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IONIC REGULATION IN FRESHWATER MUSSELS: A BRIEF REVIEW

THOMAS H. DIETZ
DEPARTMENT OF ZOOLOGY AND PHYSIOLOGY
LOUISIANA STATE UNIVERSITY
BATON ROUGE LOUISIANA, 70803, U.S.A.

ABSTRACT

Freshwater bivalves are subject to salt loss to the environment and the osmotic uptake of water. Although the gradient for water movement is low due to the reduced blood osmolality (45–60 mOsm), water influx in molluscs may be substantial since they have the highest renal filtration rates of freshwater animals. The rate of salt transport in freshwater unionid mussels is similar to other freshwater animals (1–2 μmol (g dry tissue · hr)$^{-1}$). In contrast, salt turnover in corbiculid bivalves is 5–10 times greater.

In studies on the mechanism of Na transport, we observed a coupled cation exchange (H, NH_4) and Cl transport is coupled to base (HCO_3 or OH) exchange. Thus, salt transport is intimately linked to acid-base balance. Although freshwater mussels present a large epithelial surface to the environment (gills, mantle, foot), we have found that the gills account for most of the Na and Cl uptake in Unionids.

Recently, we have shown that Na transport 'but not Cl) is stimulated 200% by a variety of biogenic "monoamines," including serotonin, when injected into the intact animal. In the isolated gills, serotonin, cyclic AMP and theophylline are effective in increasing Na influx. Bivalve gill tissue contains a serotonin sensitive adenylate cyclase system which may be involved in regulating Na transport. In addition, Na transport in freshwater mussels is inhibited by prostaglandins (PGE_2) presumably derived from the arachidonic acid present in gill tissue phospholipids.

Calcium is a major cation in freshwater mussel blood; second only to Na in pondwater acclimated animals. Calcium is abundant in the shell and in calcium concretions widely distributed in various tissues. The gills contain abundant Ca concretions, accounting for 25–50% of the dry mass. Under conditions of hypoxic stress, we have noted a reciprocal relationship between blood Na and Ca. When Na is lost from the blood, Ca usually increases and may become the principal cation. The source of Ca may be from the shell since the gill concretions were observed to increase in mass, apparently serving as Ca deposition sites.

In recent studies we have noticed a correlation between Na influx and net Ca loss from mussels. These data suggest a Na/Ca exchange mechanism involved in ionic regulation. In separate studies, we have noticed that Ca concretions disappear from the gills of gravid females during the time of shell formation in developing embryos. These data suggest the developing bivalve larvae may be obtaining "mineral nutrition" directly from the adult.

The freshwater bivalves maintain body fluid solute concentration 25–50% of that found in other freshwater animals (Krogh, 1939; Potts, 1954; Prosser, 1973; Dietz, 1977; 1979; Deaton, 1981; Burton, 1983). In addition, the specific ion concentrations in clam blood are not usually a simple dilution of body fluids since Ca and HCO_3 are typically major solutes in addition to NaCl. Although mussels have a lower solute concentration and osmotic gradient, they experience the same problems as other freshwater animals: gaining water osmotically and losing solutes by diffusion and excretion. Estimates of urine production from renal clearance of inulin or polyethylene glycol is 20–50 ml/Kg · hr (Potts, 1954; Murphy and Dietz, 1976; Dietz and Branton, 1979) which is higher than other freshwater animals (Kirschner, 1967; Prosser, 1973) and suggests that mussels have a high turnover of water even though the osmotic gradient is extremely low.

Despite the high water turnover and lower salt concentration, the freshwater unionids have salt transport rates remarkably similar to other freshwater animals. Thus, freshwater bivalves can maintain hydromineral balance in dilute media (Dietz, 1977; 1978; 1979). Salts lost across permeable epithelia and in the urine are offset by ions accumulated by epithelial transport. In our earlier studies, we showed that Na and Cl uptake occur by independent saturable pro-

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cesses (Fig. 1). The influx of Na or Cl in unfed unionids is about 1–2 μmol (g dry tissue · hr)$^{-1}$ with a transport affinity of about 0.1 to 0.2 mM/l (Dietz, 1978; Dietz and Branton, 1979). In contrast, the corbiculids transport salt 5–10 times faster than unionids and the fluxes are more characteristic of brackish water animals (Prosser, 1973; McCorkle and Dietz, 1980).

ION TRANSPORT

In earlier studies, investigators concentrated on characterizing the ionic and osmotic regulation in intact freshwater mussels (Krogh, 1939; Hiscock 1953; Chaisemartin et al., 1968; Dietz, 1979; Deaton, 1981). A major theme of our studies with the freshwater bivalves has been the independence of Na and Cl transport and the coupled cation and anion exchanged. An attractive hypothesis which has considerable supporting evidence is the coupling of ion balance with acid/base balance (see Kirschner, 1982). More recently, we have been exploring the role of Ca in Na transport. It should be noted that Na balance must occur whether or not the animal is in acid/base balance. Thus, strict coupling between Na and H could be detrimental to the animal's survival and other mechanisms must contribute to Na balance under stressful conditions.

