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SOLUTE AND WATER MOVEMENT IN FRESHWATER BIVALVE MOLLUSKS (Pelecypoda; Unionidae; Corbiculidae; Margaritiferidae)

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INTRODUCTION

All bivalve molluscs living in freshwater maintain blood osmotic pressure at concentrations above that of the medium in which they live. However, the freshwater mussels maintain blood osmolalities which are lower than that found in any other aquatic organism (Krogh, 1939; Robertson, 1964; Prosser, 1973). Nevertheless, the freshwater bivalves have the same osmo- and iono-regulatory problems as do other organisms whose blood osmolalities exceed the environment. Water continuously enters and must be excreted whereas ions must be absorbed from the medium against a substantial electrochemical gradient.

Evidence that bivalves could absorb ions from dilute media was first presented by Krogh (1939), who noted that salt depleted *Anodonta* would accumulate Cl^- from 1mM NaCl solutions. Krogh suggested Cl^- was transported by an exchange system since Cl^- was absorbed from NH_4Cl and $CaCl_2$ solutions with a net loss of cations, while Na^+ was absorbed from dilute $NaHCO_3$ solutions. Apart from Krogh's early studies, there is little information available on ionic and osmotic regulation in freshwater bivalves (Chaisemartin *et al.*, 1968; Chaisemartin, 1969; Schoffeniels and Gilles, 1972; Dietz and Branton, 1975).

ION LEVELS IN BIVALVES

The concentrations of major blood ions in representatives of three of the four families of freshwater bivalves found in the northern hemisphere are presented in Table 1. The blood is about 15-20 mM Na^+, 0.5 mM K^+ and is a saturated or supersaturates solution with respect to Ca^{++} (Potts, 1954; Burton, 1976). Inorganic phosphate is present at low concentrations (0.1-0.2 mM) and is thought to be effective in preventing crystallization of $CaCO_3$ (Burton, 1976). However, when blood is exposed to air, precipitation of some calcium and protein-carbonate complexes is noted (Potts, 1954). An alkaline blood pH is characteristic of bivalves and other molluscs (Wilbur, 1964; Bedford, 1973; Burton, 1976). The blood composition of Corbicula manilensis is significantly different from other bivalves in having NaCl as the predominant salt and little HCO_3^-.

REGULATION OF IONS

Ion balance in unfed mussels can be maintained only when salt is accumulated to offset diffusive and renal losses. Routes of ion accumulation would be across epithelia, including the gut. Drinking rates of 0.1-1.0 ml/g dry tissue - hr were measured in L. subrostrata by uptake of inulin from the bath. Uptake of $SO_4^=$ under comparable conditons is negligible. While drinking could account for 10-100% of the accumulated NaCl, it seems unlikely that this is the preferred route since it would aggrevate problems of water balance (Dietz, unpublished).

To demonstrate active transport of an ion it is necessary to show movement against electrical and concentration gradients. The in vivo transepithelial electrical potential (TEP) for L. subrostrata is shown in Figure 1. In pond water (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl) the TEP is -10 to -15 mV blood negative to the medium (Dietz and Branton, 1975). The TEP is Na^+ independent but Ca^{++} dependent. These data are in agreement with data reported for an in vitro clam mantle preparation (Kirschner, Sorenson and Kriebel, 1960; Istin and Kirschner, 1968). Kirschner and coworkers noted this TEP was a Ca^{++} diffusion potential and not due to active ion transport.

Table 1. Blood ion composition in freshwater mussels.

