Pacific Science (1982), vol. 36, no. 3

The *Nautilus* Siphuncle as an Ion Pump[1]

CHARLOTTE P. MANGUM[2] and DAVID W. TOWLE[3]

ABSTRACT: The siphuncle, which is believed to empty the newly formed chambers of the shell by a process involving the active transport of NaCl, has the metabolic, enzymatic, and morphological features of a transporting epithelium. It is capable of removing monovalent ions from solutions containing only Na^+ and no Cl^- or divalent ions, or only Cl^- and no Na^+ or divalent ions, indicating no obligatory coupling. The Na^+ and Cl^- are removed from native cameral fluid at approximately the same ratio. The levels of K^+ and the divalent ions are also lowered, but at slightly different rates. Neither H^+ nor NH_4^+ accumulate in cameral fluid to an appreciable extent.

IN THE CEPHALOPOD MOLLUSKS there is no evidence of an ability to maintain blood salts and water out of osmotic balance with the medium, even in euryhaline species (Hendrix 1980, see also Mangum 1981). The net movement of fluids across epithelia within the animal, which in a number of species plays a part in the regulation of buoyancy, can be explained by mechanisms that do not require a hypothesis of active ion transport (Denton and Gilpin-Brown 1973). Perhaps for these reasons, serious consideration has been given to hypotheses invoking passive mechanisms to explain the pumping of water out of newly formed chambers of the nautilus shell, even though the outcome of the process is a net osmotic change in the residual fluid (Denton and Gilpin-Brown 1966, 1973; Greenwald, Ward, and Greenwald 1980; Ward, Greenwald, and Greenwald 1980).

However, several observations on *Nautilus macromphalus* made by Denton and Gilpin-Brown (1966) strongly implicate the active secretion of a hypersaline solution of NaCl from cameral fluid into the blood by the siphuncle, a long tube that extends into the uninhabited chambers. Although no reliable information is available on the hydrostatic pressure and osmolality of the blood, blood pressure must be higher than ambient (see Bourne, Redmond, and Johansen 1978 for data on *N. pompilius*), and blood osmolality would be expected to be at least as high as and probably slightly higher than ambient, as in other cephalopods. If cameral fluid is initially composed of ambient seawater, then the osmotic gradient should drive permeant solutes into cameral fluid. And yet, both the osmolality and the chlorinity of cameral fluid decrease as its total volume is lowered (Denton and Gilpin-Brown 1966, Ward and Martin 1978).

In addition, the siphuncular epithelium has many of the morphological features that characterize other ion transporting epithelia, including microvilli at the site of putative NaCl absorption into the cell and, most convincing, an elaborate infolding of the basement membrane at the site of NaCl extrusion from the cell (Denton and Gilpin-Brown 1966), extending throughout the cytoplasm as a network of "canaliculi" (Greenwald et al. 1980, Ward et al. 1980). Finally, unlike the body fluids of some cephalopods, cameral fluid contains Na^+ as the most abundant cation (Denton and Gilpin-Brown 1966).

The mechanism of active ion transport by the siphuncle of nautilus seemed uniquely

[1] This research was conducted as part of the *Alpha Helix* Cephalopod Expedition to the Republic of the Philippines, supported by National Science Foundation grant PCM 77–16269 to J. Arnold. This study was also supported in part by National Science Foundation grant PCM 77–20159 (regulatory biology) to C. P. Mangum and by a University of Richmond Faculty Research grant to D. W. Towle.

[2] College of William and Mary, Department of Biology, Williamsburg, Virginia 23185.

[3] University of Richmond, Department of Biology, Richmond, Virginia 23173.

interesting to us for two reasons: First, the organ apparently survives exposure to very dilute cameral fluid for long periods, which is otherwise rare in cephalopods (Hendrix 1980). Therefore, it seemed possible that the siphuncle might also survive experimental manipulation of the ionic composition of the fluid. Important aspects of ion transport such as the coupling of Na^+ and Cl^- movements (reviewed by Towle 1981) might be elucidated. Second, cameral fluid is stagnant, ultimately as well as transiently. Unlike other ambient fluids with which animal bloods exchange inorganic ions, cameral fluid is not flushed away from the transporting epithelium by convection. If electrochemical balance across the epithelium is maintained by the efflux of counterions from the blood, they would be expected to accumulate to measurable levels in cameral fluid and thus might be clearly identified.

