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The effects of extremely alkaline water (pH 9·5) on rainbow trout gill function and morphology

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Rainbow trout that were held under control conditions, at pH 8·0, in moderately hard Hamilton tap water, had Cl^- and Na^+ influx rates (J^{Cl}_{in} and J^{Na}_{in}, respectively) of 270 and 300 μmol kg^{-1} h^{-1}, respectively. Exposure to pH 9·5 water led to an immediate 67% decline in J^{Cl}_{in} and a 45% reduction in J^{Na}_{in} at 0–1 h. Influx rates declined further and by 4–5 h the net decreases in both J^{Cl}_{in} and J^{Na}_{in} approximated 80%. By 24 h J^{Cl}_{in} had recovered to rates not significantly different from those at pH 8·0; while J^{Na}_{in} only partially recovered and remained about 50% lower than control measurements through 72 h. The complete recovery of J^{Cl}_{in} and partial recovery of J^{Na}_{in} may have been related to a fourfold greater branchial chloride cell (CC) fractional surface area observed in rainbow trout exposed to pH 9·5 for 72 h. Ammonia excretion (J_{Amm}) was about 170 μmol N kg^{-1} h^{-1} at pH 8·0 but was initially reduced by 90% over the first hour of high pH exposure. J_{Amm} rapidly recovered and by 24 h it had returned to pre-exposure levels. This recovery tended to parallel the partial recovery of J^{Na}_{in}. However, subsequent addition of amiloride (10^{-4} M) to the water at 75 h led to no change in J_{Amm}, despite a 50% reduction in J^{Na}_{in}. Thus, it does not appear that there is a linkage between Na^+ influx and the recovery of ammonia excretion under highly alkaline conditions.

Key words: chloride cells; ammonia excretion; amiloride; high pH; Na^+ influx; Cl^- influx.

I. INTRODUCTION

Numerous eutrophic lakes and ponds experience diurnal and seasonal rises in water pH as a result of high rates of algal photosynthesis (Wetzel, 1983; Hansen *et al.*, 1991). Until recently, little was known about the physiology of fish in alkaline environments, despite the fact that water pHs approaching 10 are common in eutrophic waters (e.g. Jordan & Lloyd, 1964; Barica, 1974, 1990; Wetzel, 1983; Murray & Ziebell, 1984).

Disturbances in internal ion homeostasis, acid-base balance and nitrogenous waste (N-waste) excretion have been commonly observed in salmonids exposed to high pH (pH ≥ 9·0; Wright & Wood, 1985; Heming & Blumhagen, 1988; Wood, 1989; Lin & Randall, 1990; McGeer *et al.*, 1991; Wilkie & Wood, 1991; Yesaki & Iwama, 1992; Wilkie *et al.*, 1993; Wright *et al.*, 1993; Wilkie *et al.*, 1994, in press). Wilkie & Wood (1991) suggested that the persistent decrease in plasma Na^+ and Cl^- seen in rainbow trout (*Oncorhynchus mykiss* Walbaum) exposed to pH 9·5 for 3 days was due to a decrease in ion influx and/or increased diffusive efflux of ions across the gill epithelium. Previously, in acute studies, Wright & Wood (1985) and Wood (1989) had shown reduced Na^+ and Cl^- influx in rainbow trout exposed to pH 9·5 for 3 h, whereas a number of longer term studies indicated that rainbow trout are capable of surviving at high pH for days or even weeks (Jordan & Lloyd, 1964; Murray & Ziebell, 1984; Wilkie &

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Wood, 1991; Yesaki & Iwama, 1992). Such long-term survival at high pH would be dependent upon the animals' capability to regulate ion influx and/or diffusive ion losses so as to re-establish internal Na^+ and Cl^- balance. In the present investigation we employed radiotracers ($^{22}Na^+$ and $^{36}Cl^-$) to determine the quantitative changes in unidirectional Na^+ and Cl^- influx throughout the first 72 h exposure of rainbow trout to pH 9·5.

Branchial ammonia excretion is severely inhibited at high water pH (Wright & Wood, 1985; Yesaki & Iwama, 1992) but completely recovers after 24–48 h in rainbow trout acutely exposed to pH 9·5 (Wilkie & Wood, 1991). It is often argued that NH_4^+ can substitute for H^+ on the branchial Na^+/H^+ exchanger and that a significant proportion of ammonia can be excreted via Na^+/NH_4^+ exchange on the branchial epithelium (e.g. Maetz & Garcia Romeu, 1964; Maetz, 1972, 1973; Cameron & Heisler, 1983; Wright & Wood, 1985; McDonald & Milligan, 1988). Wilkie & Wood (1991) suggested that the recovery of ammonia excretion (J_{Amm}) by rainbow trout during high pH exposure might have been due to increased Na^+/NH_4^+ exchange. This hypothesis was tested in the present study by attempting to correlate changes in J_{Amm} with those observed for branchial Na^+ influx. The Na^+ transport inhibitor, amiloride, was then used to block Na^+ influx to establish if Na^+ influx was related to J_{Amm} after adaptation to high pH.