Sodium transport in bivalves occurs in exchange for endogenous cations. We have reported an extensive Na/H and Na/NH_4 exchange and recent evidence suggests a Na/Ca antiport system as well. Chloride uptake is by a Cl/HCO_3 or Cl/OH exchange mechanism. We have partitioned the unidirectional fluxes into the various components: active transport, diffusion and exchange diffusion (see Ussing, 1949) (Table 1). Active transport is the principal component of Na influx with diffusion and Na/Na exchange diffusion being virtually non-existent in the unionids (Dietz, 1978). However, in the corbiculids exchange diffusion accounts for 67% of the ^{22}Na turnover in pondwater acclimated mussels (McCorkle and Dietz, 1980). Following salt depletion active transport accounts for 45% of the ^{22}Na turnover and exchange diffusion is reduced to 50% in *Corbicula fluminea*. Chloride isotope turnover in the unionids, however, may be up to 90% exchange diffusion in pondwater acclimated animals, not in a steady state, due to a reduced level of active transport. However, exchange diffusion is substantially reduced following salt depletion and active transport becomes the principal component of J_i^{Cl} (Dietz and Branton, 1979). Chloride transport in *C. fluminea* has not been studied extensively.

Previous studies have indicated that Na and Cl are transported from the dilute bathing medium into the animal against the electrochemical gradient (Dietz and Branton, 1975; 1979; Dietz, 1978, 1979). Pondwater is about 1 mM NaCl and blood is about 10–13 mM Cl and 13–20 mM Na. Since the body fluids are negative 10 mV, both Na and Cl are out of equilibrium. More detailed analyses of intact animals are difficult, including attempts to define the mechanism of ion transport. Never-the-less, whole animal studies delineate the problems faced by the organism and suggest specific areas of interest to be investigated using isolated tissues.

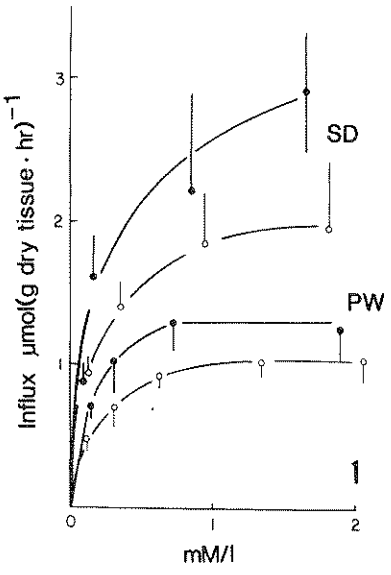

Fig. 1. The effect of Na (•) (Na_2SO_4) or Cl (o) (choline chloride) concentration in the bath on unidirectional influx in *Carunculina texasensis*. SD refers to salt depleted animals and PW refers to pondwater acclimated mussels. Vertical lines represent ±1 SE (Adapted from Dietz, 1978).

TABLE 1. Unidirectional influxes of Na and Cl partitioned into several components in pondwater (PW) acclimated or salt depleted (SD) mussels. Total influx, J_i^T was calculated from isotope uptake. Exchange diffusion, J_i^{ED}, was calculated from the decrement in efflux when the animals were transferred from dilute salt solution to distilled water. Diffusive flux J_i^D was calculated by the flux ratio equation (see McCorkle and Dietz, 1980). Active transport J_i^{AT} was calculated from $J_i^{AT} = J_i^T - (J_i^{ED} + J_i^D)$.

Species	Condition	Influx μmol (g dry tissue · hr)$^{-1}$ J_i^T =	J_i^{ED} +	J_i^D +	J_i^{AT}
Na Influx					
Corbicula	PW	8.8	5.9	0.5	2.4
fluminea[1]	SD	31.4	16.0	1.2	14.2
Ligumia	PW	1.2	< 0.05	<0.03	1.2
subrostrata[2]	SD	2.7	0.1	<0.03	2.5
Cl Influx					
Carunculina	PW	1.0	0.9	<0.02	0.1
texasensis[3]	SD	1.4	0.6	<0.01	0.8

[1]McCorkle and Dietz, 1980. [2]Calculated from Dietz, 1978. [3]Dietz and Branton, 1979.

SITES OF ION TRANSPORT

We have reported that of the several possible sites of Na and Cl uptake (gill, mantle, gut, body surface), the gill is the primary site of Na transport in freshwater mussels (Dietz and Findley, 1980; Dietz and Graves, 1981; Dietz et al., 1982). Isolated gills display saturation kinetics for both Na and Cl transport (Fig. 2). The influx of Na into isolated gills is about 12 μmol Na (g dry gill · 10 min)$^{-1}$ and the transport system has a high affinity (0.17 mM/l) Dietz and Graves, 1981). Since the gills represent about 4% of the total animal weight, then the calculated intact animal influx should be 3 μmol Na (g dry tissue · hr)$^{-1}$. Ordinarily, whole animal influx of Na is 1–2 μmol Na (g dry tissue · hr)$^{-1}$. Not only do the gills account for all the intact animal influx of Na but the results suggest the isolated gill Na transport may be stimulated. Similar studies of Cl transport in isolated gills indicate the gills can account for all Cl uptake by the bivalves (Dietz, unpublished).