In this paper we report results that are difficult to reconcile with any mechanism of emptying newly formed chambers other than active ion transport. While the mechanism of maintaining electrochemical balance remains unknown, our findings eliminate one possibility, and they suggest an area for future investigation.

METHODS AND MATERIALS

Electron Microscopy

Siphuncles were removed intact by severing the muscles attaching the animal to the shell, extending two fingers into the inhabited chamber to the most recently formed septum, and pulling gently and slightly downward on the posterior mantle. They were fixed for 45 min at room temperature in 2.6% gluteraldehyde in seawater (pH 7.2, osmolality 1003 mOsm). Then the tissue was rinsed with filtered seawater for 30 min, with two changes, and postfixed at room temperature with 1% OsO_4 in 70% local seawater for 19 min. The tissue was dehydrated with 50, 70, and 95% ethyl alcohol, and left overnight in 95%. It was then embedded in Epon 812, sectioned, and stained with lead citrate. The sections were examined with a Zeiss 9S-2 electron microscope.

Tissue Oxygen Uptake

The oxygen uptake of minced tissues was measured in 0.05 M Tris–maleate buffered seawater (pH 6.99) with a Yellow Springs Biological Oxygen Monitor Model 53. The tissue was halved; one half was added to the buffered seawater and the other to the same medium plus ouabain (10^{-3} M). After 30–45 min, ouabain was also added to the control (final concentration $2.0–2.5 \times 10^{-4}$ M) and the measurement on both aliquots of tissue repeated, to control for deterioration.

Rate of Ammonia Excretion

Animals were placed in containers with 0.05–6 liters of seawater, depending on body size, and the ammonia levels were assayed before and after 30 min incubation, using the phenol hypochlorite method (Gravitz and Gleye 1975, Solorzano 1969).

Ionic and Osmotic Composition of Blood and Cameral Fluid

Osmolality was determined with an Advanced Instruments Model 3DII freezing point osmometer, pH with a Radiometer BMS3 Blood Gas System, and ammonia as above, using 0.5-ml samples diluted with seawater to 5 ml. The activities of Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- were determined with ion-selective electrodes, using stirred, buffered dilutions of the unknown, as described by Mangum et al. (1978*b*). Due to malfunction of the Radiometer PHM4 on shipboard, it was necessary to modify the apparatus by using a Radiometer PHM 64 for the electrodes equipped with European plugs, and a Model 8040 A Fluke Multimeter connected to the input circuit of a Chemtrix 60 A electrometer for the electrodes equipped with American plugs.

Blood samples were obtained from catheterized vessels (see Lykkeboe and Johansen, this issue), and cameral fluid from small holes drilled into the shell at the sites of the two most recently formed chambers. Those chambers were aspirated until no more fluid could be obtained with a syringe fitted with a short length of polyethylene tubing (No. 60) while holding the animal in the air for 30–60 sec so

that the hole was at the lowest point of the shell, and the hole was blotted with absorbent paper. A test solution was then added to the chamber and the hole sealed with dental wax (Surgident), after the surrounding 5–10 mm was abraded. In no instance did the seal fail.

Enzymology

Tissues were frozen in homogenizing medium (0.25 M sucrose, 6 mM disodium ethylenediamine tetraacetic acid, 20 mM imidazole, pH 6.8) and shipped frozen to Virginia. Tissue samples were thawed in ice-cold homogenizing medium, briefly blotted, weighed, and homogenized in 20 volumes of fresh homogenizing medium containing 0.1% sodium deoxycholate (wt/vol) (Towle, Palmer, and Harris 1976). Homogenates were filtered through two layers of cheesecloth and were assayed immediately for $Na^+ + K^+$-ATPase activity and protein.

The $Na^+ + K^+$-ATPase activity was measured at 25°C by a coupled spectrophotometric system in a medium containing 0.1 ml filtered homogenate, 20 mM imidazole (pH 7.8), 90 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 5 mM disodium ATP, 0.1 mM NADH, 2.5 mM phosphoenol pyruvate, and 20 μl pyruvate kinase/lactate dehydrogenase mixture (Sigma 40–7) in a final volume of 2.0 ml (Saintsing and Towle 1978; Schwartz, Allen, and Harigaya 1969). Control assays contained 1 mM ouabain in addition to the above ingredients. The $Na^+ + K^+$-dependent portion of total ATPase activity was calculated as the difference between catalytic rates measured at 340 nm with and without ouabain. Protein concentrations of filtered homogenates were measured with a dye-binding assay, using bovine serum albumin as standard (Bradford 1976).

