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# SHORT-TERM CELL VOLUME REGULATION IN *MYTILUS CALIFORNIANUS* GILL

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## Summary

Long-term acclimation of *Mytilus californianus* to 60 % artificial sea water (585 mosmol $l^{-1}$; ASW) led to a 30–40 % decrease in the taurine (53.5–36.9 $\mu$mol $g^{-1}$ wet mass) and betaine (44.8–26.2 $\mu$mol $g^{-1}$ wet mass) content of gill tissue, compared with that of control animals held in 100 % ASW (980 mosmol $l^{-1}$). The $K^+$ content of gills did not change following long-term acclimation to reduced salinity. In contrast, losses of all three solutes during a brief (60 min) exposure to 60 % ASW were less than or equal to 15 %. Nevertheless, the swelling of gill cells that occurred after acute exposure to 60 % ASW was followed by a return towards the control volume. Direct optical measurement of single gill filaments confirmed that, during an acute exposure to reduced salinity, ciliated lateral cells increased in cell height (volume) and then underwent a regulatory volume decrease (RVD) with a half-time of approximately 10 min. This short-term RVD was completely inhibited by exposure to 1 mmol $l^{-1}$ quinidine, a $K^+$ channel blocker, but only when the drug was applied to the basolateral aspect of the gill epithelium. Application of 1 $\mu$mol $l^{-1}$ valinomycin relieved the inhibition by quinidine of the gill RVD. However, addition of valinomycin did not accelerate the rate of RVD observed in the absence of quinidine. These results indicate that long-term acclimation of *Mytilus californianus* gill in dilute sea water involves primarily losses of taurine and betaine, whereas short-term regulation of cell volume may involve an electrically conductive loss of intracellular $K^+$ and a counter ion.

## Introduction

When exposed for prolonged periods (days to weeks) to hypo-osmotic media, euryhaline marine invertebrates typically respond by reducing intracellular concentrations of low molecular weight organic molecules, thereby avoiding changes in the intracellular concentration of inorganic ions (Yancey *et al.* 1982). However, intertidal invertebrates may be exposed to cyclic changes in salinity that can include shifts from approximately 1000 mosmol $l^{-1}$ (i.e. sea water) to approximately 0 mosmol $l^{-1}$ (fresh water) as often as twice in a 24 h period (Stickle and Ahokas, 1975; Stickle and Denoux, 1976). For an osmoconformer, including species of the bivalve genus *Mytilus*, a short-

Key words: bivalve, *Mytilus californianus*, osmoregulation, taurine, betaine, potassium.

term change in salinity can result in a large change in the osmotic concentration of hemolymph (e.g. Shumway, 1977), necessitating a rapid compensatory volume regulatory response in the cells of these animals. Several studies have examined the response of intact bivalves, including *M. edulis* (e.g. Shumway, 1977; Livingstone *et al.* 1979; Stickle and Denoux, 1976; Hand and Stickle, 1977; Stickle and Ahokas, 1974), to brief and/or cyclic exposures to fluctuating salinity. These studies have not, however, examined the cellular mechanisms that may be activated during brief exposures to aniso-osmotic media.

The present study characterizes the response of cells in gills of *M. californianus* and *M. galloprovincialis* to acute exposure to dilute sea water. Lateral ciliated cells in both species showed a rapid, vigorous regulatory decrease in cell volume following the swelling associated with exposure to an osmotically dilute medium. However, whereas long-term exposure of gill tissue to dilute sea water was correlated with a loss of the organic osmolytes taurine and betaine, the short-term regulatory volume decrease appeared to involve electrically conductive losses of inorganic solutes.

## Materials and methods

### *Animals and sea water*

*Mytilus californianus* (Conrad) and *Mytilus galloprovincialis* (Lamarck) were purchased from Bodega Marine Laboratories, Bodega Bay, CA, and held in commercial artificial sea water (Tropic Marin, salinity 32 ‰) in recirculating aquaria at 13 °C. The animals were not fed and were normally used within 8 weeks of collection. Experiments were performed using an artificial sea water (ASW), the composition of which (in $mmol\,l^{-1}$ for 100 % ASW) was: 423 NaCl, 23 $MgCl_2$, 26 $MgSO_4$, 9 $CaCl_2$, 9 KCl and 2 $NaHCO_3$ (Cavanaugh, 1956). The osmolality of 100 % ASW (32 ‰) averaged 980 $mosmol\,l^{-1}$ with a range of 950–1010 $mosmol\,l^{-1}$ (determined using a Wescor vapor pressure osmometer, model 5500XR).

### *Long-term acclimation studies*

*M. californianus* were acclimated to 60 % ASW (19 ‰; 585 $mmol\,l^{-1}$) by transfer to 80 % ASW for 7 days followed by 14 days in 60 % ASW. Some animals were then returned directly to 100 % ASW for an additional 7 days.

### *Preparation of gill extracts*

Extracts of *M. californianus* gill were prepared for nuclear magnetic resonance (NMR) and high performance liquid chromatography (HPLC) measurements of tissue taurine and betaine content. Approximately 0.5 g (wet mass) of gill tissue was homogenized in deionized water, followed by overnight extraction at 4 °C in 6 % perchloric acid (PCA). PCA extracts were centrifuged the following day at 4 °C for 20 min at 48 000 ***g*** to remove particulate material. The pH of the supernatant was adjusted to 7 with 30 % KOH, and it was refrigerated for 2 h and centrifuged again to remove the resultant $KClO_4$ precipitate. For HPLC analysis, the neutralized extract was passed through a Sep-Pak $C_{18}$ cartridge (Millipore Corp., Bedford, MA) and stored at −20 °C until analyzed. For NMR studies, the neutralized extract was lyophilized and stored at room temperature until used.

### *Nuclear magnetic resonance spectroscopy*

Lyophilized gill extracts were reconstituted in 3.0 ml of 20 % $D_2O$ and analyzed by $^{13}C$ and $^{1}H$ NMR (nuclear magnetic resonance spectroscopy). Both types of spectra were acquired on a Bruker AMX 400 wide-bore spectrometer using a 10 mm broad-banded probe.

For $^{1}H$ NMR, the water resonance peak was suppressed with presaturation. Spectra were accumulated as the sum of 900 transients induced by a 32 ms pulse (90 °) every 2 s. Proton frequency was 400.135 MHz. The resulting free induction decays (FITS) were Fourier-transformed with a 2 Hz exponential line broadening (LB) and referenced to the water peak at 4.74 p.p.m. $^{1}H$ NMR spectra were acquired *in vivo* using the same parameters.

$^{13}C$ NMR was proton-decoupled using a WALTZ-16 sequence during the acquisition with no decoupling between acquisitions. These conditions give complete decoupling but do not establish a nuclear Overhauser effect. Spectra were accumulated as the sum of 360 transients induced by a 90 ° pulse every 20 s and referenced to the $\alpha$-$C_1$ of glucose at 96.1 p.p.m. (LB=5 Hz). $^{13}C$ NMR spectra (900 transients) were acquired *in vivo* with a 45 ° pulse every 2 s. $^{13}C$ *in vivo* measurements were proton-decoupled with a WALTZ-16 sequence, as described above. The duty cycle of decoupling was approximately 10 % and did not cause sample heating. $^{13}C$ frequency was 100.625 MHz.

### *High performance liquid chromatography*

Quantitative estimates of gill taurine and betaine content were made using the HPLC method of Wolff *et al.* (1989). Osmolytes in the gill extracts were resolved on a Waters Sugar Pak I calcium cation exchange column (300 mm×6.5 mm i.d.) heated to 84 °C, using distilled water containing approximately 0.15 mmol $l^{-1}$ ethylenediamine tetraacetic acid (EDTA) as the mobile phase. Peaks were visualized using a refractive index detector and identified from their retention times.

### *Ion-exchange chromatography*

Ion exchange chromatography measured total primary amines in *M. californianus* gill. Gill tissue was extracted overnight in 80 % ethanol. The ethanol extracts were evaporated to near dryness and then resuspended in 1.0 ml of lithium buffer (Beckman), pH 7, followed by analysis on a Beckman 7300 amino acid analyzer. Amines were visualized with ninhydrin and identified from their retention times.

