BRANCHIAL Na$^+$/K$^+$-ATPase AND OSMOREGULATION IN THE ISOPOD, *IDOTEA WOSNESENSKII*

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Summary

Pleopod Na$^+$/K$^+$-ATPase enzyme specific activity (ESA) and osmoregulatory performance were measured in isopods acclimated for 12–14 days in 100%, 75%, 50% and 25% sea water (SW). The animal was 63–65 mosmol kg^{-1} hyperosmotic to the medium in 100% and 75% SW, 214 mosmol kg^{-1} hyperosmotic in 50% SW and 239 mosmol kg^{-1} hyperosmotic in 25% SW. The five pairs of pleopods are biramous but are not similar. The two anterior pairs do not stain with silver, have low and unchanging ESA, are smaller and more rigid than the three posterior pairs and are used for swimming. The posterior three pairs are thinner and more flexible; their endopodites stain with silver and have fivefold higher ESA than their exopodites or the front two pleopods. Only the endopodites of the posterior three pleopods showed large (twofold) increases in ESA in animals acclimated in dilute media. The time course of acclimation from 100% to 50% SW was measured: osmotic equilibrium occurred after 1 day; posterior endopodite ESA was elevated after 1 day and was fully activated after 3 days. Ablation of the endopodites of the posterior three pleopods eliminated the animal's ability to hyperosmoregulate in 50% SW; ablation of the exopodites of the same appendages or of both rami of the first two pairs of pleopods had no significant effect on osmoregulation. Thus, the endopodites of the posterior three pleopods of *I. wosnesenskii* are the sites of inward ion transport in dilute media.

Introduction

Although much is known about the processes involved in salt and water balance in the more advanced (eucaridean) crustaceans, less is known about these processes in the lower crustacean groups. The Isopoda and Amphipoda (Peracaridea) are the best studied among the lower crustaceans and their osmoregulatory mechanisms appear to be similar to those of the Eucaridea (reviewed by Mantel & Farmer, 1983). Several genera in both groups have been shown to be excellent hyperosmoregulators in dilute media and adaptive changes in sodium and water fluxes in dilute media have also been reported (Harris, 1970; Percy, 1985; Sutcliffe, 1968; Thuet, 1978, 1981). In both groups acclimation to dilute

media has also been shown to involve increased affinity of the sodium uptake mechanism and reduced sodium effluxes (Croghan & Lockwood, 1968; Harris & Thuet, 1987; Lockwood, Croghan & Sutcliffe, 1976; Sutcliffe, 1968), although this is not a universal phenomenon (Harris, 1972).

The Na^+/K^+-ATPase (i.e. the biochemical manifestation of the activity of the sodium pump) has been strongly implicated in sodium transport by the gills of decapod crustaceans. The posterior gills of several species of osmoregulating crabs have been shown to have high levels of Na^+/K^+-ATPase activity (reviewed by Towle, 1984). Further, in the crab *Callinectes*, enzyme activity is high in certain areas of the gills which are specialized for ion transport and the activity in these areas increases when crabs are acclimated in dilute media (Neufeld, Holliday & Pritchard, 1980). The enzyme has been shown to have a basal–lateral location in the branchial ion transport epithelia of two crabs (Towle & Kays, 1986) and the transport steps for sodium are beginning to be elucidated in membrane vesicles prepared from the osmoregulatory tissue of crab gills (Towle & Hølleland, 1987). Several studies have implicated the gills of isopods and amphipods as the sites of active ion transport into hyperosmoregulating animals in dilute media (reviewed by Mantel & Farmer, 1983). However, little is known of the function of the Na^+/K^+-ATPase in branchial ion transport in lower crustaceans. With the exception of the large body of literature on the hypo-osmoregulating brine shrimp *Artemia* (reviewed by Conte, 1984), the reports of Thuet and his collaborators (Thuet, Thuet & Philippot, 1969; Philippot, Thuet & Thuet, 1972) on the isopod, *Sphaeroma*, are the only available studies which deal with the possible role of the branchial Na^+/K^+-ATPase in ion transport in lower crustaceans. These authors report that Na^+/K^+-ATPase activity in the gills (pleopods) of *Sphaeroma* is highest in the endopodites of pleopods 4 and 5 and that acclimation in dilute media increases the enzyme activity in these appendages.

