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SALT AND WATER BALANCE OF THE SPINY LOBSTER *PANULIRUS ARGUS*: THE ROLE OF THE GUT

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SUMMARY

1. The rate of drinking of sea water averaged $1{\cdot}5 \pm 0{\cdot}6$ ml/kg body weight per 24 h and accounts for only a minor portion of the uptake of water required to balance estimated urine production.

2. Imbibed water and ions, except Ca^{2+}, are absorbed or diffuse across the gut wall into the haemolymph. The gut appears to be a route of net loss of Ca^{2+}, derived from digestive juice and sea water, from the body.

3. The gut does not appear to be a site of regulation of ionic levels in the haemolymph or a major site of water uptake.

INTRODUCTION

Relatively little is known about the role of the gut in ionic regulation and water balance of decapod crustaceans. It has been demonstrated that decapods drink the medium (Fox, 1952; Gross, 1955). It appears that the isomotic *Homarus americanus* completely absorbs the small quantities of medium imbibed (Burger, 1957), and it is suggested also that water is absorbed across the gut wall in hypo-osmoregulators (Dall, 1967).

In only a few cases has the ionic composition of the gut fluid of decapods been analysed (Green *et al.* 1959; Gifford, 1962; Dall, 1967). Most of this work has been done on hypo-osmoregulating forms, although Robertson (1960) gives a partial analysis of the fluid in the proventriculus of the isosmotic shore crab *Carcinus maenas*. The presence of ionic or osmotic gradients between gut fluid and haemolymph has been interpreted as indicative of a salt-excretory role for the gut in hypo-osmoregulators (Green *et al.* 1959; Gifford, 1962) and in the isosmotic decapods *Scylla serrata* (Dall, 1967) and *Homarus americanus* (Dall, 1970), when these latter two species were salt-loaded. In these studies, however, water movements between gut fluid and haemolymph were not monitored. Movements of water across the gut wall may alter gradients of particular ions without those ions having moved.

The present study was undertaken to determine the possible role of the gut in ionic regulation and water balance in an isosmotic marine decapod, the spiny lobster *Panulirus argus* (Latreille). An *in vivo* technique was developed to monitor separately movements of salt and water across the gut wall.

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MATERIALS AND METHODS

Maintenance of lobsters

Panulirus argus were obtained from two geographical areas, Florida and Bermuda, and maintained as described in Malley (1977).

Five additional lobsters used for studies of ion transport in isolated hind gut were obtained from both Florida and Bermuda and similarly maintained in the laboratory except that salinity ranged from 36·3 to 37·1‰ and temperature was 16·0 ± 1·0 °C. These were not fed during the 4- to 8-week period of maintenance.

Collection of body fluids

Procedures for immobilizing lobsters and for sampling haemolymph, urine and the sea water medium are given in Malley (1977). Gut fluids were also sampled from immobilized lobsters. Proventricular fluid (PVF) flowed from the stomach when polyethylene tubing (PE 160) was inserted via the oesophagus. The tubing can be introduced into the oesophagus either in front of or behind the mandibles, but must be manually held in place to prevent severing by the mandibles. Posterior hind-gut fluid (PHGF) was obtained from the rectum and distal hind gut by inserting polyethylene tubing (PE 160) up to several centimetres into the hind gut via the anus.

As for the other body fluids, samples of PVF, taken in duplicate or triplicate, were 20 μl in volume for ion analyses except for SO_4^{2-} for which 100 μl samples were taken. Samples of PHGF were 5–20 μl in volume and were taken in duplicate where the volume permitted.

Analyses

Determinations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and SO_4^{2-} were performed on PVF and PHGF by methods for other body fluids and sea water in Malley (1977). In the determinations of SO_4^{2-} in PVF, absorption at 420 nm due to coloured components in PVF was subtracted from the turbidity produced by $BaCl_2$. Osmoconcentration of physiological saline was measured on a Mechrolab model 301 A vapour pressure osmometer using NaCl solutions as reference standards. Water content of PVF was determined as in Malley (1977) but PHGF was collected in insufficient volume for this analysis.