RESULTS

Ultrastructure of the Siphuncular Epithelium

The siphuncular epithelium of *Nautilus pompilius* is characterized by the presence of extremely elongated cells in indirect contact with cameral fluid via apical microvilli and, presumably, in contact with venous blood across a thick layer of contractile elements (Figure 1). These cells are joined in the apical region by extensive junctional complexes (Figure 1*A*), but are separated laterally in groups of two or three cells to form large intercellular drainage channels, as previously reported in light-microscopic studies of *N. macromphalus* siphuncle (Denton and Gilpin-Brown 1966). The channels contain amebocytes and other wandering cells. The blind end of each channel may lie within 15 μm of the horny siphuncle tube, whereas the channel itself measures as much as 100 μm in length. Leading into the channel are many small ducts ("canaliculi") formed by invagination of the basolateral plasma membranes of the epithelial cells (Figure 1*A*, *B*). Adjacent to these invaginations are numerous densely staining mitochondria. The base of the channel is separated from the central blood vessel by a layer of contractile elements; the layer may be up to 70 μm thick (Figure 1*C*).

A comparison of the arrangement of the canaliculi in *Nautilus pompilius* with those also observed in the siphuncle of *N. macromphalus* (Greenwald et al. 1980) indicates that the ducts seen in *N. pompilius* are more regularly arranged than those of *N. macromphalus*, producing an approximately parallel array of tubules extending well into the apical portion of the epithelial cells. The canaliculi of *N. macromphalus* appear to be shorter and less regularly arranged, but nonetheless also provide a substantial increase in membrane surface area.

Ouabain Sensitivity of Oxygen Uptake by Various Tissues

Oxygen uptake by mantle and kidney tissue isolated from *Nautilus pompilius* and *Octopus macropus* adults responded alike to the presence of ouabain. While the rate in mantle tissues was unaffected, the rate in kidney tissues decreased significantly ($P < 0.05$ according to Student's t test for paired observations; Table 1). In gill tissue, the rate decreased significantly in *O. macropus* but not in *N. pompilius*. The greatest response to ouabain, however, was found in the *Nautilus* siphuncle.