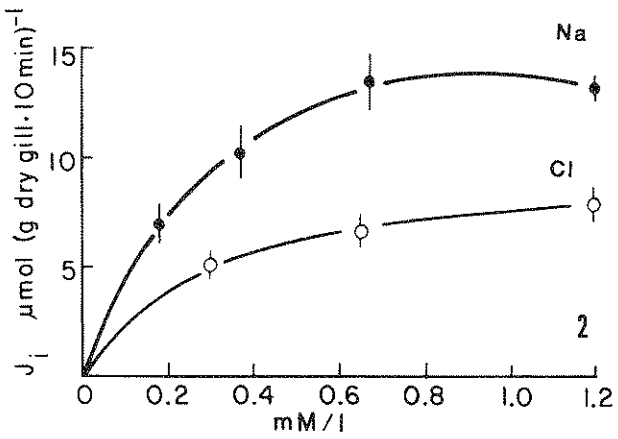

Fig. 2. Effects of Na or Cl concentration in the incubation medium on the unidirectional influx in isolated gills of pondwater acclimated *Ligumia subrostrata*. Vertical lines represent ±1 SE (Adapted from Dietz and Graves, 1981).

ION SENSITIVE ATPases

The characteristics of ion sensitive ATPases have been reported (Table 2) for mussel gill microsomes (Dietz and Findley, 1980) and others have reported on the ATPase characteristics of the mantle and kidney (Saintsing and Towle, 1978; Deaton, 1979; Wheeler and Harrison, 1982). Sodium entering into epithelial cells across the permeable apical membrane is thought to be transported to the blood by a Na/K pump which is driven by Na/K ATPase. The Na/K ATPases are notably low in most of the tissues studied. Of interest is the sensitivity of mussel enzymes to ionic strength of the assay medium. The enzymes in freshwater bivalves appear to be adapted to the dilute intracellular environment. Similarly, Cl transport across epithelial tissue may be driven by the energy supplied by a Cl/HCO_3 or HCO_3 ATPase. Both enzymes are present in gill tissue and display considerable activity. Although an anion exchange model is attractive in its simplicity and has been suggested for vertebrates as well, there is no unanimity of support for the involvement of the anion ATPases in anion transport (see De Renzis and Bornancin, 1977; Schultz, 1978; van Amelsvoort et al., 1978).

CONTROL OF ION TRANSPORT

We have observed diurnal rhythms in Na transport rates in both the unionids and corbiculids (Graves and Dietz,

Table 2. Ion sensitive ATPases in tissues of bivalve molluscs.

Species	Tissue	μmol (mg protein · hr)$^{-1}$ Na/K ATPase	Cl/HCO_3 ATPase	HCO_3 ATPase	Mg ATPase
Carunculina texasensis[1]	gill	1.5	0.9	10	12.4
Lampsilis claibornensis[2]	gill	0.8	—	—	11.3
	Mantle	0.5	—	—	6.1
	kidney	7.7	—	—	7.2
Corbicula fluminea[2]	gill	1.2	—	—	35.9
	Mantle	1.9	—	—	42.3
	kidney	0.7	—	—	11.7
Anodonta grandis[3]	mantle	0	—	16	10.7
Rangia cuneata[4]	gill	0.3	—	—	—
	mantle	0.6	—	—	
	kidney	1.0	—	—	—

[1]Dietz and Findley, 1980. [2]Deaton, 1979. [3]Wheeler and Harrison, 1982. [4]Saintsing and Towle, 1978.

1980; McCorkle-Shirley, 1982). In addition, handling some mussel species causes an immediate stimulation of Na influx lasting several hours. These data suggest a fast responding "hormonal" control mechanism regulating Na transport. Moreover, these studies also emphasize the importance of developing an isolated tissue preparation which can be studied independently from the control systems functioning in intact animals.

Some of our studies on intact animals display considerable variability because of the spontaneous changes in endogenous control over ion transport. Both Na and Cl transport are subject to separate regulatory systems. There are several lines of evidence substantiating the presence of control mechanisms for salt balance in mussels. We have reported diurnal changes in ion balance (Graves and Dietz, 1980; McCorkle-Shirley, 1982) and noted the spontaneous stimulation of Na transport in *Margaritifera hembeli* (Dietz, 1979). Salt depletion, by acclimating animals to distilled water, has been used extensively to stimulate ion transport (Krogh, 1939; Murphy and Dietz, 1976; Scheide and Dietz, 1982). Recently, we have documented that selective depletion of either Na or Cl will specifically stimulate the depleted ion transport system. When the animals are returned to the appropriate ionic solution to allow ion repletion, Na or Cl is accumulated by an accelerated ion influx until the normal blood ion concentration is achieved (Scheide and Dietz, 1982).