### *Measurement of tissue $K^+$ content*

Total tissue $K^+$ content of *M. californianus* gill was determined in a manner similar to that previously described (Wright *et al.* 1989). Briefly, disks of gill tissue (15–20 mg wet mass) were weighed and placed into 0.5 ml of 0.1 mol $l^{-1}$ $HNO_3$ and freeze-thawed three times over a 24 h period. The $K^+$ content of the tissue extracts was determined with a flame photometer (Instrumentation Laboratory).

### *Measurement of external and vascular space of intact gills*

*Mytilus* gill consists of three compartments (Fig. 1): (i) cytoplasm; (ii) vascular

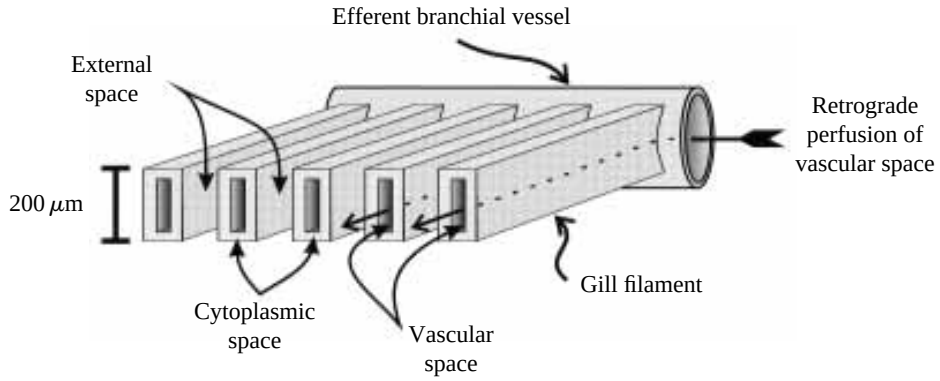


Fig. 1. Schematic representation of the cytoplasmic, vascular and external compartments of a portion of *Mytilus* gill. Individual filaments insert into the efferent branchial vessel along the distal edge of the demibranch (refer also to White, 1937) and can be perfused by means of a cannula inserted into the vessel. Scale is approximate.

(hemolymph); and (iii) 'external' (i.e. the seawater spaces between adjacent filaments, which typically are not cleared completely even after thorough blotting of tissue). The relative volume of the external compartment was estimated by incubating pieces of demibranch in ASW containing $^{14}$C-labelled polyethylene glycol ([$^{14}$C]PEG; $M_r$ approximately 4000) for 10 min. Tissue was blotted in the manner used for other assays, weighed and then extracted in 80 % ethanol for several hours before being assayed for radioactivity.

Vascular space was measured in separate experiments using a 'bolus' perfusion technique (Wright *et al.* 1989). An ASW perfusate containing [$^{14}$C]PEG was delivered in sufficient volume to distend the efferent branchial vessel (Fig. 1) to 2–3 times its original diameter of approximately 200–300 $\mu$m. The myogenic activity of the efferent branchial vessel served to perfuse evenly the gill filaments, which make perpendicular connections to the branchial vessel (Fig. 1). A total of 1.25–1.5 ml of the [$^{14}$C]PEG–ASW solution was perfused through a section of demibranch (approximately 250 mg wet mass). This represents a perfusate volume:vascular space volume of approximately 15:1. Excess perfusate flowed from the open, cut ends of the filaments. Following perfusion, the tissue was rinsed for several minutes and radioactivity was assayed as above.

The values for external and internal vascular space were used to correct for non-cell $K^+$ measured in tissue extracts:

$$[K^+]_{cell} = (K^+_{tot} - K^+_{BH+ES})/V_{cell},$$

where $[K^+]_{cell}$ is the $K^+$ concentration in cell water; $K^+_{tot}$ is the tissue $K^+$ content in $\mu$mol g$^{-1}$ wet mass; $K^+_{BH+ES}$ is the $K^+$ content of the vascular space and external space, assuming that hemolymph $K^+$ concentration is equal to that of sea water (Bayne *et al.* 1976); and $V_{cell}$ is an estimate of the volume of cell water in 1 g of gill tissue. The volume of the cellular compartment was determined by measuring total tissue water from wet/dry ratios and correcting for external and vascular space volumes.

### *Optical studies*

Morphometric measurements of relative changes in gill cell volume following exposure to sea waters of different salinity were made with a Nomarski differential interference contrast (DIC) video microscopy system. A small section of gill tissue (approximately 2 mm×8 mm) was placed (frontal surface down) on a no. 1 coverglass in a modified Dvorak–Stotler chamber (Nicholson Precision Instruments Inc.). The tissue was held in place with a 3/4 inch (19 mm) round Lucite disk [1/8 inch (3 mm) thick]. The Lucite disk had a 1/4 inch (6 mm) hole drilled through the center for light to pass through the central portion of the tissue section and was sealed to the chamber cover glass with vacuum grease. The chamber assembly was placed on the stage of an inverted Olympus IMT-2 DIC microscope and the tissue was superfused continuously at a rate of approximately 1 ml $min^{-1}$ with ASW of the appropriate composition.

The Nomarski DIC optics of our system included an Olympus 40× objective (numerical aperture of 0.55) and an ultralong working distance condenser (numerical aperture of 0.55). Video DIC images were produced with a DAGE CCD-72 video camera and displayed on an Ikegami PM175-A B/W monitor. Images were collected at pre-programmed intervals, stored on a microcomputer and analyzed off-line using IMAGE 1 software (Universal Imaging Corp.).

Optical sections of the living gill were compared with sections of fixed gill tissue. Small sections of demibranch (approximately 5 mm×5 mm each) were fixed for 90 min in 50 ml of 100 % ASW containing 3 % glutaraldehyde, pH 7.3. The tissue was then transferred to a fresh glutaraldehyde solution and held overnight at 4 °C. The following day, the tissue was rinsed for 5 min in 100 % ASW and dehydrated through an alcohol series (70, 90 and 95 % and absolute ethanol, 5 min in each solution). The tissue was embedded in a low-viscosity plastic medium (Spurr, 1969) and 1 $\mu$m sections were cut with an ultramicrotome and mounted on glass slides. Sections were stained with Toluidine Blue.

### *Transport experiments*

Gills were dissected from *M. californianus* and a piece of nylon fishing line was secured to the end of each demibranch. Prior to a transport measurement, tissue was maintained for 60 min at room temperature (23 °C) in 100 % ASW. Immediately prior to the experiments, individual demibranchs were pre-equilibrated for 1 min in a slowly stirred solution of ASW containing 10 $\mu$mol $l^{-1}$ 5-hydroxytryptamine (5-HT), to activate lateral cilia (Wright, 1979). Tissue was then suspended for 2 min in 100 ml of a slowly stirred solution of ASW of the appropriate composition containing 10 $\mu$mol $l^{-1}$ 5-HT, 37 kBq of radiolabeled taurine and sufficient unlabeled substrate to produce a total taurine concentration of 0.5 $\mu$mol $l^{-1}$.

Following the test incubation, demibranchs were rinsed for 5 min in 200 ml of ice-cold ASW containing 10 $\mu$mol $l^{-1}$ 5-HT and then blotted on tissue paper. Disks (7 mm diameter) were then cut from the demibranchs. Tissue disks were weighed to the nearest 0.1 mg and extracted for several hours in 80 % ethanol before being assayed for radioactivity. Uptake rates are expressed as pmol taurine $mg^{-1}$ wet mass 2 $min^{-1}$.

### *Data analysis*

Statistical analysis of data was made using an analysis of variance (ANOVA) coupled with paired *t*-tests. Differences were considered to be significant at the $P<0.05$ level. Data are presented as means ± standard errors of the mean.

### *Chemicals*

[$^{14}$C]taurine (3.4 GBq mmol$^{-1}$) and [$^{14}$C]PEG ($M_r$ 4000; 0.02 GBq g$^{-1}$) were purchased from New England Nuclear. All other chemicals were obtained from Sigma Chemical Corporation.