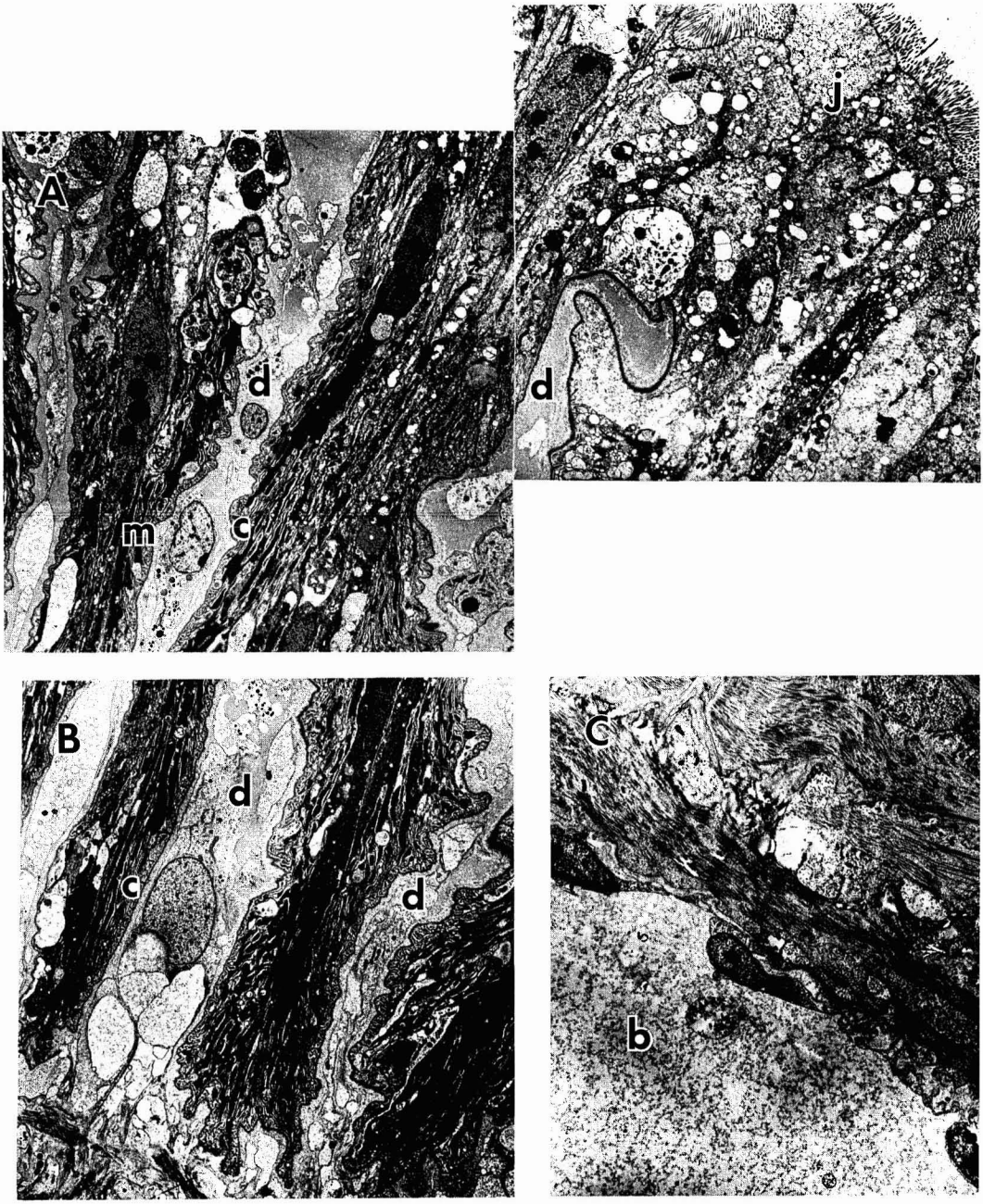

FIGURE 1. *A*, electron micrograph of the apical region of siphuncle epithelial cells. The horny siphuncle tube would be positioned at upper right, adjacent to the apical microvilli. Note the elongated epithelial cells, joined in the apical region by junctional complexes (j). Drainage channels (d), formed as large intercellular spaces, contain amebocytes and other wandering cells. Leading into the drainage channels are many canaliculi (c) formed by approximately parallel invaginations of the basolateral plasma membrane in close contact with densely staining mitochondria (m). Magnification 2550 ×. *B*, basal region of siphuncle epithelial cells approximately 100 μm from the apical end. Symbols as described in *A*. Magnification 2550 ×. *C*, electron micrograph of contractile layer enclosing a blood vessel (b). Layer of contractile elements may be as much as 70 μm thick extending medially from the basal region of the epithelial cells and drainage channels. Magnification 2550 ×.

TABLE 1

EFFECT OF OUABAIN ON OXYGEN UPTAKE ($\dot{V}_{O_2}$) OF ISOLATED TISSUES

	CONTROL RATE (μl/g-hr)	% INHIBITION BY OUABAIN (2×10^{-4} to 1×10^{-3} M)
Nautilus pompilius		
Gill	211	0–13
Kidney	594	7–17*
Mantle	92	0–2
Siphuncle	872	46–61*
Octopus macropus		
Gill	470	25–38*
Kidney	210	50–55*
Mantle	17	0–25

NOTE: At 25°C, 34‰; $N = 4$–6.
* $P < 0.05$, according to Student's t test for paired observations.

TABLE 2

SPECIFIC ACTIVITY OF $Na^+ + K^+$-ATPase

	MEAN ± SE	NUMBER OF SAMPLES N
Nautilus pompilius		
Gill	1.4 ± 0.5	6
Mantle	2.2 ± 0.9	5
Kidney	73.7 ± 10.6	3
Siphuncle	21.3 ± 3.6	7
Octopus macropus		
Gill	18.5	2

NOTE: At 25°C. Data expressed as nmole P_i/mg protein-min.

$Na^+ + K^+$-ATPase Activity of Various Tissues

The pattern of ouabain inhibition of tissue oxygen consumption is paralleled by the distribution of ouabain-sensitive, $Na^+ + K^+$-dependent ATPase activity. Homogenates of gill and mantle tissue from *Nautilus pompilius* contained low activities of $Na^+ + K^+$-ATPase, whereas kidney and siphuncular epithelium both exhibited substantial activities of this enzyme (Table 2). Gill tissue from *Octopus macropus* also showed a moderate level of $Na^+ + K^+$-ATPase activity. Although comparable data do not exist for similar tissues in other cephalopods, values reported here for *Nautilus* siphuncle and *Octopus* gill are comparable to levels of $Na^+ + K^+$-ATPase activity measured in homogenates of kidney and mantle of the euryhaline clam *Rangia cuneata* (Saintsing and Towle 1978) and in microsomal preparations from gills of the freshwater mussel *Carunculina texasensis* (Dietz and Findley 1980). The high activity noted in kidney of *N. pompilius* is similar in magnitude to $Na^+ + K^+$-ATPase activity in homogenized gills of the euryhaline teleost *Fundulus heteroclitus* (Towle, Gilman, and Hempel 1977). High $Na^+ + K^+$-ATPase activities in these and other tissues appear to be indicative of a capacity to transport large amounts of Na^+ (Bonting 1970, Towle 1981).