The purpose of the present study was to investigate the osmoregulatory abilities of the intertidal isopod *Idotea wosnesenskii* and, using the animal's large pleopods, to characterize the branchial Na^+/K^+-ATPase and investigate its role in hyperosmoregulation. Data are presented which strongly implicate the enzyme in ion transport and which show that, as in the decapods, certain areas of the isopod's gills are specialized for ion transport.

Materials and methods

Animals

Male and female *Idotea wosnesenskii* (Brandt), 20–35 mm in length, were collected from intertidal algae (*Fucus* spp.) at North Cove, Cape Arago, OR, in July and August 1985. After capture the animals were maintained in running Coos Bay sea water (32–33‰, 12–14°C) at the Oregon Institute of Marine Biology, Charleston, OR, and fed *Fucus ad libitum* until used in experiments. Animals were also collected in July 1986 and 1987, and shipped by air freight (48 h in transit)

or by car (96 h in transit) to Easton, PA, where they were maintained on *Fucus* in 100 % sea water (100 % SW, Dayno Synthetic Sea Salts, Dayno Manufacturing, Lynn, MA; $1000 \, \text{mosmol kg}^{-1} \, H_2O$) in a filtered, 400-l aquarium at 12–13°C. Mortality in transit for these animals was 10 % or less. Unless otherwise noted, animals were acclimated for 12–14 days in 100 %, 75 %, 50 % or 25 % SW before measurements of haemolymph osmolality and gill Na^+/K^+-ATPase activity were made.

Osmolality measurements

The osmolality of sea water and haemolymph samples was measured with a vapour pressure osmometer (Wescor, Inc., Model 5100 C). Animals were prepared for haemolymph sampling by blotting them dry on paper towelling. Using fine forceps, a hole was made in the dorsal exoskeleton of the head, and haemolymph was taken up by capillarity onto a standard filter paper disk used in the osmotic pressure measurement. When saturated with haemolymph, the disk was immediately placed in the osmometer and processed. Standards were similarly absorbed onto filter paper disks by capillarity and used to standardize the osmometer.

Na^+/K^+-ATPase assay

Animals were killed by decapitation and the uropods were removed to expose the five pairs of pleopods. Pleopods were removed, rinsed in ice-cold homogenizing medium ($0.25 \, \text{mol l}^{-1}$ sucrose, $6 \, \text{mmol l}^{-1}$ EDTA) and blotted dry on filter paper. Homogenates of tissues from individuals were prepared on ice using 20 twisting strokes in a hand-operated, ground-glass homogenizer (Wheaton 'Duall'). Protein concentrations in the final homogenates varied between 0·1 and $0.5 \, \text{mg ml}^{-1}$ and were measured colourimetrically using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as a standard.

The enzyme assay used to measure Na^+/K^+-ATPase activity was identical to that of Holliday (1985). Briefly, phosphate liberated from ATP by each homogenate was measured in two reaction media. One medium had optimum concentrations of all ions ($100 \, \text{mmol l}^{-1}$ Na⁺, $30 \, \text{mmol l}^{-1}$ K⁺, $5 \, \text{mmol l}^{-1}$ ATP, $10 \, \text{mmol l}^{-1}$ MgCl₂, $20 \, \text{mmol l}^{-1}$ imidazole, pH 7·2) whereas the other lacked potassium and contained $1 \, \text{mmol l}^{-1}$ ouabain ($130 \, \text{mmol l}^{-1}$ Na⁺, $5 \, \text{mmol l}^{-1}$ ATP, $10 \, \text{mmol l}^{-1}$ MgCl₂, $20 \, \text{mmol l}^{-1}$ imidazole, $1 \, \text{mmol l}^{-1}$ ouabain, pH 7·2). After incubation at 30°C for 15 min, the reaction was stopped and phosphate was measured colourimetrically as the reduced phosphomolybdate complex. Enzyme activity was calculated as the difference in phosphate liberated by each homogenate in the two media and is expressed as $\mu\text{mol phosphate liberated g}^{-1}\text{h}^{-1}$. All chemicals used for enzyme assays were reagent grade or better.