Species	Total Solute mOsm/l	Concentration mM/l (X±SEM,N) Na	K	Ca	Cl	HCO_3	pH	REF
Unionidae								
Ligumia subrostrata	47±1 (12)	20.6±0.7 (14)	0.6±0.1 (5)	3.6±0.3 (11)	12.5±1.0 (11)	11.5±0.5 (6)	7.927±0.062 (5)	1
Carunculina texasensis	45±0 (10)	15.4±0.6 (10)	0.5±0.1 (5)	4.7±0.2 (10)	11.4±0.5 (10)	11.1±0.3 (5)	7.623±0.016 (3)	2
Anodonta grandis	55±1 (6)	19.5±0.3 (6)	0.5±0 (6)	5.8±0.3 (6)	16.1±0.5 (6)	11.2±1.0 (6)	7.356±0.006 (6)	2
Anodonta cygnea	42±0 (20)	15.6±0.3 (4)	0.5±0 (5)	8.4±0.4 (17)	11.7±0.3 (14)	14.6±0.8 (14)		3
Margaritiferidae								
Margaritifera hembeli	39±0 (8)	14.6±0.3 (8)	0.3±0 (8)	5.2±0.1 (8)	9.3±0.2 (8)	11.9±0.2 (8)	8.120±0.038 (8)	2
Margaritifera margaritifera		14.4±1.7 (27)	0.5±0.1 (24)	7.8±1.0 (28)	11.4±1.9 (22)	-----	-----	4
Corbiculidae								
Corbicula manilensis	69±1 (10)	28.9±1.0 (10)	0.9±0.1 (5)	12.8±0.7 (10)	24.7±0.3 (10)	2.9±0.2 (10)	7.505±0.068	2

1. Murphy and Dietz, 1976
2. Dietz (unpublished)
3. Potts, 1954
4. Chaisemartin, 1968

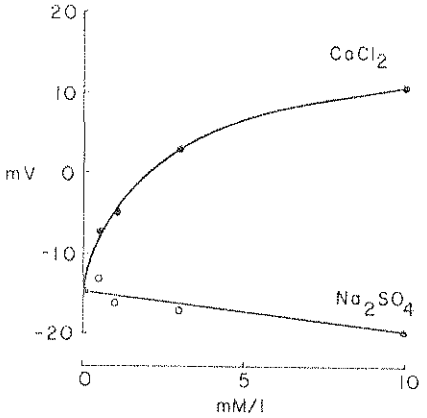

Fig. 1. In vivo transepithelial potential as a function of $CaCl_2$ or Na_2SO_4 concentration in L. subrostrata (Adapted from Dietz and Branton, 1975).

Knowledge of the electrical and chemical gradients between the animal and its environment permits calculation of passive flux ratios for diffusive ion movements using the Ussing (1949) flux ratio equation:

$$J_i/J_o = (C_o/C_i) \exp (FE/RT)$$

where J = unidirectional flux and C = concentration, F = Faraday's constant, E = the TEP, R = the gas constant and T = absolute temperature. For L. subrostrata in pond water the predicted flux ratio for both Na^+ and Cl^- is about 0.06 (Dietz and Branton, 1975).

Unidirectional fluxes for Na^+ and Cl^- were determined for mussels acclimated in pond water. The influxes (J_i) were measured by the disappearance of isotope from the bathing medium (Dietz and Branton, 1975). Net fluxes (J_{net}) were determined from ion concentration changes in the bathing solution and the efflux (J_o) was calculated by the difference ($J_i - J_{net}$). Measured influxes and effluxes were equivalent indicating that the animals were essentially in a steady state (Table 2). Since the observed J_o's contain a renal component the epithelial flux ratios (J_i/J_o) would be greater than 1. This observed flux ratio does not agree with the predicted flux ratio, therefore, both Na^+ and Cl^- must be actively transported. Although rates of ion transport in the unionid species are similar, those measured in C. manilensis are significantly higher. This difference probably reflects the more recent immigration of C. manilensis from brackish into freshwater (Sinclair, 1971). However, transport rates for C. Manilensis reported here are an order of magnitude less than those reported for M. margaritifera (Chaisemartin et al., 1968; Chaisemartin, 1969).

INULIN CLEARANCE

Inulin clearance rates in bivalves are unusually high for freshwater animals and range between 0.1 and 0.4 ml/g dry tissue - hr (Picken, 1938; Potts, 1954b; Martin et al., 1958; Kirschner, 1967;

Table 2. Unidirectional Na and Cl fluxes in freshwater mussels acclimated to pondwater.