Osmotic and Ionic Composition of the Blood and Cameral Fluid

In general, the salinity in Bindoy Bay was high throughout the month of October 1979. However, several consecutive days of rain

TABLE 3

IONIC AND OSMOTIC COMPOSITION OF THE BLOOD, NATIVE CAMERAL FLUID, AND TEST SOLUTIONS INJECTED INTO ASPIRATED CHAMBERS OF THE SHELL IN *Nautilus pompilius*

	MEAN ± SE							
	mosM	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NH_4^+	pH
Ambient seawater ($N = 6$)	1006 ± 4	445 ± 5	9.07 ± 0.20	8.25 ± 0.04	45.3 ± 0.3	525 ± 5	Trace	7.83 ± 0.05*
Blood ($N = 7$)	1011 ± 11	409 ± 4	8.17 ± 0.23	8.39 ± 0.23	41.4 ± 0.9	524 ± 11	0.097 ± 0.051	7.44 ± 0.10
Native cameral fluid	497 ± 79 ($N = 4$)	186 ± 15 ($N = 6$)	3.89 ± 0.45 ($N = 16$)	6.10 ± 0.97 ($N = 16$)	14.9 ± 2.0 ($N = 13$)	222 ± 18 ($N = 16$)	0.205 ± 0.082 ($N = 7$)	7.96 ± 0.05 ($N = 7$)
Seawater injections								
20 ml, 4 hr ($N = 5$)	1023 ± 2	460 ± 2	9.30 ± 0.54	9.11 ± 0.22	44.0 ± 0.9	552 ± 2	0.023 ± 0.003	7.74 ± 0.05
5–20 ml, 1–2 days ($N = 5$)	1004 ± 11	448 ± 6	8.53 ± 0.21	8.91 ± 0.05	44.1 ± 0.9	529 ± 13	0.054 ± 0.010	7.74 ± 0.05
3.5–5 ml, 3–5 days ($N = 4$)	958 ± 4	409 ± 9	8.60 ± 0.44	8.61 ± 0.48	41.1 ± 1.8	468 ± 21	0.170 ± 0.053	7.86 ± 0.22
5–10 ml, 7–13 days ($N = 3$)	916 ± 19	387 ± 4	7.01 ± 0.58	7.98 ± 0.25	39.7 ± 2.3	452 ± 10	0.100 ± 0.091	
Seawater + ouabain (10^{-3} M) injections								
Test solution	1050	446	8.55	8.40	44.1	5.20		7.41
3.5–5.0 ml, 5 days ($N = 3$)	988 ± 4	393 ± 7	7.16 ± 0.77	7.97 ± 0.52	3.41 ± 4	475 ± 8		7.34 ± 0.03
KCl injections								
Test solution	866					425	Trace	7.03
3.4–4.5 ml, 5 days ($N = 3$)	797 ± 13					350 ± 20		8.08 ± 0.34
3.4–4.5 ml, 7 days ($N = 4$)	797 ± 15					347 ± 16		8.52 ± 0.31
$NaHCO_3$ injections ($N = 1$)								
Test solution	907	445					Trace	9.34
3.4 ml, 5 days		410						8.74
3.4 ml, 7 days	746	390						8.82
3.4 ml, 10 days		322					0.261	8.61

NOTE: At 16–18°C. Inorganic ions and ammonia given in mM.
*At 30°C.

resulted in a measurable dilution for about a week, and hence the variation shown in Table 3. The blood of *Nautilus pompilius* is hyperosmotic to the medium, but in paired observations, only by 10–17 mOsm (Table 3). Thus, the much larger blood–medium difference in *N. macromphalus* reported by Denton and Gilpin-Brown (1966), which was based on a single observation, was probably exaggerated by the 2-week storage period, as they suggested. The blood of *N. pompilius* is significantly hypoionic in free Na^+, K^+, and Mg^{2+}, and isoionic in Ca^{2+} and Cl^- (Table 3).