Silver staining

Pleopods were removed as noted above, grasped firmly across their bases with fine forceps and secured so as to prevent leakage of haemolymph. The pleopods were rinsed in three washes of deionized water and immersed with gentle agitation in 0·5 % $AgNO_3$ for 30 s. The pleopods were again rinsed three times in deionized water and immersed for 30 s in undiluted Kodak D-19 Developer (Eastman Kodak Inc., Rochester, NY). This method identifies areas of the pleopods which have a high permeability to chloride ions; AgCl forms in these areas and the developer (or sunlight) liberates molecular silver, which appears as a black precipitate.

Pleopod ablation

Because the results of the enzyme assays and silver staining strongly implicated the endopodites of the third, fourth and fifth pleopods in ion transport, several combinations of pleopods, their endopodites or their exopodites were ablated and the effects on osmoregulation in 50 % SW were noted. Large (30–35 mm) animals acclimated for 7 days in 100 % SW were secured ventral surface up in a 7 mm i.d. rubber tube under cool sea water. The uropods extended past the end of the tube and the animal was held in position under a rubber band which passed laterally over the anterior ends of the uropods. Using fine forceps, the uropods were hyperextended laterally and were held open by the downward pressure of the rubber band. This arrangement allowed access to the pleopods, which were removed as necessary using fine forceps. Although some bleeding from the torn stumps of the pleopods occurred, mortality was low for 4 days after ablation (0–33 %). Animals were allowed to recover for 24 h in 100 % SW and were then transferred to 50 % SW for 3 days, at which time haemolymph was sampled for osmotic pressure measurements.

The following four combinations of pleopods, endopodites or exopodites were ablated: none (control), pleopods 1 and 2, the exopodites of pleopods 3, 4 and 5, and the endopodites of pleopods 3, 4 and 5. Planimetry using a photograph of the five left pleopods (silver stained) of one animal indicated that pleopods 1 and 2 together contained 33·5 % of the total pleopod surface area, while the exopodites of pleopods 3, 4 and 5 contained 32·9 % and the endopodites of the same pleopods contained 33·5 % of the total pleopod surface area. Thus, the ablations removed nearly equal areas of the pleopods and, presumably, had equal effects on respiratory gas exchange.

Statistics

The results are expressed as mean values ± standard error (S.E.). Student's unpaired t-test was used to evaluate the significance of differences between mean values. A probability (P) value of less than 0·05 was considered to be significant. In the figures, error bars on symbols indicate ±1 S.E.; S.E. values smaller than the size of the symbol are not shown.

Results

Silver staining of pleopods

Isopods have five pairs of flat, biramous gills (pleopods) located on the posterior ventral surface. *Idotea* and other valviferans differ from other isopods in that the pleopods are covered ventrally by the uropods, which are held open when the pleopods are used for swimming. Silver staining was performed on the excised left pleopods (1–5) of five animals in 100 % SW. In all five animals only the endopodites of pleopods 3, 4 and 5 took the silver stain, which was uniformly distributed over the entire endopodite (Fig. 1). This indicates that these append-ages have a high permeability to chloride ion and is an indirect indication of their importance in ion transport.

Enzyme characterization

Fig. 2 illustrates some of the characteristics of the branchial Na$^+$/K$^+$-ATPase in animals acclimated to 100 % SW. Homogenates of pleopods 3, 4 and 5 (both rami) were used for characterization. At a constant Na$^+$ concentration of 100 mmol l^{-1} the enzyme showed typical Michaelis–Menten kinetics for [ATP], [Mg^{2+}] and [K$^+$]; K_m values were 0·9, 2·1 and 4·0 mmol l^{-1}, respectively. At an ATP concentration of 5 mmol l^{-1} the optimum Mg^{2+}:ATP ratio was 2:1. The enzyme had a pH optimum of 7·2 and a K_i of 63 μmol l^{-1} for the specific Na$^+$/K$^+$-ATPase inhibitor ouabain. Based on these data, the optimum reaction medium contained final concentrations of 100 mmol l^{-1} Na$^+$, 30 mmol l^{-1} K$^+$, 5 mmol l^{-1} ATP and 10 mmol l^{-1} Mg^{2+} at a pH of 7·2 (20 mmol l^{-1} imidazole).
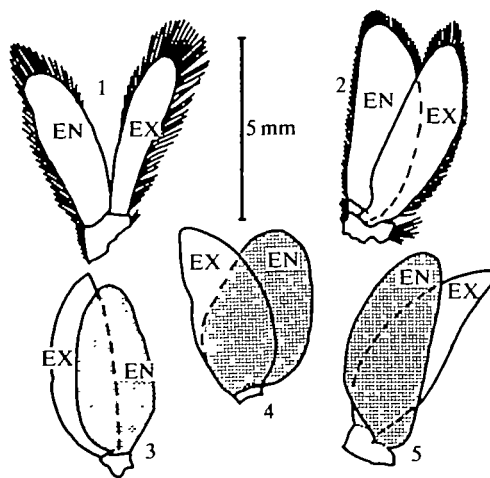