Polyethylene glycol (MW 6000–7500) was determined by the method of Hyden (1955) with modification of microsamples. Centrifugation replaced the filtration step following protein and SO_4^{2-} precipitation. Turbidity, developed by the addition of trichloroacetic acid/$BaCl_2$ reagent, was read when density was at a maximum at 410 nm in a Coleman Junior Spectrophotometer. Coloured substances in proventricular fluid did not interefere with PEG determinations.

Determination of drinking rate

Lobsters were left undisturbed in 6 l of filtered sea water for one to several hours. Then 1 l of the same sea water but containing polyethylene glycol (PEG, MW 6000–7500) was stirred into the medium to give a final PEG concentration of 14·3 mg/ml in a total volume of 7 l. The period of immersion in the PEG–sea water was from 1 to

5·25 h. Following this, lobsters were rinsed in PEG-free sea water and frozen instantaneously in acetone cooled with dry ice.

For measurement of PEG within the gut, the contents of the entire length of gut, excluding the hepatopancreas, which loses its structure upon thawing, were collected from thawing lobsters. Drinking rate was expressed as the volume of the medium (as ml/g body weight.24 h) that accounts for the amount of PEG in the gut after the immersion period. This experimental method measures both oral and anal drinking.

Polyethylene glycol introduced into the gut does not appear to cross the intestinal wall of a variety of animals (Shehadeh & Gordon, 1969), a conclusion supported here by the lack of appearance of PEG in haemolymph and urine of lobsters, whose guts had been flushed with PEG–sea water, and which had been immersed for up to 24 h in sea water containing 5 mg/ml PEG.

Experiments involving in vivo *replacement of gut fluid*

This technique was developed as a means of distinguishing between changes in the ionic composition of proventricular fluid (PVF) which are due to ion movements between this fluid and the haemolymph and those changes passively occurring because of net water movements between haemolymph and gut fluid. The ionic composition of the proventricular fluid is experimentally altered by replacing it with sea water containing a volume indicator. Changes in ionic composition and volume of the proventricular fluid are followed over time as the fluid composition returns to pre-experimental condition. This technique firstly gives an indication of the fate of sea water which is imbibed by the lobster and secondly indicates some of the fluid exchanges which take place across the gut wall of this species.

At the start of the experiment, haemolymph, and proventricular and posterior hind-gut fluid were sampled for ion analyses.

Guts were flushed with sea water containing 5·0 mg/ml of the volume indicator, polyethylene glycol (MW 6000–7500). This fluid was gravity-fed into the proventriculus through polyethylene tubing (PE 200) inserted via the mouth and oesophagus of the immobilized lobster. To prevent rupturing, most of the fluid entering the proventriculus did not pass through to the anus, but was allowed to flow instead from a second piece of PE tubing inserted into the proventriculus through the mouth and oesophagus. Flushing continued for 30–45 min during which time about 75–150 ml sea water passed through the proventriculus, which was estimated to be 15–20 ml in volume. By the end of the period of flushing, fluid coming from the mouth was colourless. In all lobsters some colourless PEG–sea water passed out of the anus, though the quantity varied with the animal. Proventricular fluid and PHGF were sampled at the end of the period of gut fluid replacement. Because of the difficulty of ensuring an effective seal, mouth and anus were not blocked. Lobsters were then placed in 16 l (6 l in one case) of sea water containing 5 mg PEG/ml, with aeration. Time at which they were immersed was designated time 0 (t_0). Proventricular fluid, PHGF and the medium were sampled periodically over the next 16–23 h. Haemolymph was sampled again at the end of the experiment.

Since the lobsters were immersed in medium identical to that used to flush the gut, drinking that may have occurred during the immersion period tends to keep concentrations of PEG and ions in the PVF at or close to t_0 values. Although drinking

would slightly retard changes in PVF composition, the advantage of the technique is that deviations in PVF composition from t_0 values that do occur are attributable solely to movements of water and ions across the gut wall and not to drinking.