The importance of branchial chloride cells (CC) for ionoregulation in freshwater teleosts is now well-established (Laurent *et al.*, 1985; Avella *et al.*, 1987; Perry & Laurent, 1989; Laurent & Perry, 1990; Perry *et al.*, 1992) but until recently, little was known about the potential role that the CC played in regulating acid-base homeostasis. Recent work by Goss and colleagues (1992*a*,*b*) suggests that changes in the exposed fractional surface area of branchial chloride cells (CC) leads to differential influx rates for Cl^- and Na^+. To investigate the potential role that branchial CCs played during exposure to high pH, we utilized scanning electron microscopy to measure the individual surface areas, densities and exposed fractional surface areas of branchial CCs in rainbow trout that were held either at pH 8·0 or pH 9·5 for 3 days.

II. MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND SET-UP

Adult rainbow trout (*Oncorhynchus mykiss* Walbaum; mean weight=278 g) were obtained from Spring Valley Trout Farm, Petersburg, Ontario and maintained in moderately hard, dechlorinated Hamilton city tap water (composition: Ca^{2+}=1·0 mmol l^{-1}; Mg^{2+}=0·3 mmol l^{-1}; K^+=0·05 mmol l^{-1}; Na^+=0·6 mmol l^{-1}; Cl^-=0·8 mmol l^{-1}; titration alkalinity=2·1 mmol l^{-1}; hardness=140 mg l^{-1} as $CaCO_3$; pH≃8·0). The fish were acclimated to the experimental temperature of 13–16° C for 1 week prior to experimentation, during which time they were starved to remove the effects that feeding might have on nitrogen metabolism (Fromm, 1963). Two days prior to sampling the fish were transferred to one of seven, darkened, 5-l, plexiglass flux boxes (see McDonald, 1983 for details) contained in a 200-l recirculating system. Water flow to each box was approximately 1·0 l min^{-1} when the boxes were opened to the re-circulating system. When the boxes were operated as closed systems during flux determinations (see below), internal aeration maintained water P_{O_2} above 130 torr and also provided vigorous mixing for flux measurements.

The incoming, dechlorinated Hamilton tap water served as the control media (mean pH = 8·06 ± 0·04). The mean experimental pH of 9·54 ± 0·05 was maintained via a pH-stat set-up. Water pH was continuously monitored with a Radiometer GK2401C combination pH electrode connected to a PHM 82 pH meter; and a TTT 80 autotitrator (Radiometer). The latter controlled a solenoid valve which regulated the drop-wise addition of 1 N KOH into the well-mixed head tank. Water then flowed from the head tank (at pH 8·0 or 9·5) into the flux boxes, which drained to a lower reservoir and was subsequently pumped back up to the head tank. Continuous additions of KOH were necessary because water replacement into the system (approximately 3 l min^{-1}) and CO_2 production by the fish titrated water pH downward (see Wilkie & Wood, 1991 for further details). Titration with KOH led to water K^+ concentrations that approximated 0·7 mmol l^{-1}.

EXPERIMENTAL PROTOCOL AND ANALYTICAL TECHNIQUES

Part I: Cl^- and Na^+ influx

The rates of unidirectional Cl^- and Na^+ influx (J^{Cl}_{in} and J^{Na}_{in}, respectively) and ammonia excretion (J_{Amm}) were determined at pH 8·0 and at 0–1, 4–5, 8–9, 24–25, 48–49, 72–73 and at 75–76 h of pH 9·5 exposure. Ten minutes prior to the start of each flux determination water flow to each box was cut off and 4 μCi of ^{22}Na (New England Nuclear) and 10 μCi of ^{36}Cl (ICN Biomedicals Inc., Canada) was added to each box to permit sufficient mixing of the isotope. For the 75 h sample period, 10^{-4} M of amiloride (Na^+ salt; Sigma) was added, along with the isotope, 10 min prior to the start of the flux period, to determine if potential decreases in J^{Na}_{in} were associated with changes in J_{Amm} (cf. Wright & Wood, 1985). At time 0 and 60 min, 50 ml water samples were taken for later determination of total γ and β counts, 'cold' Cl^- and Na^+ concentrations and total ammonia concentrations. Due to the short duration of each flux period, we were unable to obtain adequate resolution of changes in cold Na^+ and Cl^- concentrations in the water and thus, were unable to estimate net ion fluxes. These short flux periods were necessary to minimize potentially interfering changes in water chemistry caused by the fish's metabolism (e.g. decreases in water pH, increases in water ammonia) during the period of box closure.