BIOGENIC AMINE-STIMULATED SODIUM TRANSPORT

Serotonin and catecholamines are potent stimulators of Na transport in mussels (Dietz et al., 1982). We have reported that serotonin and many catecholamines are effective stimulators of Na influx when injected to achieve about 5×10^{-5} M/l blood (Fig. 3) or added to the bath (10^{-4} M/l). When gills are isolated, only serotonin stimulates Na uptake and the catecholamines are ineffective. These data suggest that serotonin stimulates the gill epithelial cells and that serotonergic neurons may be innervated by adrenergic fibers. Substantial concentrations of serotonin are found in the gills of *Ligumia subrostrata* (4 μmol/g gill tissue) and Hiripi (1968) has reported similar amounts in *Unio pictorum*. Since we have reported that injections of cyclic AMP and theophylline stimulate Na transport in mussels (Graves and Dietz, 1982; Dietz et al., 1982), these data suggest a "monoamine" sensitive adenylate cyclase is involved in Na balance (see below).

With the high concentration of serotonin present in the gill tissue we were interested in the localization of serotonin within the gills. Using Procion Yellow, a fluorescent vital stain accumulated by neurons, we identified the general gill innervation pattern. Presumptive serotonergic neurons were identified using formaldehyde-induced fluorescence of biogenic amines (Falck, 1962) (Fig. 4). These two histo-

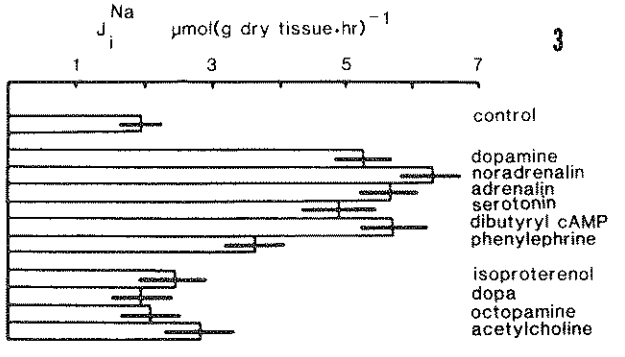

Fig. 3. The effect of injections of various drugs (~100 nmol/g dry tissue) on the sodium fluxes in pondwater acclimated *L. subrostrata* and *C. texasensis*. There were no differences between species and the data were pooled. Vertical lines represent ±1 SE (Data adapted from Dietz et al., 1982).

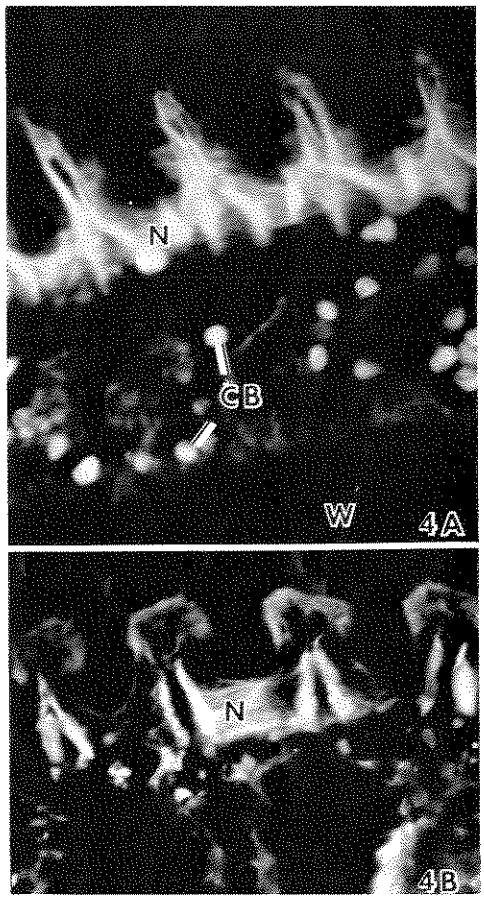

Fig. 4. Fluorescent micrographs of a gill cross-section from *L. subrostrata*. A. Animals injected with Procion Yellow. Fluorescent nerve tracts (N) between gill filaments and cell bodies (CB) are evident in the tissue between the gill filaments and the water channel (W) (field width 450 μm). B. Formaldehyde induced fluorescence having the characteristic yellow color associated with serotonin in nerve tracts (N) between gill filaments. The epithelial cells had a light green autofluorescence (Horizontal field width = 390 μm). (From Dietz et al., 1985).

chemical techniques identified the same discrete nerve tracts in mussel gill tissue (Dietz et al., 1985). We have used transmission electron microscopy to confirm the localization of nerve tract, containing dense vesicles, which branch and innervate the gill epithelia. In addition, we used ^{3}H-serotonin uptake to obtain autoradiographs which displayed high densities of silver grains in the nerve tract regions between the gill filaments and at the base of the water channel epithelium (Fig. 5).