Fig. 1. Diagram of the five left pleopods of *Idotea wosnesenskii*. EN denotes endopodites, EX denotes exopodites and shading denotes areas of pleopods 3, 4 and 5 which took the silver stain.
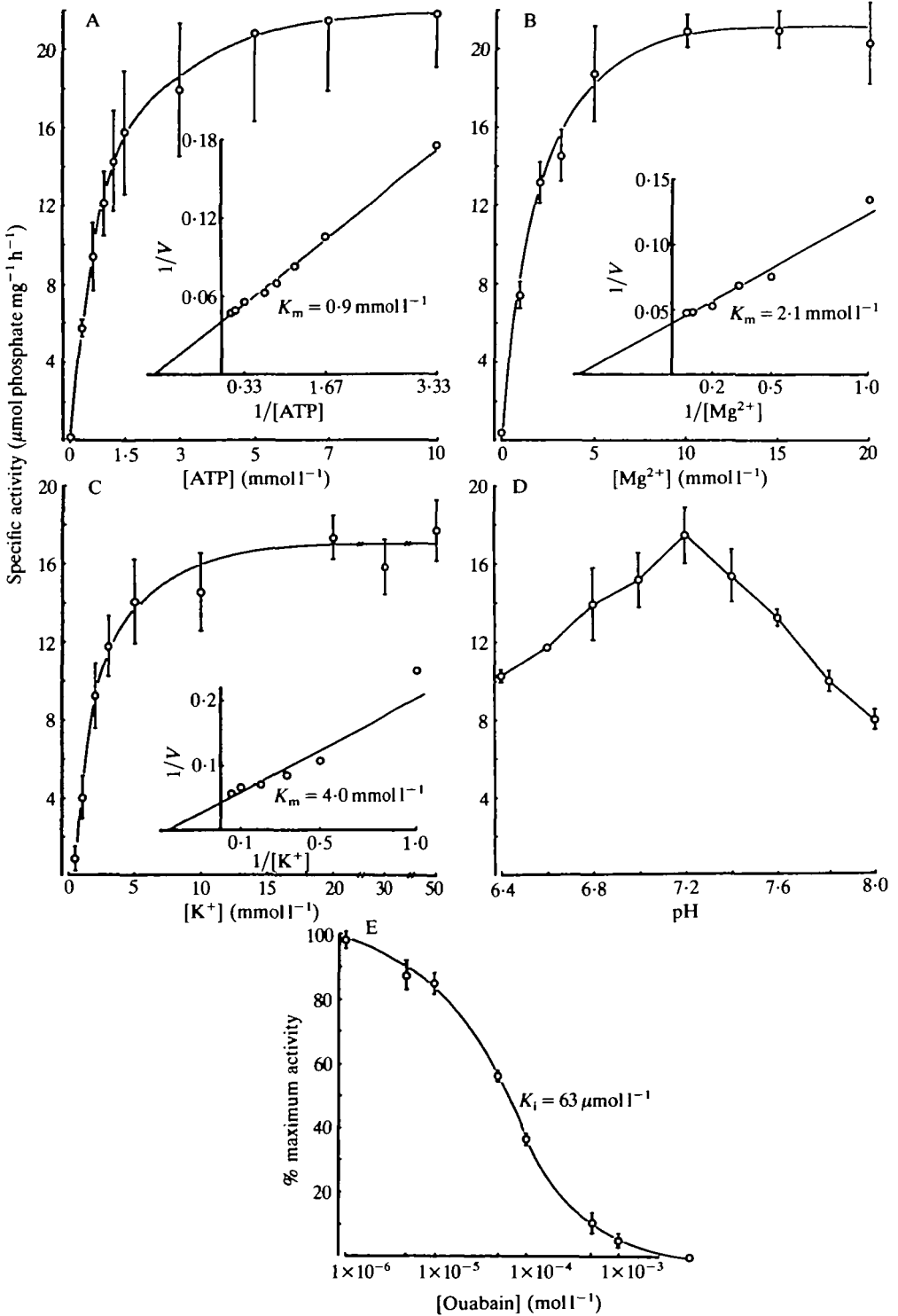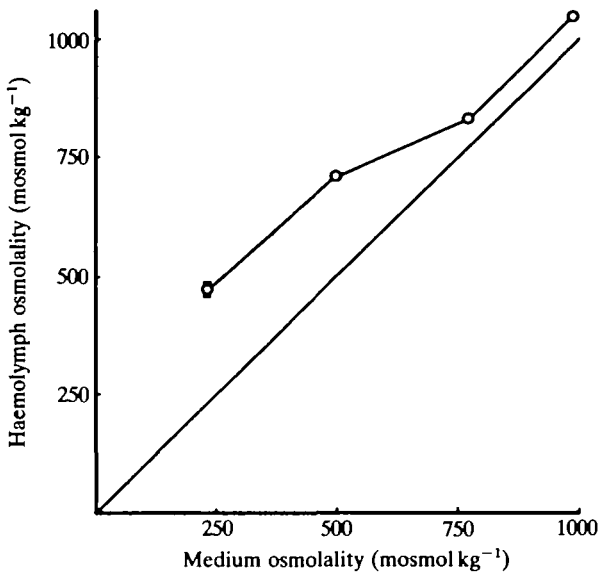
Fig. 2