^{36}Cl is a pure β emitter, while ^{22}Na emits both β and γ rays. Accordingly, each sample was analysed for total activity (on a 1217 Rackbeta scintillation counter) and then for γ activity alone (i.e. ^{22}Na; on a Packard 5000 series γ counter). The counts (β only) attributable to ^{36}Cl were determined by subtracting ^{22}Na counts from the total counts, after correcting for the different counting efficiencies of ^{22}Na on the two machines. Total water Cl^- was determined via coulometric titration (Radiometer CMT10 chloridometer) and Na^+ via atomic absorption (Varian 1275 AA). Water ammonia was determined using a micro-modification of the salicylate–hypochlorite assay described by Verdouw *et al.* (1978).

J^{Cl}_{in} and J^{Na}_{in} were calculated using the following formula:

$$J^{Cl}_{in} \text{ or } J^{Na}_{in} = (cpm_i - cpm_f)/MSA \cdot Volume/WT \quad (1)$$

where: cpm_i and cpm_f are the respective β counts (cpm ml^{-1}) at the start and end of each flux determination period; MSA is the mean specific activity of either $^{36}Cl^-$ or $^{22}Na^+$ relative to the respective cold concentrations of each ion in the water (cpm μmol^{-1}); Volume refers to the volume of water in the flux box (≃3·0 l); W and T are the weight (kg) of each animal and the duration of each flux period (h), respectively. Back-flux correction was not necessary as internal specific activity never exceeded 10% of external specific activity for either Na^+ or Cl^- (see Wood, 1988 for details).

J_{Amm} was calculated using the following formula (also see Wright & Wood, 1985):

$$J_{Amm} = ([Amm]_i - [Amm]_f) \cdot Volume/WT \quad (2)$$

where: $[Amm]_i$ and $[Amm]_f$ are the respective concentrations of total water ammonia at the start and end of each flux period; Volume, W and T have the same meanings as previously stated.

Part II: Chloride cell morphometry and morphology

The analysis was based on the methods of Laurent & Perry (1990) and Goss *et al.* (1992*a*). Briefly, separate batches of rainbow trout were held in flux boxes (described above) and exposed to pH 8·0 (controls) or pH 9·5 water for 72 h. At this time the fish were killed, one at a time, with an overdose of MS 222 anaesthetic (Sigma; 1·5 g l^{-1}). The second left gill arch was excised and the gill filaments trimmed from the arch in small pieces, rinsed in ice cold 0·15 mol l^{-1} Na^+ cacodylate buffer (BDH), then fixed in 5% glutaraldehyde for approximately 70 to 80 min. After fixation, pairs of filaments, still joined at the septum, were dissected away from one another, washed three times with buffer, and then stored at 4° C for several hours. The paired filaments were partially dehydrated via an ethanol series (35, 50, 70%) and then stored in 70% ethanol for several days. The gills were subsequently dehydrated in 95% and absolute ethanol and then taken through two successive baths (2 min each) of 1,1,1,3,3,3-hexamethyldisilazane (Aldrich) and air dried. The paired filaments were then mounted onto aluminium stubs, sputtered coated with gold and subsequently viewed on a ISI-DS130 dual stage scanning electron microscope at 2000 times magnification. The region of analysis was located on the trailing edge of the filament and next to the septum. Approximately, six non-contiguous fields (approximately 2500 μm^2 per field) were photographed per filament. A total of 18 fields per fish were subsequently analysed. Individual CC surface area was determined with a MOP-3 digitizing system (Carl Zeiss, Inc.). Chloride cell fractional surface area (CC FSA) and CC density were subsequently determined from the individual CC surface areas and total filamental surface area examined. The filamental epithelium, rather than the lamellar epithelium, was used for determination of CC surface area because the former is more appropriate for morphometry. The paired filaments can be mounted parallel to the face of the aluminum stub. As a result, the flat and relatively uniform surface topography of the filamental epithelium is readily accessible for viewing via the scanning electron microscope. The undulating nature of the gill lamellae also makes the lamellar surface less suitable for viewing and the morphometrical measurements more vulnerable to error (see Goss *et al.*, 1992*a*).

STATISTICS

All data have been expressed as means ± S.E.M. (n) where n is the number of animals sampled. The statistical significance of differences with respect to Cl^-, Na^+, and ammonia fluxes was determined via a paired t-test ($P < 0.05$) with the Bonferroni correction procedure for multiple comparisons. The significance of differences in gill morphometrical data were determined via an unpaired t-test ($P < 0.05$), after first performing an F-test to check for homogeneity of variance.