## Results

### *Organic solute content of gill tissue*

$^{13}$C and $^{1}$H NMR provided a qualitative profile of the principal organic compounds in gill tissue. Fig. 2 shows representative $^{1}$H and $^{13}$C NMR spectra obtained from PCA extracts of gill tissue. The $^{1}$H spectrum (Fig. 2A) showed three major peaks with proton ratios of 9:2:2 corresponding to the betaine trimethyl, taurine $CH_2$ and betaine $CH_2$ groups, respectively. The $^{13}$C spectrum (Fig. 2B) showed sharp peaks at 35, 45, 55 and

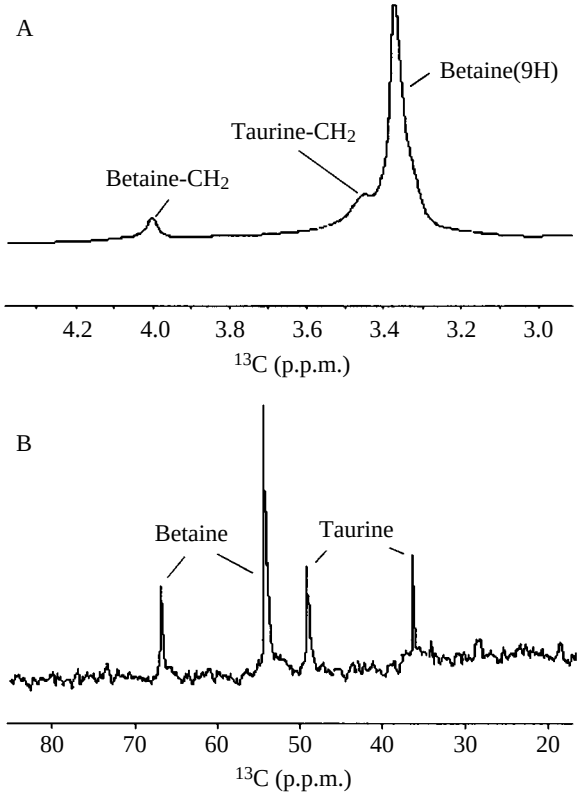


Fig. 2. Nuclear magnetic resonance spectra of PCA extracts of *Mytilus californianus* gill. See text for details.

65 p.p.m., corresponding to taurine and betaine. $^{13}C$ spectra also showed broad peaks at 25, 125 and 175 p.p.m. (data not shown), which correspond to methyl/methylene, vinyl and carboxyl carbons, respectively, in a ratio of 2.3:0.4:1. These peaks were tentatively assigned to short unsaturated acyl chains. Spectra collected from an intact living gill were qualitatively similar to spectra obtained from gill extracts (data not shown). Collectively, the NMR spectra indicate that taurine and betaine are the predominant organic solutes in *M. californianus* gill.

Quantitative measurements of taurine and betaine in the PCA gill extracts were obtained using HPLC techniques. Taurine content of gills from 10 animals maintained in 100 % ASW was 53.5±2.5 $\mu$mol $g^{-1}$ wet mass (S.E.M.) and betaine content was 44.8±2.8 $\mu$mol $g^{-1}$ wet mass.

The cytoplasmic concentrations of taurine and betaine in gill cells were calculated from a knowledge of (i) gill dry mass and wet mass, and (ii) the percentage of total tissue water occupied by vascular and external (seawater) space. Dry mass/wet mass ratios obtained from measurements using three pieces of tissue from three different animals acclimated to 100 % ASW showed gill solids to be 14.6±0.5 % of total tissue wet mass or approximately 0.15 g of solid in 1 g of wet gill tissue. Vascular space was 0.32±0.05 ml $g^{-1}$ wet mass ($N$=3) and external space was 0.29±0.02 ml $g^{-1}$ wet mass ($N$=3), which was consistent with previous reports (Wright and Stephens, 1977, external space; Wright *et al.* 1989, vascular space). Neither short- nor long-term acclimation to 60 % ASW produced significant changes in dry mass/wet mass ratio or in the volume of vascular and external fluid compartments.

Based upon the values listed above for the several tissue compartments in gills from animals acclimated to 100 % ASW, cell water in *M. californianus* gill was calculated to be 0.24±0.06 ml $g^{-1}$ wet tissue mass. This value was used to estimate the intracellular concentrations of organic solutes from measurements of total tissue solute content. For taurine and betaine, intracellular concentrations in control gills were approximately 223 mmol $l^{-1}$ and 187 mmol $l^{-1}$, respectively. These calculations are predicated on the assumption that taurine and betaine are effectively confined to the cellular compartment. This assumption is supported by reports (e.g. Zurburg and De Zwaan, 1981) that the vascular (i.e. hemolymph) concentration of amino acids in *M. edulis* is of the order of 1 mmol $l^{-1}$, which is very low compared with the amino acid content of total tissue.

The total pool of primary amines in ethanol extracts of gill tissue was measured with ion exchange chromatography. (As a quaternary amine, betaine was not identified by this protocol.) Table 1 lists the concentrations of intracellular organic solutes in gill extracts from animals acclimated to 100 % ASW. Note that taurine content measured with this procedure (49.9±4.9 $\mu$mol $g^{-1}$ wet mass; $N$=7) matched closely the value measured independently using HPLC techniques (53.5±2.5 $\mu$mol $g^{-1}$ wet mass). The total tertiary amine pool of control gill tissue (including taurine) determined using ion exchange chromatography was 75.1±7.7 $\mu$mol $g^{-1}$ wet mass. Addition of betaine content (44.8 $\mu$mol $g^{-1}$ wet mass; determined with HPLC) resulted in a total nitrogenous solute pool in *M. californianus* gill of 119.9 $\mu$mol $g^{-1}$ wet mass. Using a calculated cell water space of 0.24 ml $g^{-1}$ wet gill mass, the intracellular concentration of organic osmolytes

was, therefore, estimated to be 500 mmol $l^{-1}$. The osmolality of the 100 % ASW to which the animals were acclimated was approximately 980 mosmol $l^{-1}$. Thus, organic osmolytes in control gills constitute approximately 50 % of the intracellular osmolyte pool.

*Effect on tissue solute content of long-term acclimation to reduced salinity*

Long-term acclimation of *Mytilus* (and other bivalves) to reduced salinity is typically associated with a reduction in tissue content of nitrogenous solutes (e.g. Bricteux-Gregoire *et al.* 1964; Lange, 1963; see also Gilles, 1987). There have, however, been no studies on the response of *Mytilus* gill to long-term acclimation to reduced salinity. As expected, the nitrogenous solute content of gill tissue declined sharply following a protocol that included 7 days in 80 % ASW followed by another 14 days in 60 % ASW. Taurine and betaine content (determined using HPLC) decreased by 30 % and 40 %, from 53.5 and 44.8 $\mu$mol $g^{-1}$ to 36.9±0.28 and 26.2±3.42 $\mu$mol $g^{-1}$, respectively ($P$<0.05). In contrast, the other nitrogenous solutes declined only 10 %, from 25.2 to 22.7 $\mu$mol $g^{-1}$ (Table 1). The total tissue content of organic solutes decreased from 119.9±10.5 to 73.8±6.2 $\mu$mol $g^{-1}$. This corresponds to a calculated decrease of the intracellular organic solute concentration from approximately 500 to approximately 308 mosmol solute $l^{-1}$ cell water or a loss of approximately 200 mmol solute $l^{-1}$ cell water. When *M. californianus* were reintroduced to 100 % ASW for 7 days after 21 days in reduced salinity (7 days in 80 % ASW, followed by 14 days at 60 %), there was no significant increase in either taurine or betaine content in gill extracts. Taurine content in these animals (after 7 days in normal-strength sea water; $N$=4) was 35.8±2.8 $\mu$mol $g^{-1}$ (a change of −3 %) and betaine content was 29.5±2.3 $\mu$mol $g^{-1}$ (a change of +12.5 %).