Fig. 3. Haemolymph osmolality in *Idotea wosnesenskii* acclimated for 12–14 days in various seawater media. $N = 8$–12 animals in each medium.

Osmoregulatory performance

The osmoregulatory performance of isopods acclimated for 12–14 days in 100%, 75%, 50% and 25% SW is shown in Fig. 3. The animals are 63–65 mosmol kg^{-1} hyperosmotic to the media in 100% and 75% SW, 214 mosmol kg^{-1} hyperosmotic in 50% SW and 239 mosmol kg^{-1} hyperosmotic in 25% SW. Thus, *I. wosnesenskii* is a relatively weak osmoregulator. Animals in 25% SW for 12 days suffered higher mortality and were not as active as animals in more concentrated media; 25% SW is probably the lower salinity limit for this species.

Branchial Na$^+$/K$^+$-ATPase activity

Na$^+$/K$^+$-ATPase activities in crude homogenates of various pleopods from animals acclimated for 12–14 days in four SW media are shown in Fig. 4. Pleopods 1 and 2 had low activities which showed no significant changes on acclimation in dilute media. The posterior three pleopods (endopodites and exopodites of pleopods 3, 4 and 5 homogenized together) showed much higher activity which increased steadily on acclimation in dilute media. This salinity-dependent increase in activity was localized in the endopodites of the rear three pleopods; the

Fig. 2. Characterization of *Idotea wosnesenskii* pleopod Na$^+$/K$^+$-ATPase. (A) The effect of ATP concentration on activity; (B) the effect of Mg^{2+} concentration on activity; (C) the effect of K$^+$ concentration on activity at a constant Na$^+$+K$^+$ concentration of 130 mmol l^{-1}; (D) the effect of pH on activity; (E) dose–response curve for ouabain inhibition in the presence of 30 mmol l^{-1} K$^+$. Unless otherwise indicated, conditions were as used in the standard Na$^+$/K$^+$-ATPase assay (see Materials and methods). $N = 3$–4 homogenates from individual animals at each point.
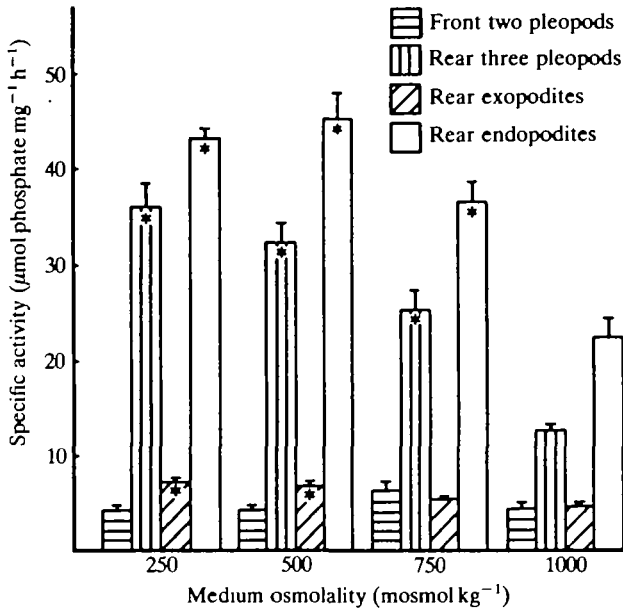
Fig. 4. Na^+/K^+-ATPase enzyme specific activities of pleopods from *Idotea wosnesenskii* acclimated for 12–14 days in various seawater media. Asterisks in histogram bars indicate a significant difference from 100 % seawater controls. $N = 7$–9 animals in each medium.