The ionic profile suggests slightly weaker Na^+ regulation than often observed in coleoids (Mangum 1981, Prosser 1973), but the most distinctive difference is the blood–medium deficit in K^+, which is found only in nautilus. In other cephalopods, total K^+ in the blood is actually hyperionic to the medium by 1.2–10.4 mM, and the discrepancy is unlikely to be due wholly to the measurement of total concentration by previous investigators rather than ion activity, as in Table 3.

Both Denton and Gilpin-Brown (1966) and Ward and Martin (1978) showed that cameral fluid taken from the shells of freshly collected animals is hypoosmotic to ambient seawater, reaching values as low as 2% seawater (Ward and Martin 1978) which, according to the Venice System, is almost fresh water. Denton and Gilpin-Brown (1966) also showed that in 30–100% seawater, Na^+ and Cl^- decrease in direct proportion to the change in osmotic concentration, suggesting that the removal of these ions is responsible for the osmotic change. Their figure 5 also indicates that the Na : Cl ratio of cameral fluid differs from that of the ambient seawater from which it is derived, even at very high concentrations that presumably reflect a recent origin from seawater. Specifically, Na^+ remains low by about 5–10%, while Cl^- is basically isoionic to seawater of the same percent concentration. This relationship suggests that in *Nautilus macromphalus* the inorganic ion profile of cameral fluid resembles that of the blood more than that of seawater.

Our data on freshly collected, negatively buoyant *Nautilus pompilius* do not agree (Table 3). The Na : Cl ratio approximates that of seawater rather than that of blood, regardless of osmolality. Even more interesting, the levels of the other inorganic ions also fall with the decrease in NaCl, although not in constant ratios. While the ratio of K^+ to Na^+ and Cl^- remains the same, Ca^{2+} becomes enriched and Mg^{2+} impoverished with the decrease in osmolality.

Osmotic and Ionic Composition of Solutions Injected into Aspirated Chambers

The data reported by Ward and Martin (1978) indicate that when the chambers of *Nautilus macromphalus* held in the laboratory are emptied of native cameral fluid and injected with various volumes of saline, the changes in chlorinity of the saline are more often positive than negative, possibly due to poor sealing of the holes drilled into the shell and consequent influx of ambient seawater, which was noted by the authors. Using only their data for injections of high-salinity (= 90%) seawater that did not increase in volume during the 5-day period, the mean Cl^- loss was about 3.5 mM/day. When they performed the same experiment and held animals of both species at various depths in nature, the rate was much faster (approx. 96–171 mM/day in *N. pompilius* held at 180–250 m for 2 days).

In *Nautilus pompilius* held in the laboratory at 0.5 m with seawater replacing native cameral fluid in the two most recent chambers, the change in total osmotic concentration and in the ions measured, with the exception of NH_4^+, was not significant for the first 2 days. At 3 and 5 days, however, there were appreciable changes (approx. −65 mM/day) in most of the ions present in the chambers injected with either large or small volumes (Table 3). After 10–13 days, the changes were greater, although the rate of change appeared to diminish to an average of about 10 mM/day. In no case did the animal gain positive buoyancy.

The ion ratios remained generally similar to those characteristic of seawater throughout the 13-day period, showing no trend of Ca^{2+} enrichment nor Mg^{2+} impoverishment. Free K^+ and divalent cations clearly decrease ($P < 0.05$) along with the change in NaCl.

The addition of ouabain to the injected

TABLE 4

RATES OF AMMONIA EXCRETION

	Nautilus pompilius	*Octopus macropus*	*Nototodarus sloani philippinensis*
Mean body weight ± SE (g)	583 ± 39	289 ± 77	102 ± 29
Temperature (°C)	18	30	30
Mean rate ± SE (μm/g wet wt-hr)	0.267 ± 0.062	1.312 ± 0.164	6.903 ± 1.373
Number of observations, *N*	6	5	3

NOTE: At 34‰.

seawater caused no mortality during the period of observation and did not slow the rate of NaCl loss from the solution. The most probable interpretation of this result is that, unlike annelid and lamellibranch epithelia (Mangum et al. 1978*a*), the siphuncular epithelium is not very permeable to this substance, and it did not enter the bloodstream where it could affect the Na^+ pump sites that are presumably located on the basolateral membranes of the epithelial cells.