Tissue $K^+$ content in gills from animals acclimated to 100 % ASW was

Table 1. *Organic amine content of* Mytilus californianus *gill*

| Solutes | 100 % ASW ($N$=7) | 60 % ASW | |
|---|---|---|---|
| | | Short-term ($N$=2) | Long-term ($N$=6) |
| Taurine | 49.9±4.91 | 54.9±2.17 | 24.9±0.94* |
| Urea | 4.96±0.75 | 5.44±0.40 | 2.39±0.59* |
| Aspartate | 4.61±0.35 | 6.18±0.19 | 2.22±0.47* |
| HLYS-A | 4.61±0.35 | 11.0±1.80* | 2.69±0.52 |
| Glycine | 2.46±0.27 | 2.20±0.01 | 0.70±0.13* |
| Glutamate | 1.50±0.10 | 1.44±0.13 | 1.55±0.22 |
| Alanine | 1.33±0.24 | 0.94±0.02 | 2.22±0.34* |
| GABA | 1.32±0.11 | 1.94±0.06* | 1.14±0.08 |
| Other amines | 4.42±1.40 | 8.06±1.19 | 9.82±3.24 |
| Total amines | 75.1±7.68 | 92.1±5.19 | 47.6±2.77* |

Primary, secondary and tertiary amines were measured in ethanol extracts of gill tissue using ion exchange chromatography.

All values are expressed as $\mu$mol solute $g^{-1}$ wet mass tissue (±S.E.M.).

*Statistically different ($P$<0.05) from 100 % ASW animals.

HLYS-A, hydroxylysine; GABA, $\gamma$-aminobutyric acid.

48±1.7 $\mu$mol g$^{-1}$. There was no significant change in tissue $K^+$ content of animals acclimated to 60 % ASW over a period of 3 weeks (46±1.8 $\mu$mol g$^{-1}$). Correction for the low concentration of $K^+$ in the external (seawater) and vascular compartments resulted in calculated cytoplasmic $K^+$ concentrations of 184±7 mmol l$^{-1}$ and 178±7 mmol l$^{-1}$ for animals acclimated to 100 % and 60 % ASW, respectively. These values are very similar to those recently measured for sarcoplasm of smooth muscle cells in *M. edulis* (200 mmol l$^{-1}$; Borseth *et al.* 1992).

*Short-term acclimation to reduced salinity*

It is apparent that the long-term response of gill cells to hypotonic stress involves preferential losses of taurine and betaine. However, it is not known whether a similar strategy is employed during acute exposures to reduced salinity. In fact, analysis of tissue extracts from gills exposed for 60 min to 60 % ASW revealed that concentrations of each of the major osmolytes (taurine, betaine and $K^+$) decreased, but none of these decreases was significant ($P$>0.05). HPLC analysis of extracts of gill tissue exposed for 60 min to 60 % ASW (paired tissues from five animals) showed that gill taurine content decreased by only 15 % (from 52.6 to 44.3 $\mu$mol g$^{-1}$) and gill betaine content decreased by less than 6 % (from 39.4 to 37.1 $\mu$mol g$^{-1}$). The decrease in tissue $K^+$ content was only 15 % (from 48±1.7 to 41±2.5 $\mu$mol g$^{-1}$). It should be noted that, after the 60 min exposure, the branchial vasculature was perfused with 60 % ASW to flush from the tissue any solutes lost across the basolateral aspect of gill cells into the vascular space. Thus, gill cells appeared to lose only some 70 mosmol l$^{-1}$ of cytoplasmic solute during a 60 min acute exposure to 60 % ASW.

*Optical characterization of the short-term volume regulation in single gill cells*

A previous study (Silva and Wright, 1992) provided evidence suggesting that *Mytilus* gill tissue is capable of a regulatory volume decrease (RVD) following the gain of water associated with acute exposure of gills to dilute sea water. Gill cells might be expected to lose solute as mechanisms to invoke an osmotically obligated loss of water. Although we could measure little loss of solute following acute exposure of gills to 60 % ASW, the errors associated with indirect measurement of the various tissue water compartments during an acute exposure to an aniso-osmotic medium may have compromised the resolution of such losses. Therefore, we elected to determine directly whether gill cells were capable of an RVD. An optical method was used to monitor changes in cell size arising from exposure of the gill epithelium to aniso-osmotic media. DIC microscopy has been used to measure cell volume changes in intact epithelia (e.g. Spring, 1985; Foskett, 1988; DiBona *et al.* 1985). The high-resolution images produced with DIC, when used in conjunction with video-imaging capabilities and computer-assisted data acquisition, provided an accurate means of monitoring rapid changes in apparent cell volume during exposure to aniso-osmotic media.

Changes in the height of ciliated lateral cells in gills from *M. galloprovincialis* and *M. californianus* provided a relative measure of change in cell volume throughout the course of exposure to aniso-osmotic media. The location of this specific cell type in the gill is shown schematically in Fig. 3A and in a cross section of fixed/stained tissue in

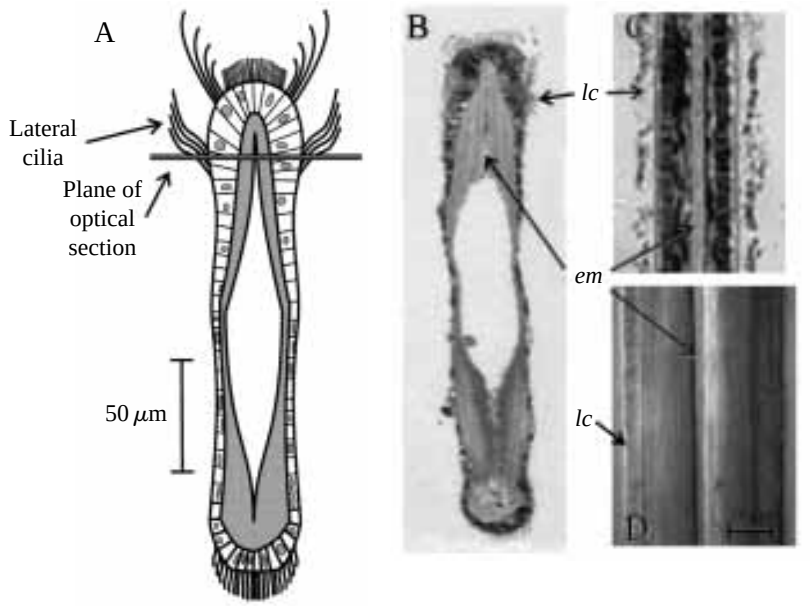


Fig. 3. Cross and longitudinal sections of a single *Mytilus californianus* gill filament. (A) Schematic representation of a filament in cross section. Epithelial cells rest on an extensive extracellular matrix (*em*) which, in turn, surrounds a hemolymph-filled branchial vessel. Lateral cilia (*lc*) arise from the lateral cells. The horizontal shaded bar represents the plane of focus used in the optical measurements of lateral cell height. (B) Cross section, stained with Toluidine Blue, of a single gluteraldehyde-fixed gill filament. (C) Longitudinal section, stained with Toluidine Blue, of a single fixed gill filament. The section was cut at the level of the lateral cells and shows these ciliated cells resting on the extracellular matrix of the filament. (D) DIC optical section of a single living gill filament. The plane of focus of the section was at the level of the lateral cells. Lateral cilia and extracellular matrix are evident. Scale bar, 20 $\mu$m.

Fig. 3B. The gill consists of thousands of individual gill filaments, each of which is an elliptical tube composed of a single layer of epithelial cells resting on an extensive extracellular matrix. The extracellular matrix, in turn, is bathed by the hemolymph contained within the vascular space of the filament. Each cell is exposed to sea water at its apical aspect and to hemolymph at its basolateral aspect. The cilia which arise from lateral cells produce the normal flow of water between adjacent filaments. Fig. 3C is a longitudinal section of a single filament from a piece of fixed tissue, cut at the level of the lateral cells. The lateral cilia are apparent, as is the extensive extracellular matrix upon which rest the lateral cells on either side of the filament. Fig. 3D is a DIC optical section of a living gill filament at the level of the lateral cells. Both the lateral cilia and the extracellular matrix are clearly evident. Indeed, the lateral cilia provided a convenient and reproducible marker for locating the lateral cells and for tracking their behavior throughout the experimental period. Note also that the apparent height of fixed lateral cells (i.e. the distance between the apical and basal aspects of the cells) is approximately 25 % less than that of cells observed using DIC microscopy. In contrast, the extracellular

matrix was virtually identical in width in both sections (5.9 $\mu$m *versus* 6.0 $\mu$m in optical *versus* stained sections). The difference in cell height probably reflects cell shrinkage occurring during the fixation process.