SEROTONIN-STIMULATED ADENYLATE CYCLASE

Since cAMP stimulates Na transport, serotonin may be influencing Na transport via adenylate cyclase-catalyzed cAMP production. Recently, we noted that salt depletion and serotonic treatment increased the cAMP content in isolated gills (Scheide and Dietz, 1983). In addition, we have reported the presence of a serotonin-stimulated adenylate cyclase in gill homogenate (Fig. 6). Addition of micromolar concentrations of serotonin significantly stimulate adenylate cyclase activity (Scheide and Dietz, 1983; 1984). Serotonin stimulates adenylate cyclase (100% or more) above base line rates of cAMP synthesis in gill tissue of several unionids and *C. fluminea* (Fig. 7). The serotonin stimulation of adenylate cyclase can be inhibited selectively by cyproheptadine.

Dopamine also increases adenylate cyclase activity in mussel gills (see Fig. 6) and this catecholamine is effectively inhibited by the antagonist, chlorpromazine (Scheide and Dietz, 1983). Of interest is the observation that dopamine has no effect on Na influx in isolated gills (Dietz et al., 1982). Dopamine may be exerting its effect on gill tissue by increasing the ciliary activity on the epithelial surface (A. Paparo, So. Ill. Univ., pers. comm.). Recently, a number of studies have addressed the biogenic amine stimulation of adenylate cyclase. Adenylate cyclase in the gills of *Aplysia* is stimulated by serotonin (Weiss and Drummond, 1981). Mendelsohn et al., (1981) reported an adrenalin/cAMP stimulation of Cl secretion in fish opercular tissue. Finally, serotonin and dopamine have been observed to stimulate adenylate cyclase in invertebrate nervous tissue (Robertson and Osborne, 1979; Stefano et al., 1981).

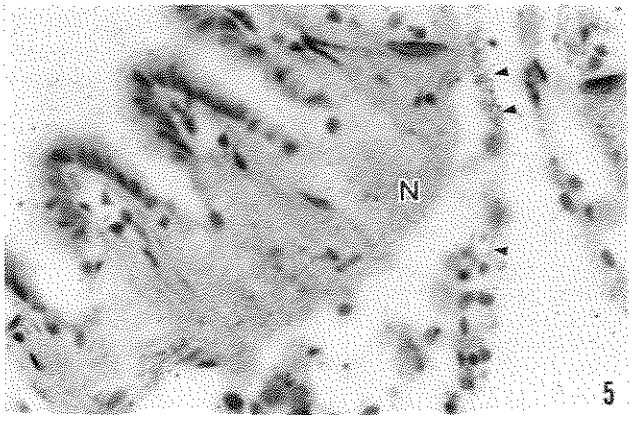

Fig. 5. Bright field autoradiograph of toluidene blue stained gill cross-section from *L. subrostrata* labeled with ^{3}H-serotonin (10^{-12} M). Silver grains are concentrated in the nerve tract (N) region and along nerve fibers at the base of the water channel epithelial cells (arrows) (Horizontal field width = 290 μm). (From Dietz et al., 1985).

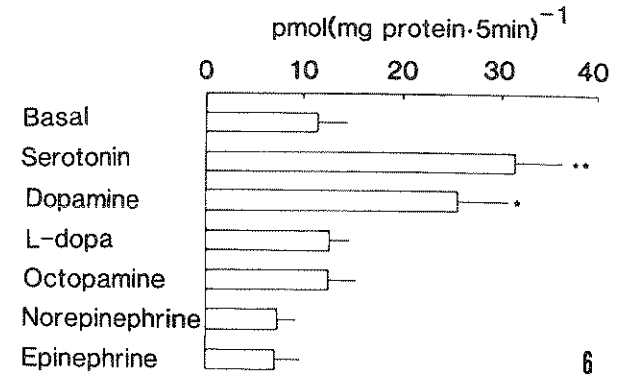

Fig. 6. Adenylate cyclase activity in gill homogenate from *L. subrostrata* exposed to 12 μm of various "monoamines." The horizontal line represents 1 SE. Dopamine (*, $P < 0.05$) and Serotonin (**, $P < 0.01$) are significantly different from basal activity (From Scheide and Dietz, 1983).

