WATER AND ION REGULATION IN *Procephalothrix spiralis* (NEMERTINA) AND *Clitellio arenareous* (OLIGOCHAETA)

Joan D. Ferraris, Bodil Schmidt-Nielsen and Joanna E. Roth, Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine and Brown University, Providence, Rhode Island

A relationship between neurosecretion and osmoregulation has been implicated in histological studies for a variety of invertebrate groups, including the Nemertina (Lechenault, C.r. hebd. Séanc. Acad. Sci. 261:4868-4871, 1965; Ferraris, Ph.D. Dissertation, Northeastern University, 1977) and the Oligochaeta (Aros and Bodnar, Symp. Biol. Hung. 1:191-202, 1960). Physiological evidence is limited but indicates a reduction in the efficiency of volume regulatory mechanisms in nemerteans lacking the cerebral ganglia and cerebral organs (Lechenault, C.r. hebd. Séanc. Acad. Sci. 261:4868-4871, 1965 and Gen. Comp. Endo. 5:695, 1965). In terrestrial oligochaetes, the cerebral neurosecretory cells may control salt and water balance through a lowering of the permeability of the body wall to water and/or by influencing regulatory mechanisms for electrolytes (Kamemoto, Gen. Comp. Endo. 4:420-426, 1964). That the neurosecretory cells influence nephridial electrolyte regulation is supported by a rise in the chloride concentration of the urine after decerebration (Kamemoto, Kato and Tucker, Am. Zool. 6:213-220, 1966) while neurosecretory control of integumental water exchange was demonstrated by Carley (Gen. Comp. Endo. 27:509-516, 1975 and Gen. Comp. Endo. 35:46-51, 1978). The present report is concerned with preliminary data on the osmo- and volume regulatory abilities of marine representatives of both phyla and the effect of decerebration on these parameters.

Table 1. Osmolality (mOsm) of entire organisms after exposure to a hypoosmotic medium (700 mOsm).

	P. spiralis		*C. arenareous*	
	Control	Ablated	Control	Ablated
0 min	963±3.19	954±7.21	943±6.70	941±6.69
5	794±6.49 (8)	812±13.50 (8)	887±6.90	875±4.52
10	746±2.50	744±5.18	856±6.39 (3)	869±8.65 (3)
15	741±8.84	748±5.62	860±7.95	844±2.90
30	734±4.33	738±1.11	807±4.33	819±2.36
1 hour	722±8.41	727±0.96	756±3.22	780±6.03
2			714±2.10	711±4.27
4	716±7.54	728±3.57	705±4.00 (2)	697±4.51 (3)
6			708±15.23	710±4.95

Means ± standard errors; n = 4 except where indicated within parentheses.

Procephalothrix spiralis (Nemertina) and *Clitellio arenareous* (Oligochaeta) were collected from under intertidal rocks and maintained at 7°C in recirculating seawater aquaria. All individuals of a given species, destined for comparison, were in the same reproductive state since a relationship between the cytology of the neurosecretory cells of nemerteans and the sexual state of these organisms has been demonstrated (Ferraris, Zoomorphologie, in press, 1979). Cerebral gnaglia were ablated by transection immediately posterior to that organ and worms returned to aquaria for a 2 day recovery period prior to use in experiments. The recovery period is sufficient for complete wound healing (unpublished observations).

Both controls and ablated animals were subjected to 75% seawater (700 mOsm; Na^+ 320 mEq/L; K^+ 7 mEq/L) at 7°C, removed from the experimental salinity, blotted and prepared for analysis after the method of Schmidt-Nielsen et al. (Bull. MDIBL 12:99-103, 1972). Duplicate specimens were placed in a small preweighed aluminum cup, weighed and dried to constant weight at 103°C for determination of water content. Osmolality, Na^+ and K^+ concentrations of entire animals were determined by vapor pressure osmometry and flame photometry, respectively. Gram H_2O/gram solute free dry weight was calculated after the method of Schmidt-Nielsen (Am. J. Physiol. 230:514-521, 1976). The significance of differences was determined using the Student t-test for unpaired samples.

Within two hours after exposure to a reduction in salinity both species are essentially isosmotic with the experimental medium (Table 1), thus, both are osmoconformers. However, the two species differ

Table 2. Gram H_2O/gram solute free dry weight of entire organisms after exposure to a hypoosmotic medium (700 mOsm).

	P. spiralis		*C. arenareous*	
	Control	Ablated	Control	Ablated
0 min	2.897±.0009*	2.912±.0020	4.013±.0027*	4.120±.0040
5	3.259±.0657 (8)	3.173±.0625 (8)	3.984±.0038*	4.325±.0029
10	3.948±.0013*	3.725±.0028	4.180±.0038* (3)	4.452±.0060 (3)
15	3.584±.0039*	3.627±.0025	4.360±.0055*	4.460±.0020
30	3.376±.0017*	3.468±.0005	4.581±.0063*	4.668±.0019
1 hour	3.669±.0040*	3.578±.0003	4.474±.0023*	4.639±.0047
2			4.205±.0015*	4.530±.0031
4	3.682±.0035*	3.620±.0015	4.785±.0030* (2)	4.821±.0035 (3)
6			4.649±.0115*	4.842±.0039

Means ± standard errors; n=4 except where indicated within parentheses; *p < .001.