Fig. 4 is a compilation of optical images collected at the level of the lateral cilia in a single gill filament from *M. californianus* acclimated to 100 % ASW. The images show changes in cell height during a salinity challenge. In Fig. 4A (control tissue in 100 % ASW), cell height was 24.8 $\mu$m. After a 2.5 min exposure to 60 % ASW, cell height had increased to 31.7 $\mu$m (Fig. 4B). After 60 min of acclimation to the reduced salinity, gill

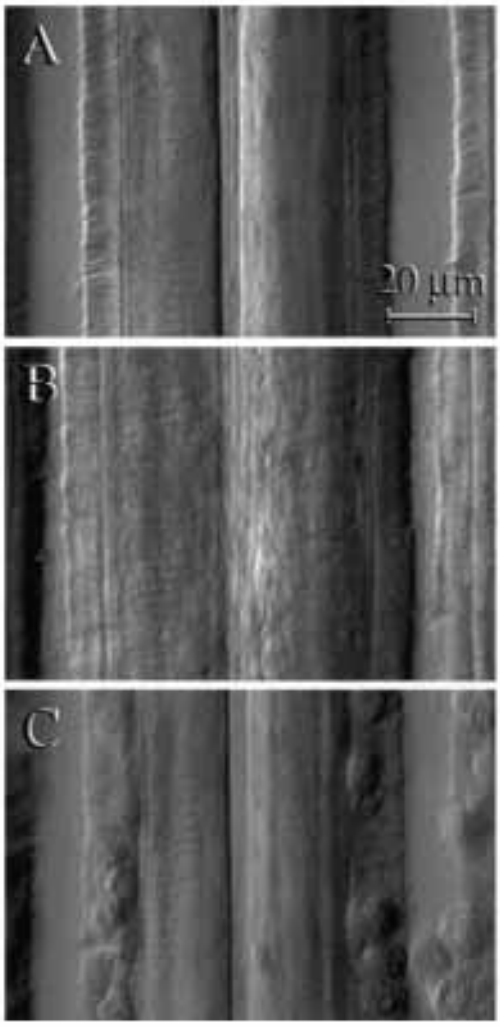


Fig. 4. DIC images of a single *Mytilus californianus* gill filament showing changes in lateral cell height recorded during exposure to aniso-osmotic ASW. (A) An image of the filament in 100 % ASW. (B) An image collected 2.5 min after a change of the chamber superfusate to 60 % ASW. (C) An image collected 1.5 min after the return of the superfusate to 100 % ASW (prior to the return to 100 % ASW, the tissue had been exposed to 60 % ASW for 60 min). Scale bar, 20 $\mu$m.

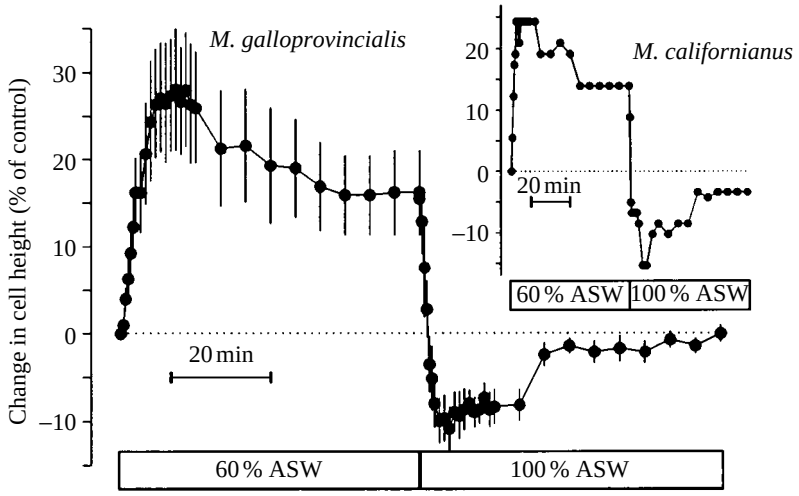


Fig. 5. Time course of change in lateral cell height in *Mytilus galloprovincialis* gill filament during a cycle of osmotic challenge. Each point represents the mean (± S.E.M.; *N*=5 animals) change, relative to time zero, of apical-to-basal height of single lateral cells determined from a video image collected at the indicated time. Inset: representative time course (from a single animal) of the change in lateral cell height in *M. californianus* gill filament during a cycle of osmotic challenge. Each point represents the change, relative to time zero, of apical-to-basal height of a single lateral cell determined from a video image collected at the indicated time.

tissue was returned to normal-strength sea water. Fig. 4C shows the decrease in cell height (to 22.3 $\mu$m) that occurred within 1.5 min of reintroduction into 100 % ASW.

Fig. 5 shows the time course of changes in the height of lateral cells in gills from *M. galloprovincialis* during 60 min of acute exposure to 60 % ASW followed by a return to 100 % ASW. Lateral cell height in animals acclimated to 100 % ASW was 23.6±0.53 $\mu$m (*N*=7). Cell height rapidly increased during the first few minutes following introduction into reduced salinity, achieving a maximum increase of 28.1±7.1 % (*N*=5) over the starting height within 5–10 min. After approximately 15 min, cell height began to decrease and, within 40 min of the initial exposure to 60 % ASW, reached a new steady-state value that was significantly below the cell height noted at the peak of cell swelling. In experiments with tissue from five different *M. galloprovincialis*, the half-time ($t_{1/2}$) for achieving the new steady-state volume (volume measured at 60 min post-exposure) was 12±1.1 min after attainment of maximal cell height. Following re-exposure of the tissue to 100 % ASW, the cells rapidly decreased in height to a value (10.8±2.5 %) below ($P$<0.05) the height at time zero. Cell height subsequently returned to the control value with a $t_{1/2}$ of 11.8±1 min (*N*=5). Observations on filaments from *M. californianus* revealed a similar pattern of response. Lateral cells from *M. californianus* had a mean control height of 22.8±0.61 $\mu$m (*N*=14), which increased by 26.5±1.5 % following exposure to 60 % ASW. The ensuing RVD had a $t_{1/2}$ of approximately 12 min. The inset of Fig. 5 shows the profile of change in lateral cell height measured for a lateral cell from a single filament from *M. californianus* during a shift from 100 % to 60 % to 100 % ASW.

If lateral cells were to behave as perfect osmometers, and if cell height were the only change in cell dimension to occur during the course of cell swelling, then cell height would increase by approximately 67 % following acute exposure to 60 % ASW. This calculation does not take into account the fact that some cell volume is osmotically inactive owing, in part, to the presence of cell solids. A typical value for the osmotically inactive fraction of cell volume is 20 % (Hoffmann, 1977), which would suggest that, upon exposure to 60 % ASW, lateral cell height should increase by approximately 53 %. In contrast, lateral cell height increased by approximately 25–30 %. The observed change in cell height could be accounted for if 55–62 % of the cell volume were osmotically inactive. It is, however, more likely that cell height was not the only cell dimension to change during cell swelling. Referring to the perspective presented in the schematic representation of lateral cells shown in Fig. 6, cells may also have changed in either the *x* or *y* dimensions, in addition to the cell height (*z*) dimension. Evidence for such changes was obtained in separate experiments, which monitored the dimensions of single filaments laid on their side, thereby facilitating the study of dimensions *x* and *y*. Following acute exposure to 60 % ASW, there was no change in the *y* dimension (which would have resulted in a change in the length of the filament). There was, however, a