The replacement of Na^+ with K^+, which also involved the removal of divalent ions and a decrease in pH, did not prevent the removal of Cl^- from cameral fluid. Although the number of observations is too few to justify a definitive conclusion on the rate of Cl^- loss, it did not clearly differ from the control, viz. slightly less than 10 mM/day for 7–10 days. Perhaps the most notable difference in the treatment of KCl was the pronounced alkalinization of the test solution. Similarly, the replacement of Cl^- with HCO_3^-, and concomitant absence of divalent ions and increase in pH, did not prevent the removal of Na^+ from cameral fluid; the rate also appears to be unchanged. In both sets of experiments, the ions normally present in cameral fluid but absent from the test solutions remained below detectable levels throughout the observation period.

Ammonia Levels and Ammonia Excretion

Even though blood Na^+ is conspicuously low in nautilus, as well as other cephalopods, there is no indication of exceptional levels of other monovalent cations, including ammonia (Table 3). Instead of the anion deficit found in the blood of many vertebrates, there is a cation deficit in *Nautilus pompilius* (Table 3).

In *Octopus macropus* adults the rate of ammonia excretion varies inversely with body size ($r = 0.976$, $P = 0.01$). The slope ($b - 1$) of a logarithmic regression line describing the data for five animals (96–485 g wet wt) is -0.372 ± 0.048 SE. The body size relationship in the other two species is less clear, probably due to the small number of observations on *Nototodarus* and the small weight range of the nautili.

Even considering the difference in experimental temperature, it is clear that the rate of ammonia excretion is very low in *Nautilus pompilius*. Assuming a temperature coefficient of 2, the rate would be only about 0.6 μM/g-hr at 30°C, and the difference may be minimized by the larger body size (Table 4).

DISCUSSION

As shown earlier in *Nautilus macromphalus* (Denton and Gilpin-Brown 1966, Greenwald et al. 1980, Ward et al. 1980), the siphuncular epithelium in *N. pompilius* looks like a site of ion transport. The present results indicate that it also has the metabolic sensitivity to ouabain and the enzymatic features of a site of active Na^+ transport. The presence of Na^+ is not necessary for Cl^- removal, and Cl^- is not necessary for Na^+ removal, suggesting that the coupling between the two processes, if any, is not obligatory.

As suggested earlier (Denton and Gilpin-Brown 1966, Ward et al. 1980), the organi zation of the siphuncular epithelium may be an important adaptation for its ion pumping activity. The parallel arrangement of mito-

chondria-lined canaliculi leading into the drainage channel between epithelial cells of the siphuncle may support a countercurrent mechanism of solute pumping, allowing the establishment of large concentration gradients across the basolateral membrane. Because these basolateral membranes are the presumed site of $Na^+ + K^+$-ATPase pumping activity, and because the direction of Na^+ pumping by animal cells is cytoplasm-to-medium, large Na^+ gradients would be produced within the canaliculi, encouraging the osmotic flow of water from the apical region of the cell (in contact with cameral fluid) to the drainage channel (in contact with the venous circulatory system).

In *Octopus macropus* as well as *Nautilus pompilius*, the metabolic and enzymatic features found in the siphuncle also occur in other organs in which the function of ion transport is unknown. The relatively high $Na^+ + K^+$-ATPase activity in the nautilus kidney might be interpreted as a mechanism to move NH_4^+ from the blood into the urine. However, Potts (1965) concluded that the NH_4^+ in the urine of *O. dofleini* arises in the kidney from precursors in the blood, and that it moves across the kidney cell membrane into the very acid urine by molecular diffusion.

Cephalopods such as the cranchid squids maintain buoyancy in the water column by replacing coelomic fluid Na^+ with the less dense NH_4^+ (Denton and Gilpin-Brown 1973), which reaches levels as high as several hundred millimolar. The metabolic basis of this astonishing phenomenon and its consequences for acid–base balance and respiratory gas exchange have not been investigated. Indeed, few data on nitrogen excretion in cephalopods are available. Nonetheless, it is clear that NH_4^+ plays no role in maintaining buoyancy in nautilus and that the very low rate of excretion is not accompanied by high levels in body fluids.

Unless it is recycled, our data indicate that the net excretion of NH_4^+ into cameral fluid is not involved in Na^+ absorption. The identity of counterions exchanged for Na^+ and Cl^- cannot be defined further from the present results. However, we suggest that the relationship between shell secretion and emptying of cameral fluid warrants further investigation. While the divalent cation activity of cameral fluid is lowered along with NaCl activity and total osmolality, the changes in monovalent and divalent ions proceed at different rates. One possible mechanism of preventing the accumulation of postulated counterions such as H^+, HCO_3^-, and OH^- in cameral fluid would be the utilization of these ions in calcification. Denton and Gilpin-Brown (1973) and Ward et al. (1980) have suggested that while the primary site of shell secretion is believed to be the mantle, the siphuncle also appears to secrete its calcareous tube.