Table 3. Calculated amounts of Na^+ and K^+ in organisms during volume regulation in µM per gram solute free dry weight.

P. spiralis	Control		Ablated	
	Na^+	K^+	Na^+	K^+
15 min	280.6±9.12**	421.5±19.55*	307.3±20.08**	408.4±28.60
30	234.7±5.34	372.2±9.54	229.8±4.71	382.9±10.13
C. arenareous				
1 hour	908.9±19.27**	273.5±2.41**	906.2±17.11*	254.9±1.81
2	745.4±13.91	243.5±2.77	783.5±30.15	256.2±4.48

Means ± standard errors; *P. spiralis* n = 8; *C. arenareous* n = 4. *p < .05; **p < .01

considerably in the time course of response. *P. spiralis* undergoes an extensive reduction in osmolality within the first 10 min, followed by further gradual decrease toward the osmolality of the medium. In contrast, *C. arenareous* loses solute and gains water more gradually and requires a full 2 hours before approximating the osmolality of the medium. These results most probably reflect the presence of a cuticle and consequent lower permeability of the body wall in *C. arenareous*.

Decerebrated *C. arenareous* volume regulate less efficiently than control animals since they invariably contain significantly more water (Table 2). In *P. spiralis* this difference only occurs during volume regulation (15 and 30 min, Table 2). During this period, *P. spiralis* control animals lose 49.6% of the water gained while ablated worms lose 31.6%. Similarly, *C. arenareous* control animals eliminate more of the water gained than do ablated worms, i.e., 66.2 and 25.2%, respectively. Neither species, however, regardless of experimental condition, is able to maintain the reduction in water content achieved beyond the time they are normally exposed to variations in salinity during a given tidal cycle ($\underset{\sim}{\sim}$ 2 hours).

In order to evaluate the solutes involved in regulation in the two species and to compare decerebrated with control animals, we will now look at the time periods during which the worms exhibit the greatest regulatory volume decrease (Table 3). In control P. *spiralis* there is significant reduction in total Na^+ and K^+ in the body during this period (16 and 12%, respectively). In contrast, in ablated animals there is a significant reduction in Na^+ content but no significant reduction in K^+ content. The findings for C. *arenareous* are identical such that control animals excrete Na^+ and K^+ during the regulatory period while ablated animals excrete only Na^+.

From these preliminary data, however, no information concerning the mebhanism of volume regulation can be deduced, since we have not yet determined cell volume and extracellular fluid volume in these worms. Experiments are in progress to determine these parameters. This study was supported by NIH grant AM15972-08.

URINARY RENAL PELVIC REFLUXES OF URINE

Bodil Schmidt-Nielsen, Monique Churchill, Bruce Sherman, Bruce Graves and Larry Reinking
Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine

When the urine exits from the Ducts of Bellini, it sometimes refluxes back into the renal pelvis (Schmidt-Nielsen, Fed. Proc. 36:2493, 1977). Most mammalian renal pelvises have elaborate extensions (Pfeiffer, J. Anat. (London) 102:321, 1968; Lacy and Schmidt-Nielsen, Am. J. Anat., in press a; Schmidt-Nielsen, Fed. Proc. 36:2493, 1977) which permit the urine to come into contact with the thin epithelium covering the outer medulla (Lacy and Schmidt-Nielsen, Am. J. Anat., in press b). The area of the outer medulla facing the pelvis in the hamster constitutes 50% of the total pelvic area, and is twice as large as that covering the inner medulla (Lacy and Schmidt-Nielsen, Am. J. Anat., in press a). When the urine refluxes, it reaches all of the pelvic extensions (Schmidt-Nielsen et al., Bull. MDIBL 17:96, 1977). Various hypotheses have been presented to explain the physiological role of these refluxes (Pfeiffer, J. Anat. (London) 102:321, 1968; Schmidt-Nielsen, Fed. Proc. 36:2493, 1977). No real understanding has been reached, however, due to lack of information concerning the physiological conditions under which urine refluxes into the pelvis.

The present investigation was undertaken to determine: 1) under which physiological conditions pelvic refluxes occur; 2) what effect refluxes have on the solute concentrations in the renal papilla and on the urine itself, and 3) to record the various types of refluxes on film.

A total of 90 successful experiments were carried out on 45 hamsters and 45 Munich Wistar rats. These animals were chosen because the renal papilla extends beyond the renal cortex at the hilus. The animals were anesthetized with 15 mg inactin per 100 g body weight. In the rats, the jugular vein and carotid artery, as well as both ureters, were catheterized. The left kidney was exposed through a flank incision and a small plastic shield was placed under the kidney to isolate it from respiratory movements. The urine was made visible by a continuous i.v. infusion of a lissamine green solution. The rate of infusion varied according to the experimental protocol. In the hamsters, two techniques were used: the kidney was either exposed and placed on a plastic shield in the same manner as described above for the rats, or (in the majority of experiments) the right kidney was exposed through an abdominal incision. It was left in situ and was isolated from respiratory movements by placing a plastic shield against the diaphragm. The lower part of the renal pelvis was cleared of surrounding fat. In both rats and hamsters the pelvis was left intact, and was illuminated with a fiber optic light. The reason for using the different techniques for exposing the pelvis was to determine if refluxes were caused by the experimental procedures of placing the kidney on a shield.