III. RESULTS

Cl^- AND Na^+ INFLUX AND AMMONIA EXCRETION

Rainbow trout, held at control pH (pH 8·0), had Cl^- and Na^+ influx rates of 270 µmol kg^{-1} h^{-1} and 300 µmol kg^{-1} h^{-1}, respectively (Figs 1 and 2). Chloride influx was dramatically reduced by 67% over the first hour at high pH (pH 9·5). By 4 h, the inhibition of J^{Cl}_{in} reached 83% to 45 µmol kg^{-1} h^{-1}. However, by 8 h Cl^- influx rates started to increase again, and by 24 h had returned to a level not significantly different from pre-exposure values. At this time, J^{Cl}_{in} was approximately 210 µmol kg^{-1} h^{-1} and by 72 h it had recovered further, to approximately 260 µmol kg^{-1} h^{-1} (Fig. 1).

Na^+ influx, on the other hand, was reduced by 45% during the first hour of high pH exposure and declined further so that by 4 h it was 80% lower than control measurements. Despite a slight recovery of Na^+ influx over the next few

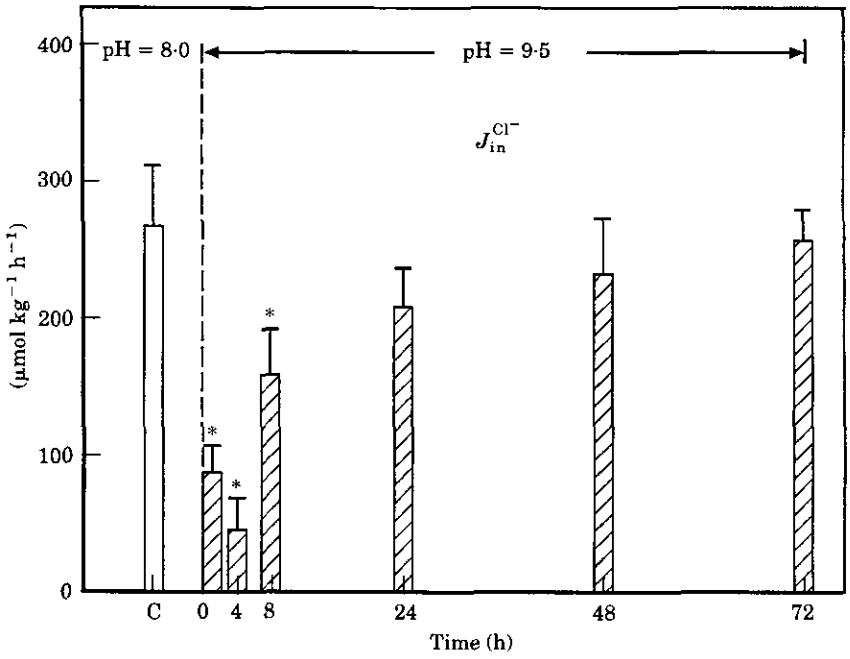

FIG. 1. The influence of severe alkaline exposure (pH=9·5) upon branchial Cl^- influx (J^{Cl}_{in}) in rainbow trout. Means ± 1 S.E.M.; n=7. *, Statistically significant differences from influx rates measured at pH 8·0 (P<0·05).

hours, J^{Na}_{in} never fully recovered and fluctuated around 150 μmol kg^{-1} h^{-1} for the remainder of the 72 h exposure (Fig. 2).

Analysis of terminal plasma samples revealed that plasma Cl^- and Na^+ concentrations approximated 116 mmol l^{-1} and 138·9 mmol l^{-1}, respectively. Neither value was significantly different from concentrations measured in fish that were held at pH 8·0 (data not shown).

Ammonia excretion was about 180 μmol kg^{-1} h^{-1} at control pH and decreased by 90% during the first hour of high pH exposure but gradually recovered; by 24 h J_{Amm} had returned to 150 μmol kg^{-1} h^{-1}, a value not significantly different from the pre-exposure rate (Fig. 3). To establish if there was a relationship between J^{Na}_{in} and J_{Amm}, the Na^+ influx blocker amiloride, was added to the water at 75 h. Despite a 50% reduction in J^{Na}_{in}, there was no effect of amiloride on J_{Amm} (compare Figs 2 and 3).

