AN ESTIMATION OF INTRA- AND EXTRACELLULAR VOLUME IN AN INTERTIDAL NEMERTEAN, *PROCEPHALOTHRIX SPIRALIS*

Joan D. Ferraris, Bodil Schmidt-Nielsen and Hilda K. Roderick, Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine

Evidence based on histological studies supports the hypothesis that neurosecretory and neuroglandular mechanisms have some function in volume regulation in intertidal nemerteans (Ferraris Gen. Comp. Endo. 39:423-466, 1979a,b,c). Preliminary physiological data (Ferraris et al., Bull. MDIBL 18:87-89, 1978) indicated a very short-term (< 30 min) influence of decerebration on volume regulation in *P. spiralis*. The present study tested, over a longer exposure period than in the previous study, the ability of control, decerebrated and sham-operated *P. spiralis* to volume regulate in a diluted medium under similar conditions. Further, an attempt was made to determine intra- and extracellular volume using an *in vitro* method for extracellular space determination.

Procephalothrix spiralis were collected from under intertidal rocks and maintained at 7°C in recirculating seawater aquaria. Animals were decerebrated by transection immediately posterior to the cerebral ganglia (ablated) while a cut was made in the body wall of the cephalic region of sham-operated worms. Both groups were then returned to aquaria for a five day recovery period. Worms were subjected to 70% seawater (650 ± 4.05 mOsm) for varying time periods, drained of adhering seawater, weighed, dried at 100°C, reweighed and heated (98°C, 3 min) in 50 µl distilled deionized water. Following diffusion at 4°C, osmolality was determined on the supernatant, and, in conjunction with wet weight/dry weight determination, was used to calculate grams H_2O/gram solute free dry weight (g H_2O/g s.f.d.w.) after the method of Schmidt-Nielsen (Am. J. Physiol. 230: 514-521, 1976). Extracellular space (PEG space) was determined *in vitro* using C^{14}-polyethylene glycol M.W. 4000 as a marker for extracellular fluid. Intact worms which were either maintained in 100% seawater (947 ± 4.15 mOsm) or pre-exposed to 70% seawater for 2,6,10,16 or 22 hours were briefly drained and then cut into 2 mm pieces with a razor blade. Tissues were incubated (total 2 hours, 7°C) in open vials with shaking at 30 min intervals. Each vial contained 5-8 mg tissue and 0.5 ml incubation medium [100 or 70% seawater containing 0.4 mg C^{14}-PEG (0.25 µCi) plus 4.5 mg PEG M.W. 4000]. Following incubation, tissues were briefly rinsed, weighed, dried, and heated, as above, but in 20 µl distilled-deionized water. Following diffusion, 10 µl of the tissue supernatant and 5 µl of the incubation medium were each pipetted into 5 ml scintillation fluid (Biofluor). Data were compared for significant differences using a one-way analysis of variance (Freund, 1962) followed by Student-Newman-Keul's test (Steele and Torrie, 1960) for separation of significant means ($p \leq 0.05$).

Results of exposure of all groups to 70% seawater demonstrated a significant, maximum increase in total water content at one hour (Table 1). Mean water content decreased gradually in control, ablated and sham-operated animals through 24 hours, but in no group was water loss statistically significant. Further, there was no significant difference among the three groups tested during such exposure. Limited volume regulatory ability was, however, indicated since in no group of animals did hydration occur to the degree expected by changes in tissue osmolality (Table 2). *In vitro* determination of PEG space in *P. spiralis* demonstrated that the extracellular compartment comprises only 5% of the total body water in these worms (Table 3). Further, the percent of the total body water which extracellular did not change significantly during a 0 to 24 hour exposure to 70% seawater. Thus, the increase in water content which occurs during such exposure affects the volume of both the extra- and intracellular compartments; but much more water enters the intracellular space.

TABLE 1

TOTAL WATER CONTENT (g H_2O/g s.f.d.w.) DURING EXPOSURE TO 70% SEAWATER

Hours in 70% Seawater	Control	Ablated	Sham-Operated
0 (n=20)	3.24 ± 0.06	3.39 ± 0.13	3.23 ± 0.05
1 (n= 7)	4.51 ± 0.16[a]	4.42 ± 0.10	4.24 ± 0.17
4 (n=11)	4.19 ± 0.13	4.20 ± 0.12	4.10 ± 0.12
8 (n=10)	4.10 ± 0.18	3.94 ± 0.11	3.98 ± 0.16
12 (n=13)	3.98 ± 0.10	4.20 ± 0.08	4.02 ± 0.10
18 (n=13)	3.95 ± 0.07	4.03 ± 0.08	3.88 ± 0.06
24 (n=13)	3.96 ± 0.12[b]	4.05 ± 0.09	3.94 ± 0.13

Mean ± S.E. [a]n = 6, [b]n = 12.