exopodites showed only slight, although significant, increases in activity in 25 % and 50 % SW. These data strongly implicate the endopodites of pleopods 3, 4 and 5 in osmoregulatory ion transport.

Fig. 5 shows the time courses of changes in pleopod homogenate Na^+/K^+-ATPase activities in the rear three pleopods and in haemolymph osmolality after abrupt transfer of animals from 100 % to 50 % SW. Changes in haemolymph osmolality were essentially complete after 1 day. The rear three pleopods and their endopodites showed significantly increased enzyme activities after 1 day, but the changes were not complete until 3 days after transfer. The three rear exopodites showed no significant changes in enzyme activity after 3 days and the activities were only slightly, but significantly, higher after 12–14 days. Thus, the endopodites of pleopods 3, 4 and 5 are again shown to be important in osmoregulatory ion transport.

Pleopod ablation

The effects of ablation of various combinations of pleopods, their exopodites or their endopodites on the osmoregulatory abilities of animals transferred from 100 % to 50 % SW are shown in Table 1. Ablation of the first two pairs of pleopods or the exopodites of pairs 3, 4 and 5 had no significant effects on mortality, haemolymph osmotic pressure or the osmotic gradient maintained after 3 days in 50 % SW. Ablation of the endopodites of pleopods 3, 4 and 5, however, caused the
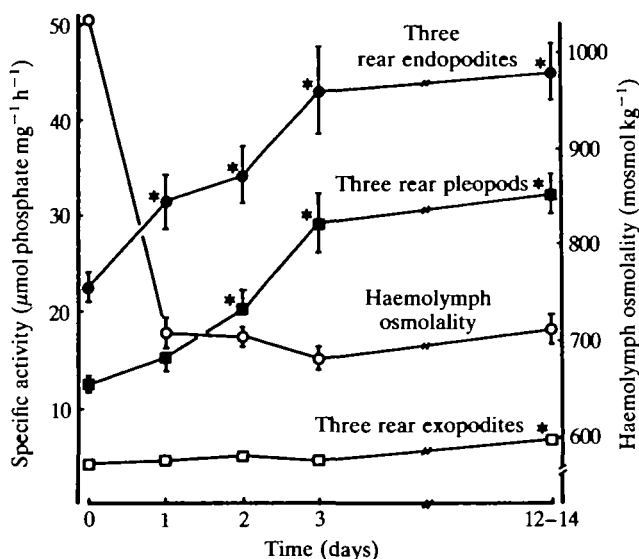
Fig. 5. Time course of changes in pleopod Na$^+$/K$^+$-ATPase activity and haemolymph osmotic pressure in *Idotea wosnesenskii* abruptly transferred from 100% to 50% sea water. Asterisks beside a symbol indicate a significant difference from values at time zero.

Table 1. *The effects of pleopod ablations on the osmoregulatory ability of* Idotea wosnesenskii *3 days after transfer from 100% to 50% sea water*

Treatment	Mortality (%)	Haemolymph osmotic pressure (mosmol kg^{-1})	Osmotic gradient between haemolymph and medium (mosmol kg^{-1})
Control	0	681 ± 13 (6)	169 ± 13 (6)
Pleopods 1 and 2 ablated	0	677 ± 8 (11)	178 ± 8 (11)
Exopodites of pleopods 3, 4 and 5 ablated	0	651 ± 9 (8)	162 ± 10 (8)
Endopodites of pleopods 3, 4 and 5 ablated	33*	539 ± 2 (6)*	41 ± 2 (6)*

Mean values ± s.e. (number of animals in parentheses).
* Significantly different from control values.

animals to be nearly isosmotic with the medium and three of the nine animals died. Because equal pleopod surface areas were ablated in the three experimental groups (approximately 33% in each case, see Materials and methods), the effects of reduced pleopod surface area on respiration were apparently negligible. These data show that the endopodites of pleopods 3, 4 and 5 are the sites of osmoregulatory ion transport.