CHLORIDE CELL MORPHOMETRY

Examination of the branchial filamental epithelium of rainbow trout held at pH 8·0 *vs* pH 9·5 for 72 h suggested that the differential alterations in Na^+ and Cl^- influx might be explained by high pH induced increases in branchial CC fractional surface area. Those exposed to pH 9·5 for 72 h had fourfold greater CC fractional surface areas of about 280 000 μm^2 mm^{-2} [Fig. 4(a)]. This higher CC FSA was due to the twofold greater CC surface area and twofold greater CC density in the fish held at high pH [Figs 4(b),(c)]. The individual CC surface area in these fish approached 50 μm^2 and CC density was approximately 6000 CC mm^{-2}.

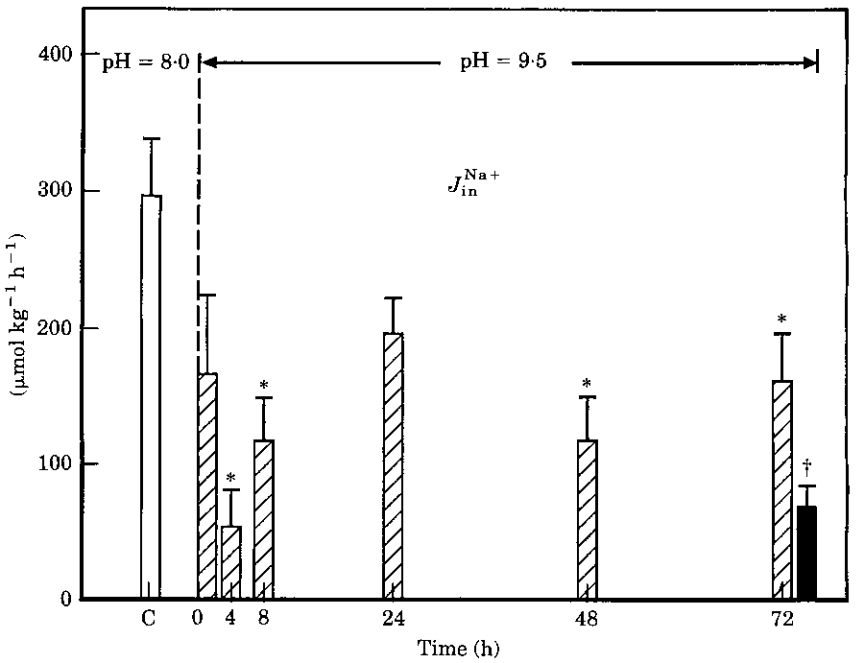

FIG. 2. The influence of severe alkaline exposure (pH=9·5), and subsequent amiloride treatment at 75 h (■), upon branchial Na^+ influx (J^{Na}_{in}) in rainbow trout. Means ± 1 S.E.M.; n=7. *, Statistically significant differences from rates measured at pH 8·0 (P<0·05). †, The changes in J^{Na}_{in} following amiloride treatment, were significantly different from J^{Na}_{in} at 72 h (P<0·05).

The greater CC FSA in the pH 9·5 fish is readily apparent in the representative scanning electron micrographs that are presented in Figs 5(a) and 5(b). Note the larger surface area and increased numbers of individual CCs in the fish that were held at pH 9·5. Furthermore, the CCs of the treatment fish were often aggregated, whereas CCs in the control fish were more or less solitary. The surface morphology of visible filamental CCs varied widely in fish at high pH. For instance, there were qualitative differences in the appearance and density of apical microvilli both amongst and within individual fish; frequently CCs that appeared stunted or worn, were in close association with CCs that possessed numerous, prominent apical microvilli [Figs 5(a), (b)].

IV. DISCUSSION

Previous studies have reported persistent, sometimes lethal, reductions in plasma Cl^- and Na^+ concentrations in rainbow trout exposed to alkaline water (pH ≥ 9·5; Heming & Blumhagen, 1988; Wilkie & Wood, 1991; Yesaki & Iwama, 1992). The present study suggests that some of these reductions might have been caused by an initial inhibition of branchial Cl^- and Na^+ influx. However, the ability of rainbow trout to survive at pH 9·5 for up to 5 weeks (Wilkie & Wood, 1991; M. P. Wilkie, H. E. Simmons & C. M. Wood, unpubl. obs.), suggests that the fish are able to offset these initial ionoregulatory disturbances. It appears that the fish in the present study were effectively regulating their internal ionic status because plasma Cl^- and Na^+ levels were normal. Furthermore, these