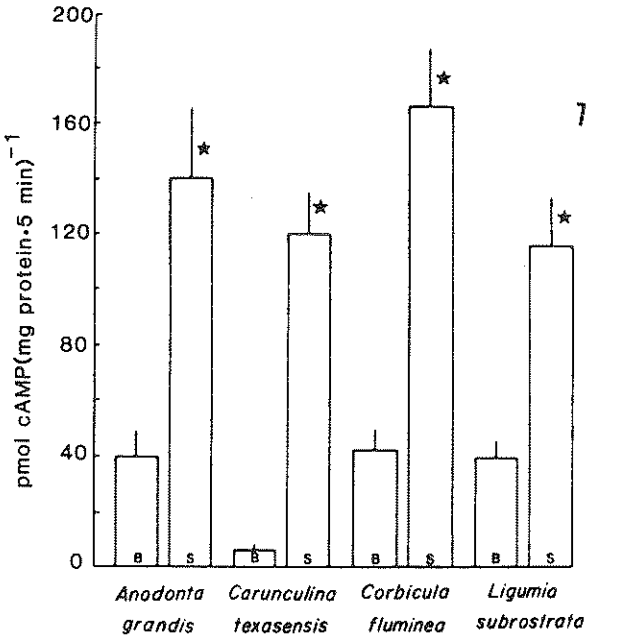

Fig. 7. Basal (B) and serotonin (S) (60 μM/l) stimulated adenylate cyclase activity in the homogenates of gills from 4 species of freshwater mussels. The vertical lines represent 1 SE. * = significantly different from basal activity $P < 0.01$. (From Scheide and Dietz, 1984).

PROSTAGLANDIN INHIBITION OF NA TRANSPORT

Prostaglandins of the diene series (PGE_2 and PGF_{2a}) are synthesized from arachidonic acid by a cyclooxygenase pathway. Prostaglandin E_2 is an effective inhibitor of Na influx in *L. subrostrata* but PGF_{2a} is not (Table 3). We have found that inhibitors of cyclooxygenase (indomethacin, meclofenamate) (Flower and Blackwell, 1976; Flower, 1974) block prostaglandin production in mussels and cause a stimulation in Na transport (Graves and Dietz, 1979; 1982; Saintsing and Dietz, 1983; Saintsing et al., 1983) (Table 3). Prostaglandins are widely distributed in animals and have been extensively implicated in modulating ion transport in gills, toad bladder and kidney (Nomura and Ogata, 1976; Zusman et al., 1978; Orloff and Zusman, 1978; Freas and Grollman, 1980, 1981; Korff and Jarabok, 1980; Zusman and Keiser, 1980).

We have developed radioimmuno-assay techniques for measuring prostaglandins in mussel blood using specific antibodies (Saintsing, et al., 1983) and Freas and Grollman (1980) have measured prostaglandins in the gills of *Modiolus demissus*. Both PGE_2 and PGF_{2a} are present and preliminary data suggest prostacyclin is synthesized but thromboxane is not detectable in mussel blood. Arachidonic acid is a major fatty acid in the mussel gill phospholipids as measured by gas chromatography (Pollero et al., 1981a, b; Saintsing et al., 1983; A. Hagar and T. Dietz, unpublished). We have initiated studies of the prostaglandins synthesized by the cyclooxygenase pathway in mussel gill tissue (Saintsing et al., 1983; A. Hagar and T. Dietz, unpublished). In addition, we have noted lipoxygenase metabolites of arachidonic acid are produced by gill homogenate. However, we do not know what role the hydroperoxyeicosatetraenoic acids (HPETE's, leukotrienes?) play in the physiology of bivalves (Fig. 8).

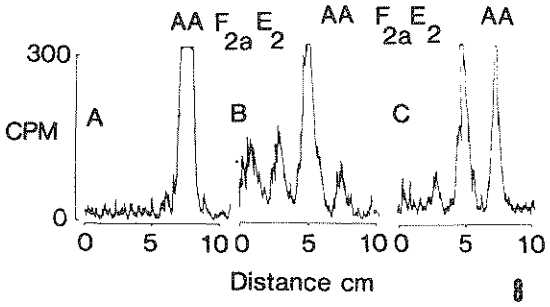

Fig. 8. Metabolism of ^{3}H-arachidonic acid (AA) by gill homogenate from pondwater acclimated *L. subrostrata*. A. ^{2}H-arachidonic acid incubated 60 min with boiled homogenate. B. ^{3}H-arachidonic acid incubated with homogenate. C. ^{3}H-arachidonic acid incubated with homogenate in the presence of 1 mM meclofenamate. Prostaglandin E_2 and F_{2a} standards are indicated at the top of the chromatograms (From Saintsing et al., 1983).

ROLE OF CALCIUM CONCRETIONS IN BIVALVE GILLS

Recently, we have observed a substantial amount of calcium concretions in the gill tissue of mussels (Silverman et al., 1983; 1985; Steffens et al., 1985) (Fig. 9). Since gill tissue is not a direct component of the shell forming tissue, it is probable that the concretions are serving as a Ca reservoir. We have documented that Ca concretions increase in mass under hypoxic conditions (Silverman et al., 1983). Our hypothesis is that the concretions in the gill are serving as a Ca source possibly for osmoregulation by cation exchange and as a Ca source for shell formation of larvae in reproductively active females (see below). Morphologic studies of unionid gills have demonstrated an extensive neuronal network in parallel association with calcium concretions (Dietz et al., 1985; Silverman et al., 1983; 1985; Steffens et al., 1985). Similar appearing concretions have been reported in other mollusc tissues and in the mantle tissue where they may play a role in shell formation (Abolins-Krogis, 1970; Petit et al., 1980; Davis et al., 1982).