I. wosnesenskii swims using its pleopods. It is of note that only animals with pleopods 1 and 2 ablated could not swim, indicating the importance of these appendages in locomotion.

The first two pleopods may also serve to ventilate the other pleopods; ablated animals had an average ventilatory rate of 239 ± 11 beats min^{-1} at 12°C, while intact controls averaged 207 ± 2 beats min^{-1} ($N = 6$ in each group, $P < 0.05$). However, this role in ventilation is apparently not a critical one because there was no mortality or impairment of osmoregulation in 50 % SW when pleopods 1 and 2 were ablated (Table 1).

Discussion

Idotea wosnesenskii, like the other species of *Idotea* that have been studied (Todd, 1963; Brusca, 1966), is slightly hyperosmotic to the medium in 100 % and 75 % SW and is a relatively weak osmoregulator in 50 % and 25 % SW (Fig. 3); the latter medium appears to be the lower lethal limit (Brusca, 1966). In contrast, isopods of the genera *Cyathura* (Segal & Burbanck, 1963), *Gnorimosphaeroma* (Riegel, 1959), *Jaera* (Jones, 1972; Forbes, 1974), *Mesidotea* (Lockwood & Croghan, 1957; Percy, 1985) and *Sphaeroma* (Charmantier & Thuet, 1969; Charmantier & Trilles, 1973; Riegel, 1959) can hyperosmoregulate well in very dilute media. Although *I. wosnesenskii* occurs more commonly in the exposed intertidal zone (Menzies, 1950; Ricketts & Calvin, 1968), it also occurs in quieter bay waters where it may experience moderately dilute media (Kozloff, 1974; Rudy & Rudy, 1983). Thus, the relatively weak osmoregulatory abilities of *I. wosnesenskii* are consistent with its distribution in nature.

Using silver staining, several authors have shown that isopod pleopods are very permeable to chloride ions (Babula, 1979; Babula & Bielawski, 1981; Bubel & Jones, 1974; Croghan & Lockwood, 1968; Hrabě, 1949), indicating that they may be the site of ion transport. Some of these authors do not state whether the pleopods stain uniformly with silver or if only certain parts of them stain, as shown in the present study. With one exception, where such information is provided, the appendages which stain are the endopodites of pleopods 3, 4 and 5 (Babula, 1979; Babula & Bielawski, 1981; present study). Hrabě (1949) found that in *Asellus* the endopodites of pleopods 3, 4 and 5 and certain portions of the exopodites of pleopods 2 and 4 stained with silver. Hrabě's data may indicate that a larger surface area is available for ion transport in the pleopods of this freshwater isopod. Babula & Bielawski (1981) have described ultrastructural details typical of crustacean branchial ion transport epithelia in the endopodites of pleopods 3, 4 and 5 of *Mesidotea*; the same appendages of *Asellus* (Babula, 1979) also show these features. The exopodites of the same appendages and pleopods 1 and 2 had much thinner epithelia which did not show these ultrastructural features. Thus, the endopodites of the posterior pleopods of isopods appear to be similar in their silver-staining characteristics and ultrastructure to the patches of ion transport epithelia found in the gills of crabs (Copeland & Fitzjarrell, 1968; Finol &

Croghan, 1983; Barra, Pequeux & Humbert, 1983). It is also interesting that the posterior pleopods of isopods have elevated Na^+/K^+-ATPase activities, as do the posterior gills of several crabs (Towle, 1984).

Na^+/K^+-ATPase is widely accepted to be the driving force for ion transport in crustacean gills (reviewed by Towle, 1984). The results of the present study show that the pleopod Na^+/K^+-ATPase of *I. wosnesenskii* is similar in its ionic requirements to the branchial Na^+/K^+-ATPases of other crustaceans (reviewed by Holliday, 1985). Of particular note is the relatively high K_i for ouabain ($63 \mu moll^{-1}$, Fig. 2), indicating a much lower affinity for the inhibitor than in vertebrate tissues (Neufeld *et al.* 1980).