Preliminary experiments on ion movements across the isolated hind gut

The abdomen was severed from the cephalothorax. The abdominal portion of the hind gut, about three-quarters of its total length, was excised minus the rectum. Contents of the excised hind gut, called hind-gut fluid, were expelled by a stream of air and later analysed for ions. This fluid is to be distinguished from posterior hind-gut fluid, which was sampled from the rectum and the posterior 2–3 cm of the hind gut. The hind gut was thoroughly flushed with physiological saline. At time 0 (t_0) the two ends of the hind gut were tied with surgical thread to form a closed bag containing physiological saline. This bag was incubated for several hours in identical, aerated, saline. Dissection and incubation were performed at room temperature (22·0–25·0 °C) except for one experiment where temperature was 15·5–17·0 °C. After 2·25–4 h of incubation, the internal, or luminal, fluid was emptied from the hind gut bag and analysed for ions. The gut was flushed again with fresh saline, re-incubated and re-sampled once or twice more. In several experiments the luminal saline contained polyethylene glycol at 2 mg/ml, so that volume changes of the preparation could be measured. In initial experiments, physiological saline was based on figures given in Prosser & Brown (1961, p. 60, table 5) and was 545·2 mM-Na^+, 10·3 mM-K^+, 13·4 mM-Ca^{2+}, 16·6 mM-Mg^{2+}, 574·4 mM-Cl^- and 20·5 mM-SO_4^{2-}. This fluid was buffered with 8·84 mM borate buffer (H_3BO_3 and NaOH). Anhydrous dextrose (12 mg%) was also added. Later the composition of the saline was based on analysis of haemolymph from *P. argus* and was as follows: 503·7 mM-Na^+, 9·9 mM-K^+, 16·4 mM-Ca^{2+}, 21·1 mM-Mg^{2+}, 549·5 mM-Cl^-, 19·3 mM-SO_4^{2-}. It was buffered and contained dextrose as above. The pH of these salines varied from 7·46 to 7·50. Hind guts immersed in these salines contracted peristaltically throughout the experimental period.

RESULTS

Ionic composition of proventricular fluid

Proventricular fluid (PVF) was obtained from nearly all lobsters sampled. It varied in colour from yellow-green to very dark brown; in viscosity from watery to fairly viscous. Typically it was transparent, though small amounts of solid, flocculent material were occasionally present. The colour indicates the presence of digestive juice secreted by the hepatopancreas.

Ionic analysis of PVF from nine Bermuda lobsters sampled within 1–3 days of their collection from the field gave the following results (mean mM concentrations ± S.E.) 440 ± 7 Na^+, 10·8 ± 1·1 K^+, 16·2 ± 0·9 Ca^{2+}, 24·2 ± 2·5 Mg^{2+}, 461 ± 9 Cl^- and 10·4 ± 2·4 SO_4^{2-}. The number of determinations was nine except for SO_4^{2-} where N was five. The average of seven determinations from these nine lobsters of water content of PVF as % of wet weight was 86·3 ± 2·2. These values can be compared with analyses from the same nine individuals for haemolymph, urine and the sea water in which the animals were maintained by referring to Malley (1977, Table 1). Composition of sea water, PVF and haemolymph is compared in Fig. 1. This shows the

Table 1. *Ionic concentrations (molal) in proventricular fluid (PVF) compared with those in haemolymph and sea water for* Panulirus argus

Comparison	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}
PVF (% haemolymph)	95·0 ± 0·7	92·0 ± 5·9	122·9 ± 3·7	207·3 ± 18·5	98·7 ± 1·2	23·7 ± 14·7
N	18	19	19	19	19	8
P	< 0·01	> 0·1	< 0·01	< 0·01	> 0·2	< 0·01
PVF (% sea water)	104·1 ± 0·9	98·2 ± 6·6	156·3 ± 5·0	49·4 ± 3·8	94·6 ± 1·3	11·7 ± 6·8
N	19	19	19	19	19	9
P	< 0·01	> 0·5	< 0·01	< 0·01	< 0·01	< 0·01

Values are means ± S.E. of *N* ratios. *N* = number of determinations from 9 Bermuda and 14 Florida lobsters sampled under both field and laboratory maintenance conditions. *P* = probability that the mean is equal to 100 using Student's *t* test.