TABLE 2

COMPARISON OF CHANGES IN WATER CONTENT IN TISSUES (V_1/V_2) WITH VALUES EXPECTED BY CHANGES IN TISSUE OSMOLALITY (Π_2/Π_1) AFTER ONE HOUR IN 70% SEAWATER

	Π_2/Π_1		V_1/V_2
Control	0.700 ± 0.010	(6)	0.724 ± 0.026
Ablated	0.703 ± 0.011	(7)	0.769 ± 0.017
Sham-Operated	0.711 ± 0.008	(7)	0.768 ± 0.029

Mean ± S.E. (n).

TABLE 3

C^{14}-PEG SPACE DURING EXPOSURE TO 70% SEAWATER

Hours in 70% Seawater	PEG Space (as % of Total Water Content)
0	4.95 ± 0.453 (12)
1	6.44 ± 0.654 (12)
4	4.91 ± 0.572 (24)
8	4.39 ± 0.563 (12)
12	5.53 ± 0.705 (12)
18	5.38 ± 1.091 (12)
24	4.56 ± 0.534 (12)

Mean ± S.E. (n).

According to our findings, the water content of these worms appears to be independent of the presence or absence of the cerebral ganglia and the cephalic gland during a 1 to 24 hour exposure to 70% seawater. This may be linked with the fact that, to our knowledge, neuroendocrine control has only been demonstrated for mechanisms of extracellular (e.g., Grimm-Jørgensen, J. Exp. Zool. 212: 471-473, 1980) rather than intracellular volume regulation in invertebrates. In nemerteans, extracellular volume regulation may be obscured because such a small fraction of the total water is extracellular fluid. However, since neuroendocrine influences on intracellular volume have not been well studied (Gilles, 1979. In: Mechanisms of Osmoregulation in Animals, Gilles (ed.), Wiley, N.Y.), and since the intracellular compartment in this species is of significant magnitude in the intact animal, mechanisms involving the control of intracellular volume lend themselves to study in this species. Support was provided by NIH awards GM 07047, AM 15972 and AM 15973.

DRILLING FLUID EFFECTS ON TELEOST AND ECHINODERM DEVELOPMENT

Richard B. Crawford and Jonathan D. Gates, Department of Biology, Trinity College, Hartford, Connecticut

As a consequence of petroleum drilling offshore, large quantities of drilling fluids (muds) are introduced into the marine environment. These drilling fluids are aqueous suspensions of a variety of components pumped down the center of the drill bit. The fluid composition is varied as needs of the drilling operation are encountered, such as lubrication, cooling, antibacterial action, suspension of drill cuttings, prevention of intrusion of seawater into the bore hold, and capture of H_2S. Portions or all of the drilling fluid may be discharged into the surrounding waters during the drilling program and especially at its completion. This discharge can amount to significantly large quantities (e.g., 2,000 tons per hole) in the vicinity of a drilling platform.

The impact of drilling fluids on marine and estuarine environments is unknown due to the paucity of toxicological studies which have been conducted. In this report we describe the effects of five different drilling fluids on fertilization and early embryo development in a teleost and an echinoderm.

Five drilling fluid samples were tested, all obtained from a Mobile Bay drilling rig and supplied by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fla. These samples were lignosulfonate-mud types containing barium sulfate. They are identified by the date the sample was taken in 1979: March 12, April 24, May 29, June 26, and October 11. Stock materials were kept at 4° and solutions or suspensions prepared from the stock were kept at the temperature of the developing embryos. Standardization of the drilling fluid solutions was based on analyses of dry weight. For example, the dry weight of one of the stock solutions was 262 mg/ml which was then indicated as 262 ppt. The test solutions were dilutions of this to make 10 ppt, 1 ppt, 100 ppm, 10 ppm and 1 ppm. Upon making these solutions with seawater, copious precipitates developed. Therefore following dilution the fluids were filtered through Whatman #1 paper. Therefore the drilling fluid components tested were those which remain soluble in sea water.

Embryos of Fundulus heteroclitus were used as the model for teleost development studies. Adult fish were obtained from estuarine waters of Frenchman Bay and kept in floating live cars in seawater. Eggs were stripped from females and fertilization was initiated by adding a sperm suspension obtained by mincing dissected testes. Filtered seawater kept at 18° was the normal incubation medium.

For these studies, embryos were placed in the drilling fluid solutions 1 min after fertilization and they were maintained at those concentrations for the duration of their development. The incubation dishes contained 10 ml of medium, changed daily, and from 50 to 100 embryos. The particularly susceptible events to environmental toxins observed with the dissecting microscope included early cleavage, blastulation, gastrulation, formation of the body