ACKNOWLEDGMENTS

We are grateful to J. M. Arnold for fixing the material and to J. Thomas for preparing the electron micrographs of the siphuncle.

LITERATURE CITED

BONTING, S. L. 1970. Sodium-potassium activated adenosinetriphosphatase and cation transport. Pages 257–363 *in* E. E. Bittar, ed. Membranes and ion transport. Vol. 1. Wiley, London.

BOURNE, G. B., J. R. REDMOND, and K. JOHANSEN. 1978. Some aspects of hemodynamics in *Nautilus pompilius*. J. Exp. Zool. 205:63–70.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.

DENTON, E. J., and J. B. GILPIN-BROWN. 1966. On the buoyancy of the pearly nautilus. J. Mar. Biol. Assoc. U.K. 46:723–760.

———. 1973. Flotation mechanisms in modern cephalopods. Adv. Mar. Biol. 11:197–268.

DIETZ, T. H., and A. M. FINDLEY. 1980. Ion-stimulated ATPase and NaCl uptake in the gills of freshwater mussels. Canad. J. Zool. 58:917–923.

GRAVITZ, N., and L. GLEYE. 1975. A photochemical side reaction that interferes with the phenol hypochlorite assay for ammonia. Limnol. Oceanogr. 20:1015–1017.

GREENWALD, L., P. WARD, and O. E. GREENWALD. 1980. Cameral liquid transport and buoyancy control in the chambered nautilus (*Nautilus macromphalus*). Nature 286: 55–56.

HENDRIX, J. P. 1980. Osmoregulation and salinity tolerance in the bay squid *Lolliguncula brevis* (Blainville). M.S. Thesis, Florida State University, Tallahassee. 25 pp.

MANGUM, C. P. 1981. The influence of inorganic ions and pH on HCO_2 transport systems. Pages 811–822 *in* J. Lamy and J. Lamy, eds. Invertebrate oxygen-binding protein. Structure, active site and function. Marcel Dekker, New York.

MANGUM, C. P., J. A. DYKENS, R. P. HENRY, and G. POLITES. 1978*a*. The excretion of NH_4^+ and its ouabain sensitivity in aquatic annelids and molluscs. J. Exp. Zool. 203: 151–157.

MANGUM, C. P., M. S. HASWELL, K. JOHANSEN, and D. W. TOWLE. 1978*b*. Inorganic ions and pH in the body fluids of Amazon animals. Can. J. Zool. 56:907–916.

POTTS, W. T. W. 1965. Ammonia excretion in *Octopus dofleini*. Comp. Biochem. Physiol. 14:339–355.

PROSSER, C. L. 1973. Comparative animal physiology. Vol. 1. W. B. Saunders, Philadelphia.

SAINTSING, D. G., and D. W. TOWLE. 1978. $Na^+ + K^+$-ATPase in the osmoregulating clam *Rangia cuneata*. J. Exp. Zool. 206: 435–442.

SCHWARTZ, A., J. C. ALLEN, and S. HARIGAYA. 1969. Possible involvement of cardiac Na^+, K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. J. Pharmacol. Exp. Ther. 168:31–41.

SOLORZANO, L. 1969. Determination of ammonia in natural waters by the phenol hypochlorite method. Limnol. Oceanogr. 14:799–801.

TOWLE, D. W. 1981. Role of $Na^+ + K^+$-ATPase in ionic regulation by marine and estuarine animals. Mar. Biol. Lett. 2:107–122.

TOWLE, D. W., M. E. GILMAN, and J. D. HEMPEL. 1977. Rapid modulation of gill $Na^+ + K^+$-dependent ATPase activity during acclimation of the killifish *Fundulus heteroclitus* to salinity change. J. Exp. Zool. 202:179–186.

TOWLE, D. W., G. E. PALMER, and J. L. HARRIS, III. 1976. Role of gill $NA^+ + K^+$-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. J. Exp. Zool. 196:315–321.

WARD, P., and A. W. MARTIN. 1978. On the buoyancy of the pearly nautilus. J. Exp. Zool. 205:5–12.

WARD, P., L. GREENWALD, and O. E. GREENWALD. 1980. The buoyancy of the chambered nautilus. Sci. Amer. 243(4):190–203.