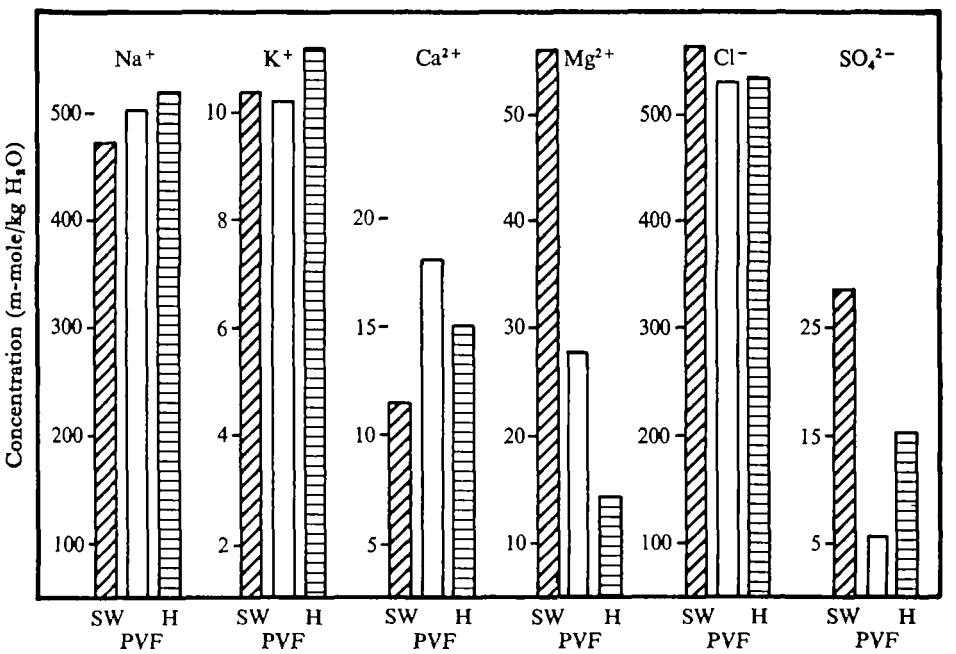

Fig. 1. Histogram of ionic concentrations (millimolal) in sea water (SW), proventricular fluid (PVF) and haemolymph (H) of *Panulirus argus*. Values are means of *N* determinations from nine Bermuda and six Florida lobsters sampled under both field and laboratory maintenance conditions. *N* = 20 for haemolymph ions, 19 for PVF ions, and 25 for sea-water ions, except for SO_4^{2-}, where *N* = 11 for haemolymph, 10 for PVF and 19 for sea water.

relative molal ionic compositions of these three fluids averaged over all determinations on Florida and Bermuda lobsters. Concentrations of Na^+ and Mg^{2+} in PVF are intermediate between respective levels in haemolymph and sea water (Table 1). Proventricular levels of Ca^{2+} exceed those in both the haemolymph and sea water. Concentrations of Cl^- in PVF are less than in sea water but statistically the same as those in haemolymph. Puzzling are the low concentrations of SO_4^{2-} in PVF; these are well

Table 2. *Comparisons of ionic concentrations (molar) in hind gut fluid with those in haemolymph and sea water for* Panulirus argus

Comparison	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-
Hind gut fluid (% haemolymph)	102·4 ± 4·8	123·7 ± 13·8	97·1 ± 6·6	232·7 ± 35·0	105·7 ± 4·5
N	4	4	4	3	4
Hind gut fluid (% sea water)	106·4 ± 2·6	117·2 ± 9·0	139·6 ± 1·4	47·5 ± 6·5	93·1 ± 1·2
N	4	4	4	4	4

Values are means ± S.E. of *N* ratios. *N* = number of determinations from four lobsters.

below levels of this ion in both haemolymph and sea water. In PVF from five of nine lobsters sampled, no SO_4^{2-} at all could be detected by the method used. The other four PVF samples contained average concentrations of 12·76, 12·28, 2·07 and 0·51 millimolal SO_4^{2-}. Haemolymph, PVF and sea water all have similar K^+ levels (Table 1). The striking features of PVF are thus its high Ca^{2+} levels (123% of haemolymph) and low SO_4^{2-} (24% of haemolymph). Magnesium is intermediate in concentration between sea water and haemolymph and markedly different from both.

None of these ionic gradients suggest that the proventriculus is a site of the active excretion of Mg^{2+} or SO_4^{2-}, or of the active uptake of Na^+, Ca^{+2} and Cl^-, which are required for the maintenance of the haemolymph/sea-water ionic gradients exhibited by this species (Malley, 1977).