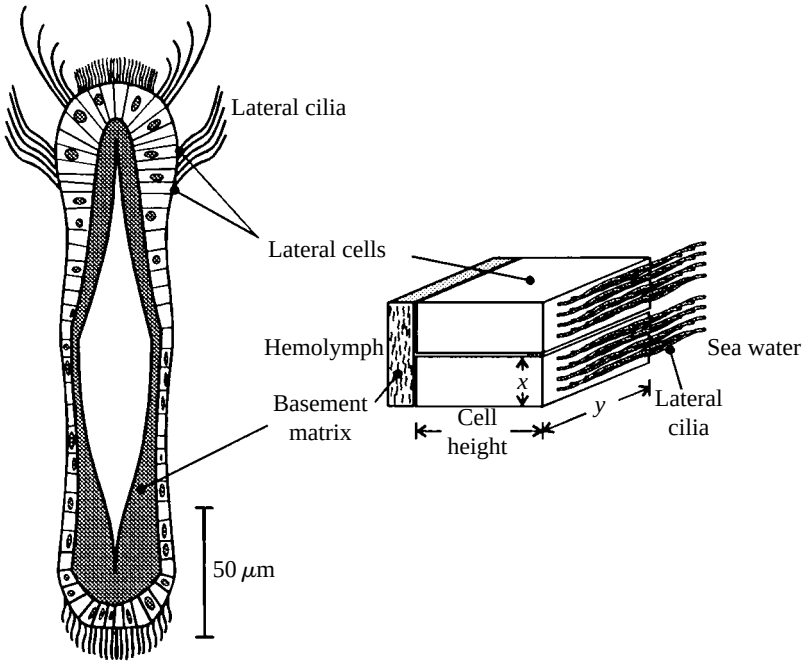


Fig. 6. Schematic representation of the three dimensions of lateral ciliated cells of *Mytilus* gill. On the left is a cross section of a single gill filament. On the right is a three-dimensional representation of two lateral cells. The apical, ciliated aspect of the cells is exposed to sea water; the basal aspect rests on an extracellular matrix. The DIC optical sections recorded changes in 'cell height', i.e. the apical-to-basal dimension. Changes in the *x* dimension of gill cells would increase the frontal to abfrontal dimension of the filament. Changes in the *y* dimension of gill cells would increase the overall length of the filament.

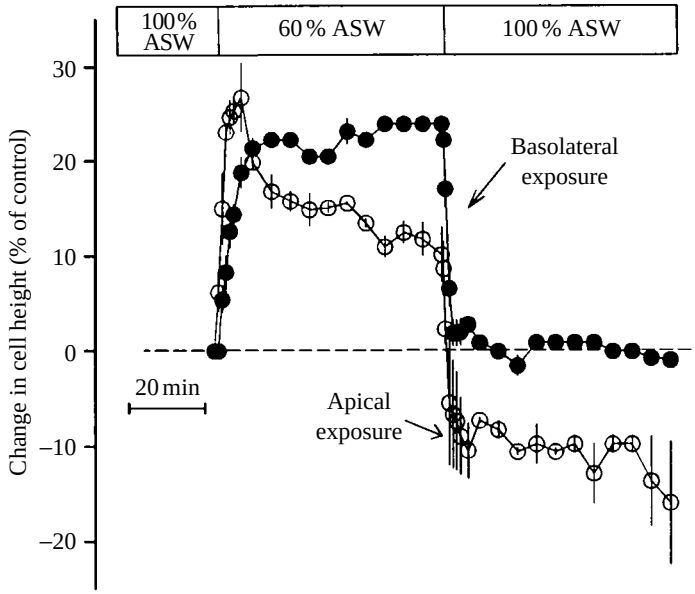


Fig. 7. Effect on lateral cell height of apical *versus* basolateral exposure to 1 mmol $l^{-1}$ quinidine. Exposure to quinidine was limited to the basolateral aspect of lateral cells by perfusing demibranchs from *Mytilus californianus* with 100 % ASW containing 0.5 mmol $l^{-1}$ quinidine sulfate, then exposing individual gill filaments to quinidine-free ASW (first 60 % followed by a return to 100 %; filled circles). Exposure to quinidine was limited to the apical aspect of lateral cells by simply exposing gills to sea water (first 60 % then 100 %; open circles) containing 0.5 mmol $l^{-1}$ quinidine sulfate. Each point represents the mean (± S.E.M.) of the change in cell height noted in tissue from three different animals.

12–14 % increase in the *x* dimension (i.e. the distance from frontal to abfrontal surface of the filament) of a single filament. Taken together with the 25–30 % change in cell height, the increase in *x* dimension of the filament suggests a 40–48 % increase in total cell volume, which is in reasonable agreement with the prediction of a 53 % increase in volume noted above. Thus, the lateral cells from *Mytilus* gill appear to behave as osmometers following acute hypotonic stress.

### *Effect of quinidine and valinomycin on short-term volume regulation in single lateral cells*

Loss of cell volume after osmotic swelling typically follows a loss of osmotically active solute (Lang *et al.* 1990). Although our measurements did not indicate a loss of solute from gill tissue following acute exposure to dilute sea water, the observations presented in Fig. 5 suggest that lateral cells (at least) invoke a volume-sensitive loss of solute during exposure to 60 % ASW. The mechanisms leading to RVDs in many cells include activation of a volume-sensitive $K^+$ channel (Chamberlin and Strange, 1989; Lang *et al.* 1990), which is typically blocked by millimolar concentrations of quinidine (e.g. Civan *et al.* 1992). As shown in Fig. 5, the RVD of the gills appear to be qualitatively and quantitatively similar in *M. galloprovincialis* and *M. californianus*. In

the studies examining the effect of quinidine on the gill RVD, we used tissue from *M. californianus* because it is more amenable to perfusion than is *M. galloprovincialis* and, therefore, to an examination of the response to the drug of the basolateral aspect of the epithelium. When gill filaments were placed in 60 % ASW containing 1 mmol $l^{-1}$ quinidine, thereby limiting exposure of the drug to the apical aspect of lateral cells, there was no change in the observed volume regulatory response ($t_{1/2}$ of 10±1.5 min; Fig. 7, open circles). However, when gills were first perfused with 100 % ASW containing 1 mmol $l^{-1}$ quinidine, thereby exposing the basolateral membrane to the drug, the volume regulatory response associated with exposure to 60 % ASW (either with or without 1 mmol $l^{-1}$ quinidine in the external seawater solution) was completely blocked (Fig. 7, filled circles).

Although quinidine has been shown directly to influence processes associated with volume regulation in other cells, it was important to determine the effect of quinidine on the general metabolic status of *Mytilus* gill. One assay for gill cell function is integumental transport. Fig. 8 shows that quinidine does inhibit the taurine transporter found in the apical (seawater-facing) membrane. The measured $K_{50}$ for quinidine inhibition of taurine uptake (Fig. 8 inset) was 192 $\mu$mol $l^{-1}$. Significantly, the inhibition of taurine transport by quinidine was completely reversed by rinsing the tissue for 5–30 min in quinidine-free ASW (data not shown). These data suggest that the inhibitory

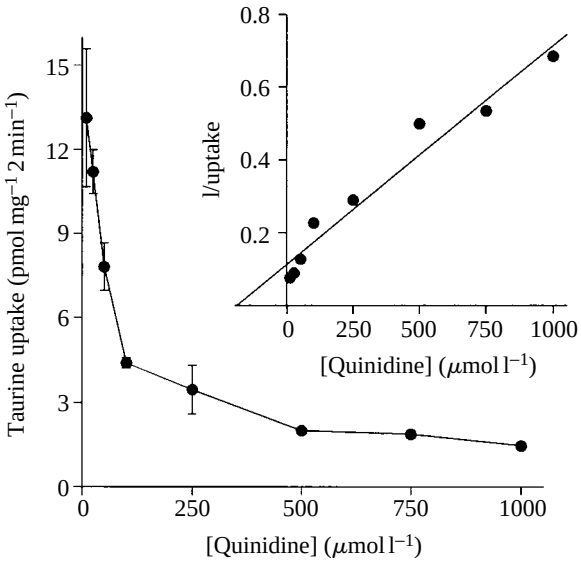


Fig. 8. Effect of quinidine on uptake of taurine into intact, isolated *Mytilus californianus* gill. Gills were incubated in 100 % ASW containing 10 $\mu$mol $l^{-1}$ 5-HT, 37 kBq of [$^{14}$C]taurine, sufficient unlabeled taurine to produce a final taurine concentration of 0.5 $\mu$mol $l^{-1}$ and 0.5 $\mu$mol $l^{-1}$ to 1 mmol $l^{-1}$ quinidine. Each point is the mean ± S.E.M. of taurine uptake measured in three different animals. Inset: modified Dixon plot (1/uptake *versus* inhibitor concentration). The line was fitted using a least-squares regression. The *x*-intercept represents $-K_{50}$, i.e. the quinidine concentration that blocked 50 % of the taurine uptake. The apparent $K_{50}$ for quinidine was 192 $\mu$mol $l^{-1}$.