Species	ueq/g dry tissue - hr				Reference
	J_i^{Na}	J_o^{Na}	J_i^{Cl}	J_o^{Cl}	
Ligumia subrostrata	1.13±0.16 (8)	1.32±0.25 (8)	1.48±0.36 (13)	1.65±0.49 (13)	Dietz & Branton, 1975
Caruncalina texasensis	1.36±0.10 (10)	1.14±0.14 (10)	1.18±0.08 (15)	1.56±0.25 (15)	*
Corbicula manilensis	10.52±1.02 (10)	9.48±1.61 (10)	6.19±1.08 (15)	6.81±1.01 (15)	*

*Unpublished

Chaisemartin et al., 1970: Murphy and Dietz, 1976). This level of inulin filtration indicates a daily water filtration rate of about 100% of the total tissue weight. However, most filtered water is apparently reabsorbed (Little, 1965). To check for possible convection salt movements the fluxes of Na^+ and Cl^- were determined before and after the bathing medium was made isosmotic using 50 mM mannitol (J_i^{Na} before 0.96 ± 0.20 μeq/g dry-hr, after 80 ± 27% of control, N = 8; J_i^{Cl} before 1.36 ± 0.21 μeq/g dry-hr, after 75 ± 10% of control, N = 10). Although the fluxes tend to be slightly depressed the reduction is not significant and convective salt movement is minimal (Dietz, unpublished).

KINETICS OF Na AND Cl TRANSPORT

Unidirectional influxes were determined for Na^+ and Cl^- in pond water acclimated *L. subrostrata* exposed to a range of NaCl concentrations between 0.1-2.0 mM (Figure 2). Transport systems for both Na^+ and Cl^- are saturable. The capacity (V_{max}) for Na^+ transport is about 2 μeq/g dry tissue - hr and the V_{max} for Cl^- is 1 μeq/g dry tissue - hr. The affinity (K_s) of the transport systems for both Na^+ and Cl^- is between 0.1 and 0.15 mM. These transport rates and affinities are similar in magnitude to a variety of freshwater animals (Shaw, 1963; Chaisemartin, 1969; Alvarado and Moody, 1970; Dietz and Alvarado, 1970; Kirschner, 1973; Dietz and Alvarado, 1974; Dietz, 1974a).

A classic technique for stimulating ion transport is to subject the animals to distilled water (Krogh, 1939). Prolonged exposure of *L. subrostrata* to deionized water leads to a rapid decline in blood NaCl (Figure 3). However, there is a simultaneous increase of Ca^{++} and HCO_3^- which tends to minimize the decline in total blood solute (Murphy and Dietz, 1976). When salt depleted mussels are returned to 0.5 mM Na_2SO_4 or 1 mM choline chloride solutions

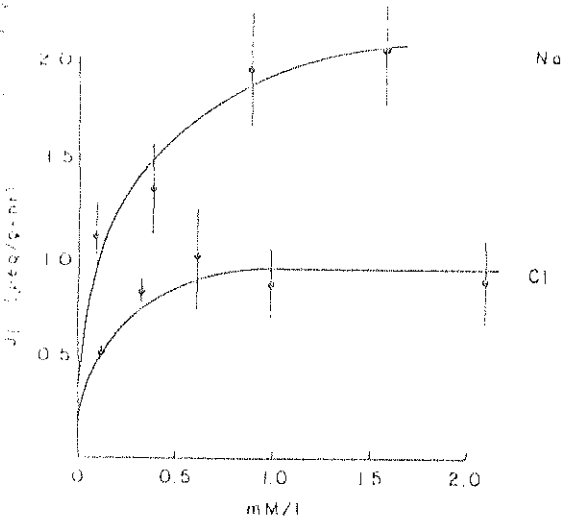

Fig. 2. Unidirectional Na and Cl influx for pond water acclimated L. substrata exposed to various NaCl concentrations (Dietz and Braun, unpublished).

