in the gill diffusion pathway. The thickness of the water-to-

Fig. 8. A model of successive steps in changing chloride cell function during transfer of *O. a. grahami* from normal Lake Magadi water (pH 10) to Lake Magadi water acidified to pH 7 with HCl. (*a*) At pH 10: the fish is flooded with Na^+ plus $CO_3^{2-} - HCO_3^- - OH^-$ via drinking and diffusive entry. The basolateral Na^+,K^+ pump (ATPase) creates an electrochemical gradient for Na^+ entry into the chloride cell (CC) from the extracellular fluid. Na^+ and K^+ plus CO_3^{2-} or $2HCO_3^-$ or $2OH^-$ enter the cell on a neutral cotransporter. K^+ leaves passively through selective basolateral apical channels, and basic equivalents are extruded passively through apical channels. Na^+ moves out via the paracellular pathway across leaky junctions between the chloride cell and the accessory cell (AC). The small arrow points to a tight junction. It is not known whether the accessory cell plays a direct role in transport, but note the presence of one of the numerous processes of the accessory cell encrusted in the apical membrane of the chloride cell. This will serve to increase the junctional area available for the transport process, as illustrated. PVC, pavement cell. (*b*) At pH 7 (2–3 h): the fish is no longer flooded with basic equivalents and an internal metabolic acidosis develops. The chloride cell pit is closed, being covered over by pavement cells (PVC). Ion exchange is temporarily slowed or stopped. (*c*) At pH 7 (24 h): the fish is now flooded with Na^+ plus Cl^- and the chloride cell functions in a typical "seawater" fashion. The pavement cells (PVC) pull back and the apical pore is reopened. Na^+ and K^+ move as in *a*, but Cl^- is extruded passively through apical channels. In addition, the apical Cl^-/HCO_3^- exchange may operate in the opposite direction so as to take up HCO_3^- and excrete Cl^-.
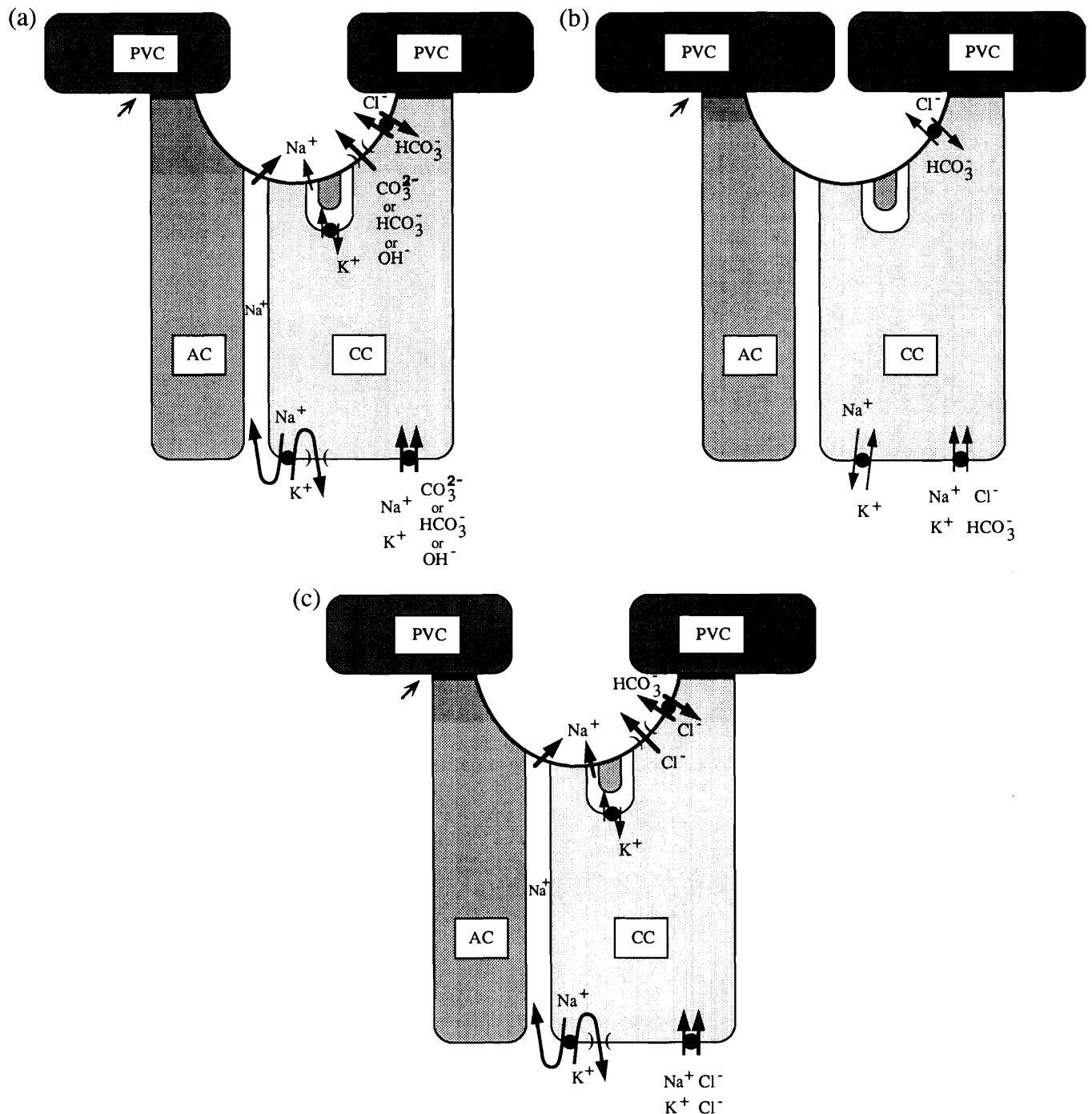
blood barrier decreased rather than increased after short-term exposure at pH 7.

Finally it is noteworthy that the population of mucous cells had almost completely disappeared after 24 h at pH 7. Mucus secretion generally plays the role of protecting the gills when the fish is subjected to a variety of physical and chemical stresses (Handy and Eddy 1991). The presence of mucus adds to the efficiency of the permeability barrier to ion loss or gain (Handy 1989). In *O. a. grahami* subjected to pH 7, the lack of mucous is a sign of severe maladaptation.

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