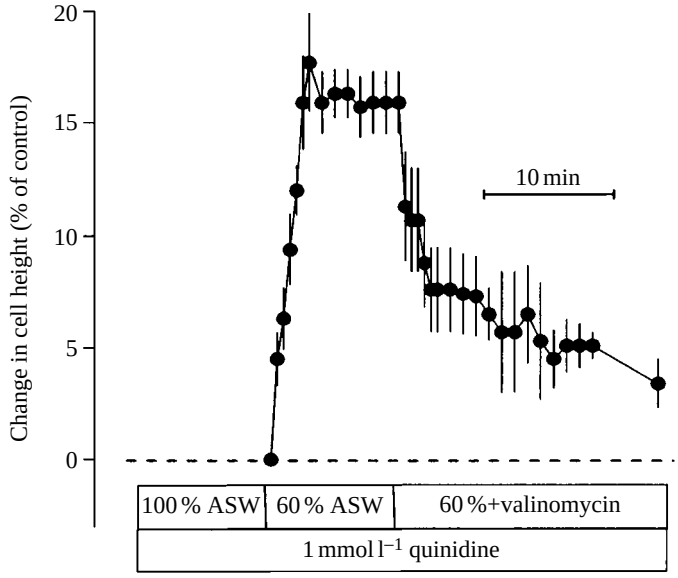


Fig. 9. Effect of valinomycin on the inhibition by quinidine of the lateral cell RVD. Demibranchs from *Mytilus californianus* were perfused with 1 mmol $l^{-1}$ quinidine after which individual filaments were exposed to 60 % ASW (also containing 1 mmol $l^{-1}$ quinidine). These conditions completely blocked the lateral cell RVD. After 10 min, 1 $\mu$mol $l^{-1}$ valinomycin was added to the bath. The presence of valinomycin completely reversed the inhibition of the RVD. Each point is the mean (± S.E.M.) of the changes in lateral cell height noted in tissue from three different animals.

effects of quinidine are transient and do not compromise the functional integrity of the tissue.

If the inhibition of the RVD by quinidine was the result of inhibition of a conductive $K^+$ channel (Germann *et al.* 1986), then treating the gill with valinomycin, an electrogenic $K^+$ ionophore, should reverse the inhibitory effect. To test this hypothesis, gills were first perfused with 1 mmol $l^{-1}$ quinidine in 100 % ASW. Ten minutes after exposing the tissue to 60 % ASW containing quinidine (resulting in apical and basolateral exposure to the drug), 1 $\mu$mol $l^{-1}$ valinomycin was added to the bath. Prior to the addition of valinomycin, the gill RVD was completely blocked (Fig. 9). Shortly after addition of the ionophore, cell volume decreased, indicating that a conductive loss of $K^+$ can play a significant role in cell volume reduction. The electrogenic loss of $K^+$ implies an equivalent electrogenic loss of a counter anion. Thus, lateral cells may invoke a substantial anion conductance during exposure to 60 % ASW.

If cation (i.e. $K^+$) conductance is the rate-limiting aspect of the lateral cell RVD, then the addition of valinomycin to gills undergoing an acute exposure to 60 % ASW should accelerate volume recovery by providing an additional pathway for a conductive loss of $K^+$. However, 1 $\mu$mol $l^{-1}$ valinomycin added to the perfusate 5 min after exposing the gill tissue to 60 % ASW did not accelerate the rate of recovery (Fig. 10). Cell height returned to its new steady state within 20 min of exposure to the dilute sea water, with a half-time

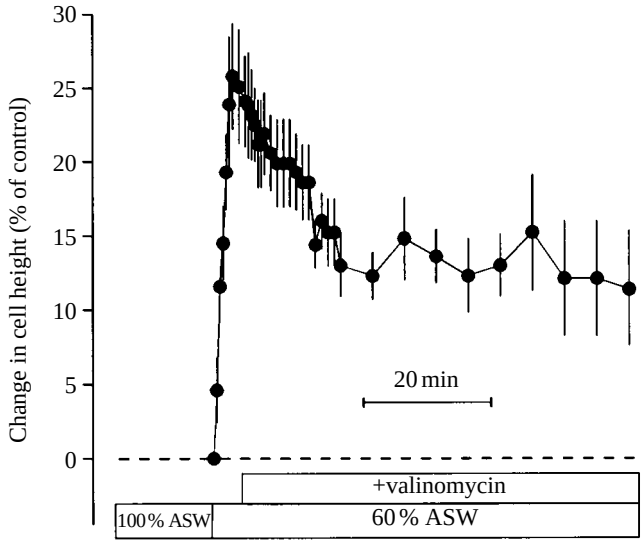


Fig. 10. Effect of valinomycin on the rate of recovery of lateral cell volume following exposure to 60 % ASW. Gill filaments from *Mytilus californianus* were exposed to 60 % ASW to induce the RVD response. Five minutes after the onset of the RVD, 1 $\mu$mol $l^{-1}$ valinomycin was added to the bath. The presence of valinomycin did not change the rate of RVD. Each point is the mean (± S.E.M) of the changes in lateral cell height noted in tissue from three different animals.

not different from that of control tissues ($t_{1/2}$=10±1.2 min), suggesting that anion, rather than cation, conductance limits the rate of the gill RVD.

## Discussion

The present study examined the response of *Mytilus californianus* gill to both long-term and short-term exposure to dilute sea water. As expected, nitrogenous compounds, particularly taurine and betaine, constituted a substantial amount of the total intracellular solute pool. The nitrogenous solute pool of *M. californianus* gill was approximately 500 mmol $l^{-1}$ cell water in animals acclimated to 100 % ASW (approximately 1000 mosmol $l^{-1}$), and taurine and betaine made up almost 80 % of this total. Nitrogenous solutes in gills from animals acclimated over a period of 3 weeks to 60 % ASW decreased from 500 to 300 mmol $l^{-1}$, almost entirely as a result of decreases in taurine and betaine content. Cytoplasmic [$K^+$], which was 184 mmol $l^{-1}$ in animals acclimated to 100 % ASW, was unchanged in animals acclimated to 60 % ASW (178 mmol $l^{-1}$). In this reliance on manipulation of organic osmolytes to meet the stress of chronic exposure to dilute sea water, the response of the gill parallels that of intact *M. edulis* (Bricteux-Gregoire *et al.* 1964; Lange, 1963) and other euryhaline molluscs (Gilles, 1987).

Although taurine and betaine were lost from gill tissue during the process of long-term acclimation to dilute sea water, neither of these osmolytes appeared to be synthesized during the process of reacclimation to full-strength sea water. This observation is in

accord with previous studies on the pattern of solute accumulation in both *M. edulis* (Livingstone *et al.* 1979) and *M. demissus* (Baginski and Pierce, 1975) during the period of reacclimation to full-strength sea water following long-term exposure to dilute media. Indeed, it has been suggested (Bishop *et al.* 1983) that the accumulation of the large concentrations of taurine and betaine that characterize the tissues of many molluscs may require an input of exogenous material from the diet.

Whereas a great deal is known about the response of euryhaline molluscs to long-term exposure to dilute media, comparatively little is known about the mechanisms used by animals during the short-term, transient exposures to low salinities that characterize many intertidal habitats. The present results show that lateral cells from *M. californianus* and *M. galloprovincialis* gill can invoke a vigorous regulatory volume decrease (RVD) in response to the swelling that follows abrupt exposure to dilute sea water. On the basis of the observed changes in apparent volume of cells in filaments, lateral cells appeared to respond as near-perfect osmometers to abrupt exposure to aniso-osmotic media. However, within minutes of the swelling induced by exposure to dilute sea water, lateral cells began to regulate their volume back towards the control value (Fig. 5). This RVD was blocked by application of quinidine to the basolateral, but not to the apical, aspect of gill cells (Fig. 7), suggesting a polarity to the response of the gill epithelium to aniso-osmotic media.