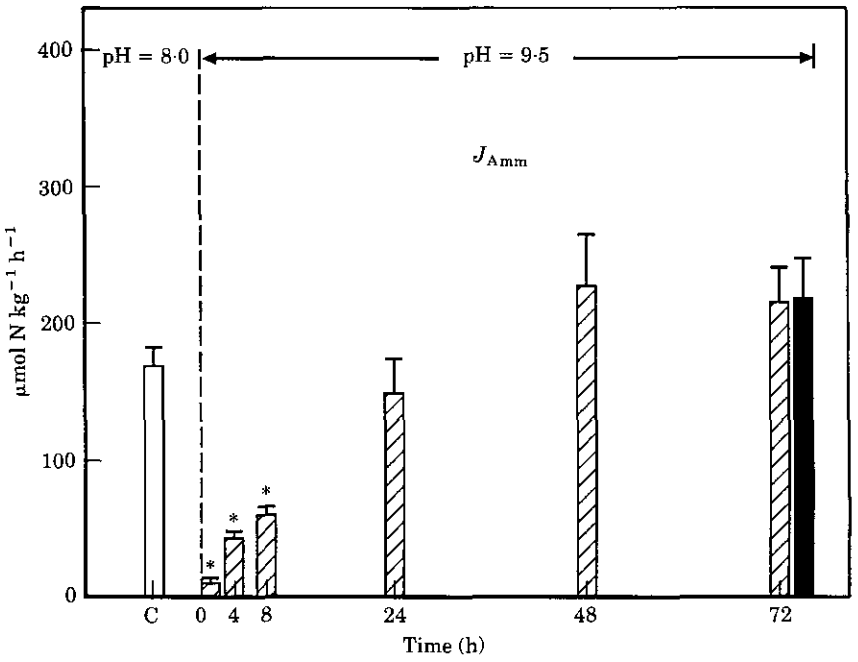

FIG. 3. The influence of severe alkaline exposure (pH=9·5), and subsequent amiloride treatment at 75 h (■), upon the rates of ammonia excretion (J_{Amm}) by rainbow trout. Means ± 1 S.E.M.; n=7. *, Statistically significant differences from J_{Amm} at pH 8·0 (P<0·05).

levels approximated to values that were previously reported for rainbow trout that survived at pH 9·5 for 72 h (Wilkie & Wood, 1991). The present study suggests that some correction of internal Cl^- and Na^+ balance might have resulted from complete re-establishment of J^{Cl}_{in} and partial recovery of J^{Na}_{in}. Future studies should focus on how these two parameters are modulated during long term high pH exposure and also establish if there are significant reductions in the efflux components of Cl^- and Na^+.

The fourfold increases in CC FSA, observed at pH 9·5 in the present study, are probably indicative of an adaptive response of salmonids to highly alkaline environments. Elevations in CC surface area and/or number have previously been observed in Lahontan cutthroat trout (*Oncorhynchus clarki henshawi* Gill and Jordan; formerly *Salmo clarki henshawi* Richardson) acutely exposed to, or *living* in highly alkaline Pyramid Lake, Nevada (pH=9·4; Galat *et al.*, 1985; Wilkie *et al.*, in press). Potentially, the increased CC FSA, observed in the present study, might have accounted for the complete recovery of J^{Cl}_{in} and the partial recovery of J^{Na}_{in} observed at high pH. Studies on fish in ion poor water indicate that both Na^+ and Cl^- influx are positively correlated with increases in branchial CC FSA (Laurent *et al.*, 1985; Avella *et al.*, 1987; Perry & Laurent, 1989). However, when acid-base disturbances are experimentally induced, the correlation between J^{Na}_{in} and CC FSA breaks down while that between J^{Cl}_{in} and CC FSA is accentuated (Goss *et al.*, 1992*a,b*). Indeed, Goss and colleagues have suggested that only J^{Cl}_{in} is directly mediated via the CCs, whereas J^{Na}_{in} may occur through the pavement (' respiratory ') cells of the branchial epithelium.