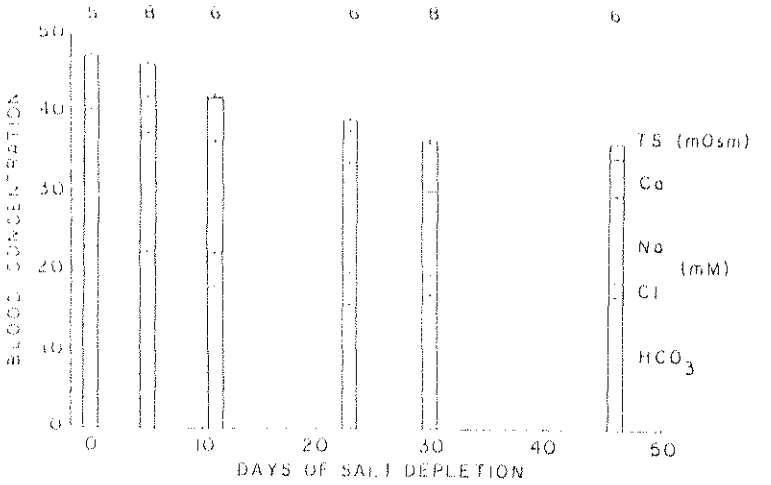

Fig. 3. Blood solute composition in L. substrata subjected to deionized water. The column height represents total solute (TS) and the specific ion concentrations are represented by the subdivision. Vertical lines represent one standard error of the mean and the number of animals is given above each bar (Murphy and Dietz, 1976).

There is a significant net uptake of Na^+ or Cl^-, respectively (Table 3). The elevated J_{net} is due to both an increased J_i and a decrease in J_o. The reduced J_o is probably due to enhanced renal reabsorption of salts and lower diffusive losses across the epithelia. Preliminary experiments have indicated the increase in

Table 3. Effect of salt depletion on unidirectional fluxes of Na from Na_2SO_4 and Cl from Choline Cl.

Species	Ion	N	ueq/g dry tissue-hr J_i	J_o	Reference
Ligumia subrostrata	Na	5	2.24±0.22	0.56±0.15	Murphy and Dietz, 1976
	Cl	6	1.82±0.25	0.59±0.14	Dietz and Branton, 1975
Carunculina texasensis	Na	5	3.37±0.25	0.50±0.06	unpublished
	Cl	9	2.33±0.40	1.35±0.37	unpublished

NaCl transport is due to an elevated V_{max} with no change in affinity (Branton, unpublished). It is noteworthy that Na^+ and Cl^- transport are independent, apparently Na^+ is transported in exchange for an endogenous cation (NH_4^+ or H^+ while Cl is exchanged for HCO_3^- or OH^-. Ligumia subrostrata is ammonotelic (Dietz, 1974b) and has high concentrations of blood HCO_3^- for ion exchange. However, these exchange mechanisms have not been studied.

EFFECTS OF PHARMACOLOGICAL AGENTS

Although ion transport in mussels can be stimulated it is remarkably refractory to inhibition by a number of pharmacological agents (Table 4). The following substances had no effect on chloride fluxes when dissolved in the bathing medium: Furosemide, Amiloride, 4-acetamino-4'-isothiocyano stilbene-2, 2' disulfonic acid. Tiocyanate caused a 40% reduction in J_i but only at a high concentration. Furthermore, "stilbene" injection had no effect on either J_i or J_o. Acetazolamide injection had no effect on J_i but resulted in a 300% increase in J_o. This effect may be due to inhibition of renal Cl^- reabsorption.

Preliminary experiments of Na^+ fluxes indicate acetazolamide injection was without effect on J_i or J_o. Amiloride (0.5 mM) in the bathing medium reduced J_i to 20% of controls with no apparent effect on J_o. These data point out that important differences in Na^+ and Cl^- transport properties exist between vertebrates and invertebrates (Epstein et al., 1973; Kirschner, 1973; Dietz, 1974a; Garcia-Romeu and Ehrenfeld, 1975; Alvarado et al., 1975).

CONCLUSION

These data indicate freshwater mussels possess well developed transport systems for maintaining ion balance while living in dilute solutions. In bivalves the rates of transport and the affin-

Table 1. Effect of various drugs on Cl fluxes in unionids.

Drug	Conc.	Location	N	Flux as % of Control J_i^{Cl}	J_o^{Cl}
SCN	1mM	Bath	7	60*	90
Furosemide	1mM	Bath	8	98	170*
Aufloxide	0.1mM	Bath	5	71	76
Stilbene	0.15mM	Bath	6	67	89
Stilbene	54mg/g dry	Inj	3	89	128
Acetazolamide	7mg/g dry	Inj	9	114	336**

*P < 0.05
**P < 0.01

ity of the transport mechanism toward Na^+ and Cl^- are indistinguishable from those of other freshwater animals. However, the mussels are relatively insensitive to selected drugs. Nevertheless, the bases for maintaining unusually low concentrations of blood solutes is not due to a deficiency of ion transport capacity. It is possible that the low blood solute levels may be due to the limited capacity of the bivalve kidney to excrete water.

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