The identities of the solutes involved in the observed short-term regulatory volume decrease of gill cells were not clear. On the basis of the degree of volume recovery apparent in lateral cells following exposure to 60 % ASW (i.e. approximately 40 %; Fig. 5), approximately 160 mosmol $l^{-1}$ cell water should have been lost. However, there was no statistically significant loss of solute from gills exposed for 1 h to 60 % ASW. The structure of the gill offers a potential explanation for this paradox. If solute losses were largely restricted to efflux across the basolateral membrane into the vascular space (i.e. branchial hemolymph), and the observed polarity of the inhibitory effect of quinidine supports this suggestion, then some of the solute lost from the cells could have been trapped within the vascular compartment during the preparation of tissue extracts and the subsequent measurement of tissue solute content. Although the branchial vasculature was flushed with 60 % ASW immediately prior to extraction of tissue solutes, it is possible that this action was insufficient to remove solutes in the substantial unstirred layer within the extracellular matrix that separates the blood space from the basolateral membrane of the gill epithelium (see Fig. 3). In support of this hypothesis, there is evidence from electron microscope studies that extracellular matrix in vertebrate tissues can contain very large concentrations of monovalent cations, presumed to be bound (and therefore osmotically inactive) to fixed anionic charges on the matrix (Hargest *et al.* 1985).

Despite the failure to measure a significant loss of solutes from whole tissue, the pharmacological manipulation of the RVD noted in the intact gill and in single cells suggests that loss of $K^+$ is a principal element in the short-term regulation of cell volume in *Mytilus* gill. Quinidine (and its stereoisomer, quinine) is a known blocker of $K^+$ channels and an inhibitor of the RVD reported to occur in many different cell types (e.g. Civan *et al.* 1992; Hoffmann *et al.* 1986; Germann *et al.* 1986; see Hoffmann and Simonsen, 1989). Quinidine blocked the reduction of lateral cell height (Fig. 7) that

normally occurred within minutes of the swelling brought on by acute exposure to 60 % ASW. The ability of valinomycin to reverse the inhibitory effect of quinidine (Fig. 9) indicates that a significant component of the solute loss associated with the short-term gill RVD involves conductive pathways and thus argues against the electroneutral loss of organic solute of the type reported to occur in some renal cells (e.g. Garty *et al.* 1991; Yamauchi *et al.* 1991).

Indirect evidence thus supports the contention that the short-term RVD in the gill involves a conductive loss of $K^+$ from gill cells. A conductive loss of $K^+$ would necessitate a parallel conductive loss of one or more different anionic species from the gill as well. The effectiveness of valinomycin in relieving the effect of quinidine indicates that there must, in fact, have been a substantial anionic conductance in the gill epithelium during the RVD. It is interesting to speculate on the identity of the anion(s) that may be involved in the short-term RVD of gill cells. In most cells that invoke a set of parallel conductive pathways to effect the loss of solute required for cell shrinkage, an efflux of $Cl^-$ is the primary means of balancing the parallel efflux of $K^+$ (Hoffmann and Simonsen, 1989). It is, however, unlikely that gill cells contain enough $Cl^-$ to serve as a quantitatively significant solute for a volume decrease. The concentration of $Cl^-$ in sea water and in *Mytilus* hemolymph is approximately 550 mmol $l^{-1}$ (Potts, 1958; Bayne *et al.* 1976). If $Cl^-$ were distributed at electrochemical equilibrium across the gill cell membrane, the inside-negative 60 mV electrical potential of lateral cells (Murakami and Takahashi, 1975) would result in a cytoplasmic $Cl^-$ concentration of only approximately 55 mmol $l^{-1}$. Estimates of intracellular $Cl^-$ activity in the sarcoplasm of *M. edulis* smooth muscle range from 73 to 84 mmol $l^{-1}$ (Borseth *et al.* 1992), and the only measurements of intracellular $Cl^-$ in a molluscan epithelial cell are those of Gerencser and White (1980), who reported a value of 10 mmol $l^{-1}$ for cell $Cl^-$ activity in intestinal cells from *Aplysia*. As noted earlier, the extent of volume recovery in lateral cells following the swelling associated with acute exposure to 60 % ASW implies a loss from the cell of approximately 160 mmol solute $l^{-1}$ cell water. Whereas it is possible for $K^+$ to provide as much as half of this solute, $Cl^-$ probably cannot.

There are a limited number of candidates for the anion(s) that can be lost, in conjunction with $Cl^-$, to counter the loss of $K^+$ from lateral cells. Taurine has been found to serve as a conductive substrate, along with $Cl^-$, for anion channels involved with cell volume regulation (Banderali and Roy, 1992). Assuming that the pH of gill cytoplasm is of the order 7.6, then the concentration of anionic taurine would be approximately 15 mmol $l^{-1}$, given a $pK_2'$ for taurine of 8.7 (Budavari, 1989) and a total taurine concentration of 200 mmol $l^{-1}$. [There are no reports of cytoplasmic pH in molluscan epithelia. However, animal cell cytoplasm pH is typically 0.2–0.5 units below extracellular pH. The assumption, for purposes of this discussion, of a cytoplasmic pH in gill cells of 7.6 is based upon the fact that gill cells are exposed to both sea water (pH 8.0–8.2) and hemolymph (pH 7.7; Bayne *et al.* 1976).] The loss of anionic taurine would lead to its continued regeneration within the cell from the large cytoplasmic pool of total taurine. Nevertheless, the comparatively low concentration of mobile anionic solutes ($Cl^-$ and taurine) in gill cells could explain why anionic, rather than cationic, conductance appears to be rate-limiting in lateral cell volume regulation.

When the gill was reintroduced to full-strength sea water, lateral cells initially shrank below their starting volumes (indicative of a loss of solute during the RVD) and then recovered their control volume (Fig. 5). The nature of this 'regulatory volume increase (RVI) following RVD' (Hoffmann and Simonsen, 1989) has not been explored in bivalves. Although this phenomenon is commonly observed in many cell types (Gilles, 1987), Pierce (1971) reported that intact *Modiolus* (several species) failed to recover volume once tissue shrinkage had occurred, even the shrinkage noted as part of the apparent whole-animal RVD that followed brief exposure to hypo-osmotic media. We have no evidence upon which to speculate what solutes may be employed by *Mytilus* gill to produce the 'RVI following RVD', other than to note that in vertebrate cells this increase in cell volume typically involves an accumulation of $Cl^-$ and $Na^+$ (the latter a preface to the reaccumulation of $K^+$ *via* $Na^+/K^+$-ATPase activity; Lang *et al.* 1990). If this proves to be the strategy employed by *Mytilus* gill, it imposes an interesting set of problems for this tissue. Accumulation of $Na^+$ would, of necessity, result in a decrease of the $Na^+$ electrochemical gradient across the apical and basolateral membranes of the gill epithelium. This gradient provides the energy for maintaining the large outwardly directed gradients for amino acids and other organic solutes that exist in high concentrations in these cells (Wright and Pajor, 1989) and which are central to the maintenance of normal cell volume. The present results indicate that the gill is capable of undergoing repeated cycles of cell volume regulation; it is not known whether the repeated exposure to fluctuating salinity has any influence on the ability of the integument to accumulate organic solutes. Livingstone *et al.* (1979) found that the concentration of several amino acids in tissues of intact *M. edulis* decreases in animals exposed to fluctuating salinity. They did not, however, examine amino acid concentrations in specific tissues, such as the gill. It is reasonable to speculate, however, that changes in the $Na^+$ electrochemical gradient arising from either short-term or long-term exposures to dilute sea water would ultimately influence the ability of molluscan tissues to maintain the high intracellular concentrations of organic solutes that characterize tissues acclimated to full-strength sea water.

In summary, the long-term volume regulatory response of *Mytilus* gill to reduced ambient salinity includes changes to the intracellular pool of organic osmolytes, primarily a loss of taurine and betaine. No significant changes occur in tissue $K^+$ content following long-term acclimation to reduced salinity. Upon acute exposure of gill tissue to dilute sea water, ciliated lateral cells are capable of a rapid short-term volume regulation that presumably comes into play during routine tidally driven changes in ambient salinity. The short-term RVD probably includes the loss of inorganic solutes from the gill. The inhibition (and subsequent reversal with valinomycin) of the short-term RVD produced by basolateral exposure to quinidine suggests that the response is mediated by the efflux of intracellular $K^+$ and a counter anion(s) across the basolateral membrane of gill cells.

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