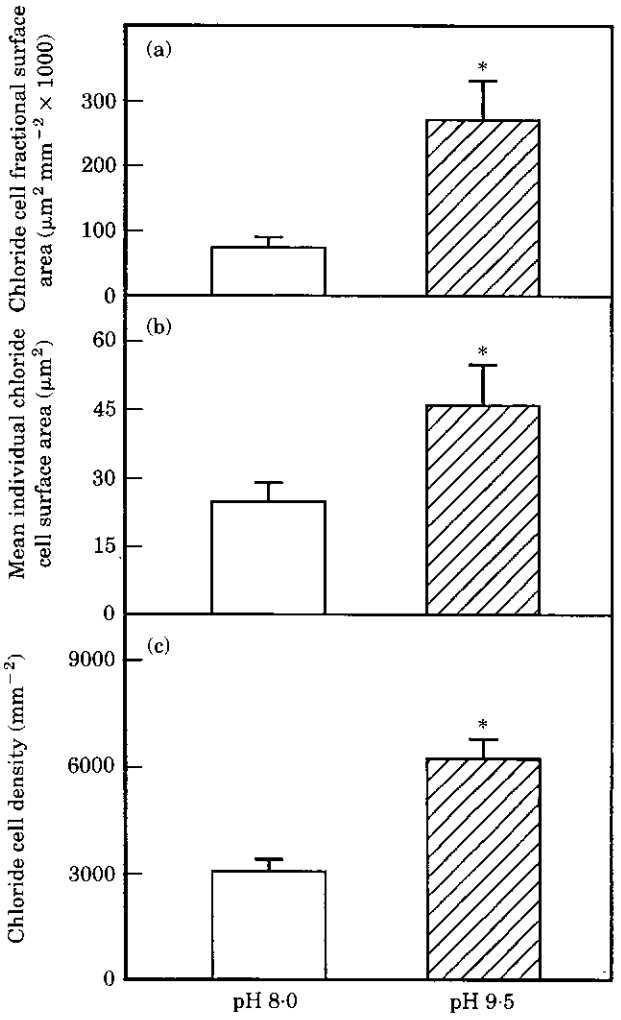

FIG. 4. Differences in branchial (a) chloride cell fractional surface area; (b) mean individual chloride cell surface area; and (c) chloride cell density of rainbow trout exposed to pH 8·0 or pH 9·5 dechlorinated Hamilton tap water. Means ± 1 S.E.M.; n=4 at pH 8·0 and n=5 at pH 9·5. *, Significant differences from pH 8·0 values (P<0·05).

Based on our earlier work (Wilkie & Wood, 1991) it is highly likely that the rainbow trout exposed to pH 9·5 in the present study were suffering from a respiratory alkalosis (see also Wright & Wood, 1985; Lin & Randall, 1990; Yesaki & Iwama, 1992). It is therefore possible that increased CC FSA was also related to the maintenance of acid-base homeostasis. Goss and colleagues (1992*a*,*b*) have suggested that increased CC FSA, induced in rainbow trout and bullheads (*Ictalurus nebulosus* Lesueur) via metabolic alkalosis, leads to a stimulation of Cl^- influx and a relative decrease in Na^+ influx which results in a net excretion of basic equivalents (=retention of acidic equivalents). The differential recovery of Cl^- and Na^+ influx in the present study (Figs 1 and 2) may be explained by the fact that increased CC FSA would favour Cl^- influx