We have qualitatively identified Ca in the concretions histochemically on sectioned frozen gill tissue. In addition, we have separated the concretions in pure form (as determined by electron microscopy) by homogenizing the tissue and either digesting the tissue with 1 N NaOH at 60° C for 1 hr (concretion appearance is not affected) or layering the

Table 3. Effects of injections of prostaglandin (PG) biosynthesis inhibitors, 5HT and cAMP on blood PG concentrations and J_n^{Na} in pondwater acclimated *Ligumia subrostrata* (from Saintsing et al., 1983).

Treatment	N	Dose μmol/g dry tissue	PGE_2 ng/ml	J_n^{Na} μmol (g dry tissue · hr)$^{-1}$
Control	50	—	0.39	−0.02
Meclofenamate	9	0.81	0.18**	1.76**
Indomethacin	10	0.28	0.09**	2.41**
Serotonin	10	0.12	0.14**	1.85**
cAMP	10	1.88	0.21*	2.57**

Significantly different from corresponding controls, *$P < 0.05$; **$P < 0.01$.

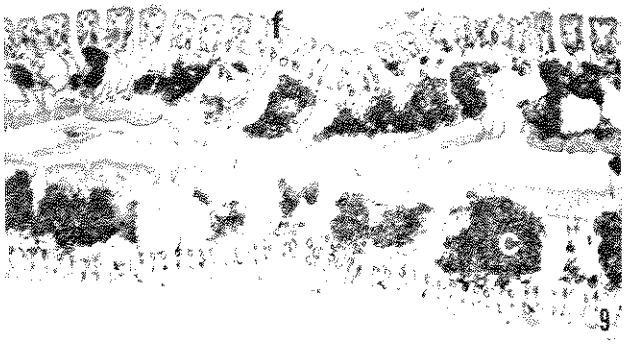

Fig. 9. Light micrograph of toluidene blue stained freeze-dried *Anodonta grandis* gill. The cross-section demonstrates the dense accumulation of concretions (C) below the gill filaments (F) (From Silverman et al., 1985) (Horizontal field width = 3.7 mm).

homogenate over 2.5 M sucrose and centrifuging the concretions into the discontinuous gradient. The purified concretions amount to 25–50% of the gill weight and by extracting the concretions in acid we have found 20–30% of the mass was Ca (determined by atomic absorption) (Table 4). The chemical analyses were confirmed by energy dispersive X-ray spectroscopy where Ca and P were the principal elements and Fe and Mn were found to be minor components (Fig. 10) (Steffens, unpublished).

The concretions in *Ligumia subrostrata* contain 25% volatile (at 450° C) organic material. The nature of this material is unknown, but it does not contain appreciable amounts of either oxalate (M. Hatch, La. State Univ., unpublished) or carbonate. By histochemical analyses, glycoprotein appears to be present. Recently, we prepared polyclonal antisera against the concretion organic material (Steffens et al., 1985). Antisera against concretions from one unionid species cross-react with all other species of unionids examined; indicating the conservative nature of at least one organic component. Such conservation may indicate an important function for these concretions and their calcium binding ability.

Most studies of ion regulation in aquatic animals have focused on Na and Cl and their potential exchange ions NH_4, H, or HCO_3. However, in our previous studies, we noted the importance of Ca in osmo-regulation in freshwater mussels.

Table 4. Chemical composition of concretions isolated from pondwater acclimated *L. subrostrata* (from Silverman et al., 1983).

Concretion content	(g/g dry gill)	0.25
Ash (450° C)	(g/g dry concretion)	0.75
Volatile organic	(g/g dry concretion)	0.25
Calcium	(g/g dry concretion)	0.25
Phosphorous	(g/g dry concretion)	0.13
(calculated as PO_4 or P_2O_7)		0.37–0.40
HCO_3	(g/g dry concretion)	0.03
Unidentified	(g/g dry concretion)	0.07

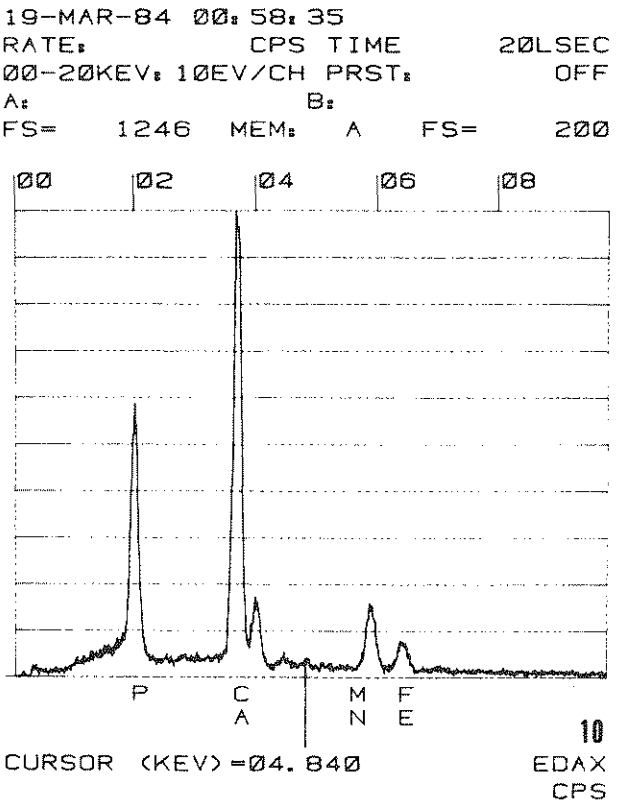

Fig. 10. Energy dispersive X-ray spectrum of purified concretions from the gill of *L. subrostrata*. Calcium and phosphorous are the major components with manganese and iron being minor constituents (Steffens, unpublished).