Thuet *et al.* (1969) found high levels of Na^+/K^+-ATPase in the endopodites of pleopods 4 and 5 in *Sphaeroma*. These authors also reported that Na^+/K^+-ATPase activity increased in pleopods from animals acclimated in 20% SW, indicating a role for the enzyme in osmoregulatory ion transport. However, sodium concentrations used in the assay media were high and were different; pleopods from 100% SW animals were assayed in $500 \, mmoll^{-1}$ sodium, whereas those from 20% SW animals were assayed in $300 \, mmoll^{-1}$ sodium. Philippot *et al.* (1972) characterized the pleopod Na^+/K^+-ATPase of the same species; their results (see their fig. 5) show that the difference in pleopod enzyme activity between the two groups in the 1969 study can be accounted for by the difference in sodium concentrations in the assay media. In the present study a constant assay medium was used and the endopodites of pleopods 3, 4 and 5 clearly showed increased enzyme activity in animals acclimated in dilute media (Fig. 4) and are, thus, likely to be involved in osmoregulatory ion transport. In this regard these appendages are similar to the ion transport patches of *Callinectes* (Copeland & Fitzjarrell, 1968); these patches have high enzyme activity and increase in size on acclimation in dilute media (Neufeld *et al.* 1980; Aldridge & Cameron, 1982).

Although the evidence reviewed above implicates the endopodites of the rear two or three pairs of pleopods in osmoregulatory ion transport by isopods, it does not prove it. This proof is provided by the ablation experiments summarized in Table 1. Endopodites 3, 4 and 5 are necessary for hyperosmoregulation. Ablation of equal pleopod surface areas, either the exopodites of the same appendages or pleopods 1 and 2, had no significant effect on osmoregulatory ability.

It has been suggested that changes in branchial Na^+/K^+-ATPase activity in euryhaline fish may be associated with protective processes of cellular volume regulation in epithelia exposed to osmotic stress, rather than with transepithelial ion transport (Kirschner, 1977; Evans, 1979). This possibility is excluded in *I. wosnesenskii* by the data shown in Figs 4 and 5. Although all the pleopods are presumably subjected to equal osmotic stress in the dilute media, only the endopodites of pleopods 3, 4 and 5 show large increases in enzyme activity in dilute media; the other pleopods show little or no change.

In the present study the time course of changes in posterior endopodite enzyme activity after acute transfer from 100% to 50% SW (Fig. 5) is relatively fast; activities are significantly elevated after 1 day and activation is complete after

3 days. In contrast, the crabs *Callinectes* (Neufeld *et al.* 1980) and two species of *Uca* (Holliday, 1985; D'Orazio & Holliday, 1985) took 7–10 days to activate completely their branchial Na^+/K^+-ATPases in dilute (10–20 % SW) media. These long periods are consistent with the synthesis of new enzyme and proliferation of new ion transport tissue (Copeland & Fitzjarrell, 1968; Aldridge & Cameron, 1982) in the crabs' gills. Other studies (Towle, Palmer & Harris, 1976; Savage & Robinson, 1983) have found rapid (20 min to 1·5 h) increases in branchial Na^+/K^+-ATPase activity in *Callinectes* transferred to dilute media. These rapid changes are consistent with the activation of previously existing but nonfunctional enzyme. In the present study the time course of activation (1–3 days) is such that either synthesis of new enzyme or activation of existing enzyme or both could be occurring. As discussed by Neufeld *et al.* (1980) and Holliday (1985), it seems likely that both processes occur, since osmotic equilibrium in the three species of crabs and in *I. wosnesenskii* is essentially complete in 1 day.

In summary, the endopodites of pleopods 3, 4 and 5 of *I. wosnesenskii* have high Na^+/K^+-ATPase enzyme activity which further increases on acclimation in dilute media in which the animal hyperosmoregulates. As shown by the ablation experiments, these appendages are necessary for hyperosmoregulation and, thus, are the sites of inwardly directed ion transport. In preliminary studies using excised pleopods from *I. wosnesenskii* in 100 % SW, I have found that initial rates of $^{22}Na^+$ influx are nearly twice as high in endopodites as in exopodites of pleopods 3, 4 and 5. Further, initial rates of $^{22}Na^+$ influx in excised endopodites increased in animals acclimated to 50 % SW. Unlike the morphologically complex gills of the decapods, the biramous isopod pleopod provides a unique opportunity for the study of ion transport mechanisms in intact crustacean gills. Such studies are now in progress in this laboratory.

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