Ionic composition of hind-gut fluids

Fluid from the abdominal portion of the four hind guts used in the *in vitro* hind-gut experiments was analysed for ions and compared with haemolymph from the same lobsters and with the sea water in which the animals were living. The hind-gut fluid differed principally from haemolymph in having higher Mg^{2+} concentrations. Relative to sea water, the hind-gut fluid contained more Ca^{2+}, less Mg^{2+} and slightly less Cl^- (Table 2).

Posterior hind-gut fluid (PHGF), from the rectum and last 1–3 cm of the hind gut, was obtained in sufficient quantities for analysis from about three-quarters of all animals sampled. In appearance PHGF was more variable than PVF. It was usually less highly coloured than PVF of the same animal and viscosity ranged from a watery to a thick mucus-like consistency. Occasionally PHGF samples contained small amounts of particulate or flocculent solids which were not removed to avoid the possibility of evaporation during handling. Only one PHGF sample was of sufficient volume to permit SO_4^{2-} analysis.

Ionic composition of PHGF from eight lobsters sampled shortly after collection from the field in Bermuda in mean concentrations, mM ± S.E. followed by the number of determinations in parentheses is 477 ± 16 Na^+ (7), 11·6 ± 0·5 K^+ (8), 13·5 ± 1·6 Ca^{2+} (8), 53·2 ± 2·2 Mg^{2+} (8), 559 ± 16 Cl^- (8) and 33·7 SO_4^{2-} (1). In ionic composition, PHGF more closely resembles sea water than it does haemolymph, except that concentrations of all ions exceed those in the sea water (Table 3). This is most pronounced for Ca^{2+} (22% higher). Although these Ca^{2+} levels in PHGF are approximately equal to haemolymph levels on a molar basis, they may be higher on a molal basis depending

Table 3. *Comparisons of ionic concentrations (molar) in posterior hind-gut fluid (PHGF) with those in haemolymph, proventricular fluid (PVF) and sea water for* Panulirus argus

Comparison	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^{2-}
PHGF (% haemolymph)	99·8 ± 2·0	106·7 ± 3·8	96·3 ± 5·1	440·3 ± 22·8	114·0 ± 2·3	185·5
N	18	19	19	20	20	1
P	> 0·5	> 0·05	> 0·4	< 0·01	< 0·01	–
PHGF (% sea water)	104·9 ± 2·0	110·3 ± 3·6	122·0 ± 6·5	101·3 ± 8·4	104·7 ± 2·2	118·1
N	19	19	19	20	20	1
P	< 0·05	< 0·02	< 0·01	> 0·5	< 0·05	–
PHGF (% PVF)	112·2 ± 2·8	131·3 ± 8·3	89·9 ± 6·3	262·5 ± 22·3	124·0 ± 3·1	371.9
N	13	13	13	13	14	1
P	< 0·01	< 0·01	> 0·05	< 0·01	< 0·01	–

Values are means ± S.E. of *N* ratios. *N* = number of determinations from nine Bermuda and eight Florida lobsters sampled under both field and laboratory maintenance conditions. *P* = probability that the mean is equal to 100 using Student's *t* test.

upon the relative water contents of the two fluids. Unlike PVF, PHGF on the basis of one sample appears to contain substantial SO_4^{2-}.

These data provide no direct evidence of a role of the hind-gut in ionic regulation except perhaps for the posterior hind gut, the fluid of which contained Cl^- in concentrations greater than in either haemolymph or sea water. The data suggest that anal drinking occurs since the PHGF resembles sea water, particularly with regard to Mg^{2+} and SO_4^{2-}. The high Ca^{2+} concentration is probably due to passage of PVF fluid along the gut mixing with the anally imbibed water. Some water resorption may occur in the rectum for faecal pellet formation, and the elevated Cl^- levels may occur incidentally. There is no evidence here for specific secretion of Cl^- into the hind gut for the purpose of ion regulation.

Drinking rates

Rate of drinking of the medium was measured in three lobsters weighing 338, 370 and 382 g and with carapace lengths of 7·5–7·9 cm. By assessing the amount of polyethylene glycol (PEG) in the gut after the lobsters imbibed medium with known concentrations of PEG during the experimental period, these lobsters appeared to have drunk the medium at the rates of 1·40, 1·53 and 1·62 ml/kg body weight.24 h, respectively. Mean ± S.E. is 1·52 ± 0·62. Fluid contents of the hepatopancreas were not included in the PEG measurement.