There is an inverse relationship between blood Na and Ca. When the animals are salt depleted in de-ionized water, selectively depleted of Na by acclimation to choline chloride or subjected to anoxia, the blood Na declines from 15–20 mM to < 10 mM while Ca increases from 3–5 mM to 8–20 mM (Dietz, 1974; Murphy and Dietz, 1976; Scheide and Dietz, 1982; Silverman et al., 1983). Recently, we determined the net flux of Ca while measuring the net flux of Na and noted a direct relationship between the loss of Ca and the gain of Na (Scheide and Dietz, unpublished). The relationship between Na/Ca exchange is $J_n^{Ca} = -0.5\ J_n^{Na} - 1.51$ ($r - 0.55$; $P < 0.001$). The relationship pertained to pondwater acclimated controls, salt depleted or serotonin treated animals. The source of Ca for the Na/Ca exchange is unknown but the concretions in the gill would be in a suitable location for Na/Ca exchanged in both intact animals and isolated gills.

Recently, we have observed the disappearance of calcium concretions when the animals enter the reproductive period (Silverman, et al., 1985). Calcium concretions are X-ray dense in relation to other soft body parts and a loss of density coincides with the mobilization of the concretions during the early reproductive period in female *Ligumia subrostrata* (not shown) and *Anodonta grandis* (Fig. 11). Possi-

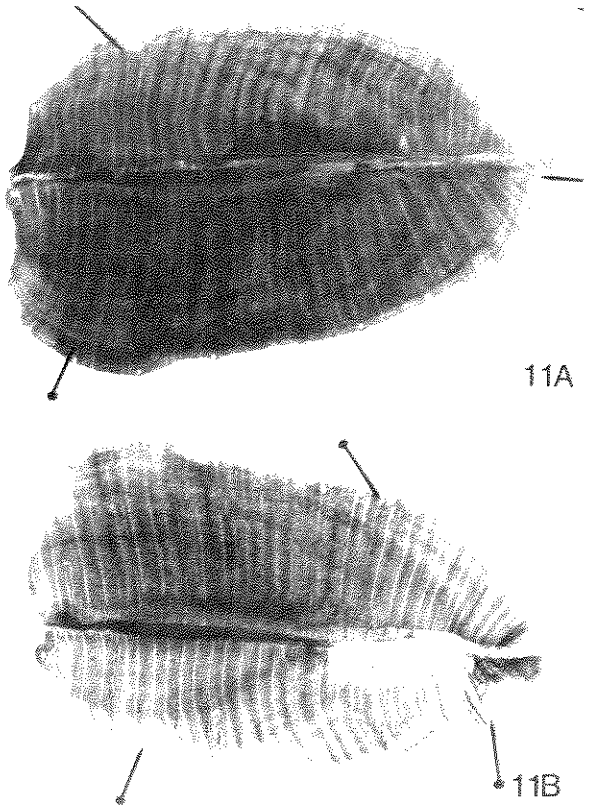

Fig. 11. X-ray radiograms of a pair of gills from *A. grandis* before and during reproductive activity. Both A and B were exposed to the same X-ray parameters and photographic development conditions. A. A gill from an animal before reproduction. The parallel lines of dense concretion material extend nearly to the leading edge of the gill. B. A gill from an animal containing early larval glochidial stages with calcified valves. The concretions have been mobilized mostly from the lateral marsupial gill but loss of concretions is evident from the medial gill. A small rectangle of one surface of the gill was removed to demonstrate that the concretions were present on both lamellae (From Silverman et al., 1985) (Horizontal field width = 8.3 cm).

bly the liberated calcium is available for shell growth in these animals. However, the calcium is mobilized in the female gills after the fertilized eggs begin developing in the gill marsupium and may provide a source of Ca for the embryonic shell formation. We have observed that *A. grandis* broods substantially more embryos in the gills and has twice the calcium concretions when compared to *L. subrostrata*. In addition, preliminary evidence indicates radioactivity appears in the glochidial shells when concretions labelled with ^{45}Ca are mobilized by the females. Further studies are required to understand the mechanism of Ca transfer and the role of Ca in Na balance. Calcium may be important in the transepithelial Na transport process in bivalves, and may be particularly important when the animal is under stress: reproductive, anoxic and/or osmotic.

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