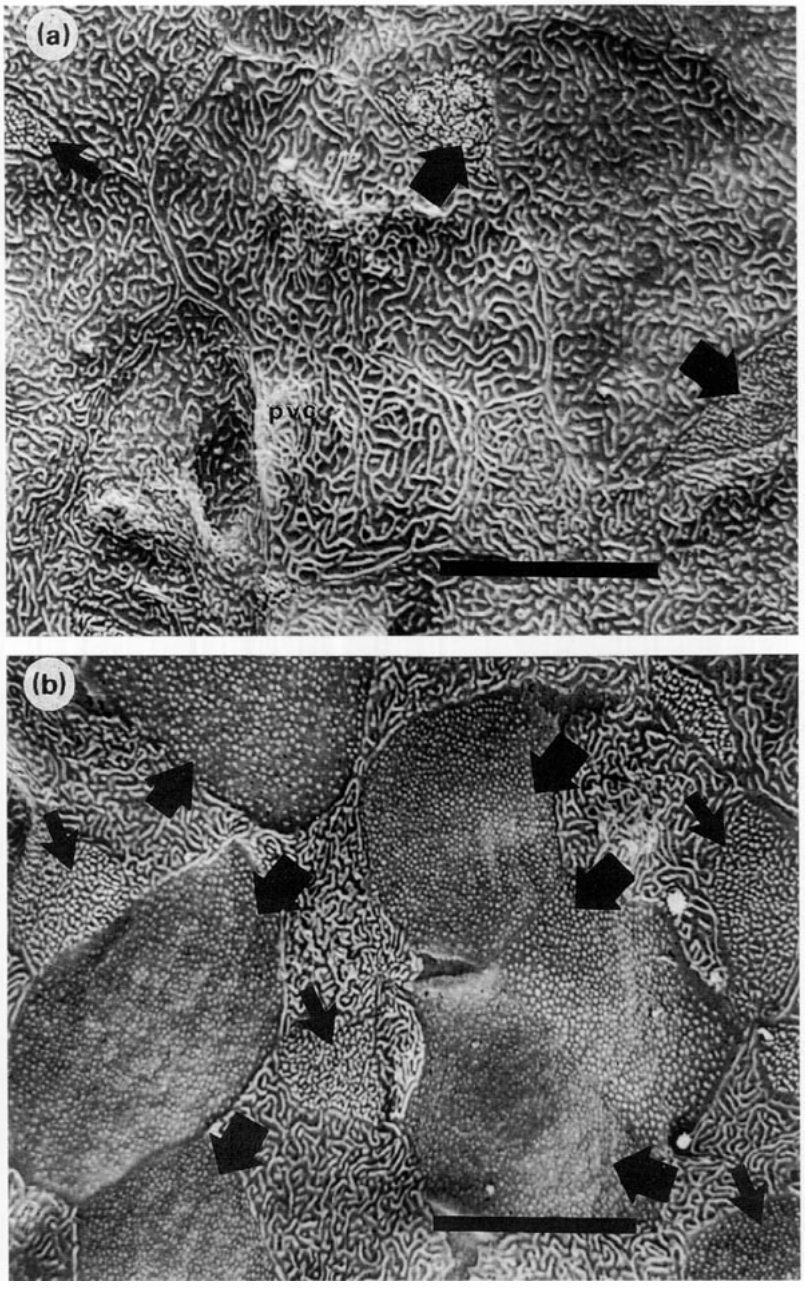

FIG. 5. Representative scanning electron micrographs of the filamental epithelium of rainbow trout exposed to pH 8·0 (a) or pH 9·5 (b) dechlorinated Hamilton tap water. Note the greater density and individual surface areas of chloride cells (CCs) (indicated by arrows) in the pH 9·5 trout. Also, note the differences in ornamentation of the CCs and the tendency of CCs to form aggregations in the pH 9·5 fish. pvc, Pavement cell; scale bar 10 μm. Magnification: (a) 2650 × ; (b) 2850 × .

over Na^+ influx, and therefore a net excretion of basic equivalents according to the theory of Goss and colleagues (1992*a*,*b*). Indeed, the blood acid-base measurements of Wilkie & Wood (1991) indicated a compensatory loss of basic

equivalents from the blood stream by the third day of exposure to pH 9·5. The possibility that changes in CC FSA differentially affects Cl^- and Na^+ uptake, to offset alkalosis in trout at high pH, is an intriguing possibility that deserves further study.

High pH exposure induces elevations in internal NH_4^+ (e.g. Wright & Wood, 1985; Lin & Randall, 1990; Wilkie & Wood, 1991; Yesaki & Iwama, 1992) and accordingly, several researchers have proposed that NH_4^+ can replace H^+ as the internal counterion for the Na^+/H^+ exchanger under these conditions (Wilkie & Wood, 1991; Yesaki & Iwama, 1992). In the present study, the observation that both Na^+ influx and ammonia excretion (J_{Amm}) declined and recovered (though J^{Na}_{in} only partially) over a similar time frame might suggest a functional linkage between these two fluxes (compare Figs 2 and 3). However, Na^+/NH_4^+ exchange seems unlikely at high pH because addition of the Na^+ uptake blocker amiloride to the water at 75 h had no effect on J_{Amm}, despite a 50% reduction in J^{Na}_{in} (compare Figs 2 and 3). These observations are in contrast with those of Yesaki & Iwama (1992) who reported that addition of amiloride to pH 10 water inhibited J_{Amm} by rainbow trout. Unfortunately, rates of J^{Na}_{in} were not determined by Yesaki & Iwama. At least in *alkaline* Hamilton tap water, it does not appear that NH_4^+ is a counterion for Na^+.

It is likely that simultaneous increases in blood pH and ammonia led to a steady rise in the blood P_{NH3} of the rainbow trout exposed to high pH (e.g. Wilkie & Wood, 1991; Yesaki & Iwama, 1992; Wright *et al.*, 1993). Potentially, this may have increased the blood to water P_{NH3} diffusion gradient and accounted for the recovery of J_{Amm} that was observed in this study and those previously mentioned. Future studies should address this possibility in more detail.

In summary, the differential recovery of Cl^- influx, relative to Na^+ influx, observed following exposure of rainbow trout to pH 9·5, may have been related to greater branchial CC FSA. Furthermore, the present study suggests that NH_4^+ is not a counterion for Na^+ under alkaline conditions. Accordingly, future investigations should attempt to deduce the mechanisms behind high pH-induced alterations in ion influx patterns and also establish how such changes affect overall ionic balance, ammonia excretion, and acid-base regulation, in rainbow trout exposed to alkaline pH. Determining the role that branchial chloride cells play in mediating ion influx at high pH may also provide clues that describe how some salmonids are able to tolerate alkaline environments indefinitely (Galat *et al.*, 1985; Wilkie *et al.*, in press).

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