In vivo *replacement of gut fluid*

The experiment was performed on four lobsters of both sexes (carapace length, 7·8–10·6 cm; weight, 417–905 g). The proventriculus was thoroughly flushed by the gut fluid replacement technique. PVF concentrations at t_0 averaged, as % of those in PEG–sea water: Na^+ 99·6%, K^+ 100·5%, Ca^{2+} 99·3%, Mg^{2+} 97·8%, Cl^- 100·0%, SO_4^{2-} 104·7%, PEG 100·2%. The technique did not completely flush the gut along its length since PHGF at t_0 contained 0·66, 2·37, 3·08 and 4·19 mg PEG/ml in the four animals compared with 5·0 mg PEG/ml in the replacement fluid.

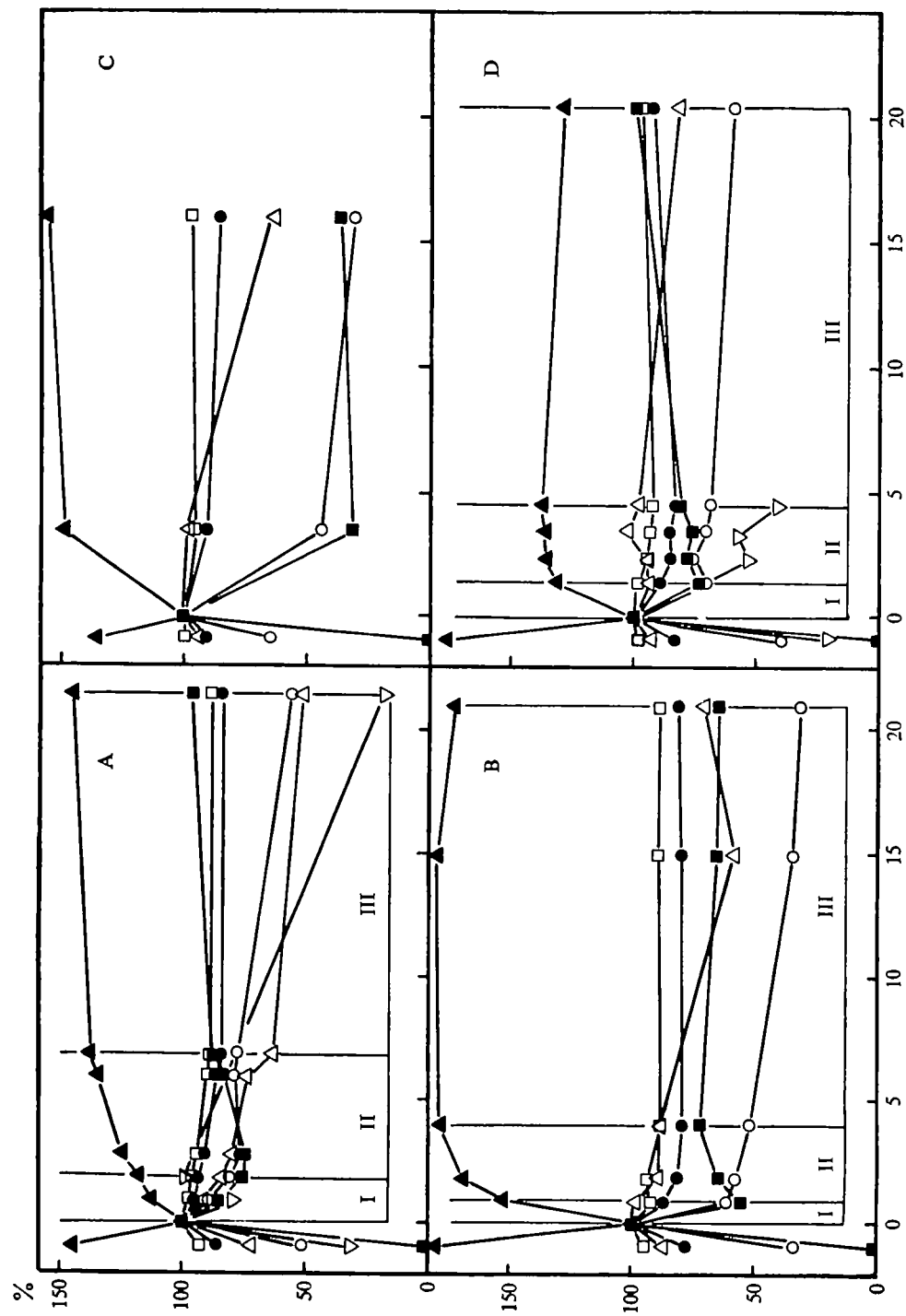

Fig. 2. Changes with time in concentrations of six ions (molar) and PEG (mg/ml) in proventricular fluid as percentage of their respective concentrations at t_0 following replacement of the gut fluid with PEG-sea water. Values to the left of t_0 are pre-experimental concentrations. Periods labelled I, II and III are defined in the text. A, B, C and D are four lobsters. ■, PEG; □, Na^+; △, K^+; ▲, Ca^{2+}; ○, Mg^{2+}; ●, Cl^-; ▽, SO_4^{2-}.

Filling the proventriculus with PEG–sea water caused the concentrations of all ions in the PVF to deviate from the pre-experimental values, shown in Fig. 2 to the left of time 0; the deviations were greatest for Ca^{2+}, Mg^{2+} and SO_4^{2-}. Overall, the ion concentrations in PVF returned to approximately pre-experimental values by five hours following flushing.

Initially after t_0, PEG concentrations in PVF dropped to between 33 and 73% ($\bar{X}$ = 58·4%) of their concentration at t_0 (Fig. 2, period I). Thereafter in A, C and D, PEG concentrations increased, more rapidly at first (Fig. 2, period II) and more slowly during the remainder of the experiment (period III). Decrease in PEG concentration (period I) indicates the movement of PEG-free fluid into the proventriculus. The PVF sampled at t_0 was colourless, whereas at the next sampling it was considerably coloured, indicating that period I is dominated by the secretion of digestive juice. Two processes, drinking and absorption of fluid from the gut, could contribute to the increase in PEG concentration in PVF during period II. Drinking alone, at the rates given earlier, could account for only about 8% of the observed increase. Therefore, there must predominately be a net uptake of water from the gut to the haemolymph during this period and a corresponding decrease in the volume of PVF. During period III, the concentration of PEG in PVF slowly increased or levelled off.

None of the sequences of changes in ionic concentrations in PVF can be explained by water movements alone (Fig. 3). There are net movements of all ions into and/or out of gut lumen during the experimental period. During period I (Fig. 3), concentrations of all ions, except SO_4^{2-} and Mg^{2+} in lobster D, were above values accounted for by water movements alone; Ca^{2+} was particularly elevated. Therefore, the digestive juice which is secreted in period I appears to be a NaCl solution hypoionic to sea water and containing K^+ in concentrations less than in sea water, very little Mg^{2+} and SO_4^{2-}, and levels of Ca^{2+} considerably above those in sea water. Lobster B (Figs. 2, 3) was probably moribund during the experiment since it died 2 days later. Evidently, after flushing of the gut, it secreted a large amount of digestive juice. Following this, there was little drinking or absorption of fluid from the gut. The data from this animal support the conclusions about the composition of digestive juice. Generally during period II, concentrations of all ions, except Ca^{2+}, fell, both absolutely (Fig. 2) and relative to PEG levels (Fig. 3). Only those of Ca^{2+} approximately paralleled changes in PEG concentrations. Thus, it appears that during Period II, Na^+, K^+, Mg^{2+}, Cl^- and SO_4^{2-} moved out of the PVF presumably into the haemolymph. The movements of Na^+ and K^+ are against concentration gradients. Chloride also may move against concentration gradients since terminal PVF Cl^- concentration in lobster C was 93·5% of haemolymph on a molal basis. Changes in PVF Cl^- closely paralleled those in Na^+, although relative to their t_0 concentrations, Cl^- values were consistently lower than those of Na^+. Magnesium, and SO_4^{2-} initially, move into the haemolymph down sharp concentration gradients. But SO_4^{2-} in the PVF falls to extremely low levels, much below those in either haemolymph or sea water. In the absence of data on electrical potential differences between haemolymph and PVF it is not possible to distinguish between the possibilities that individual ions are being actively transported as opposed to diffusing down electrochemical gradients. The data suggest that the gut is relatively impermeable to the Ca^{2+} secreted in the digestive juice or alternatively that the ion is bound.

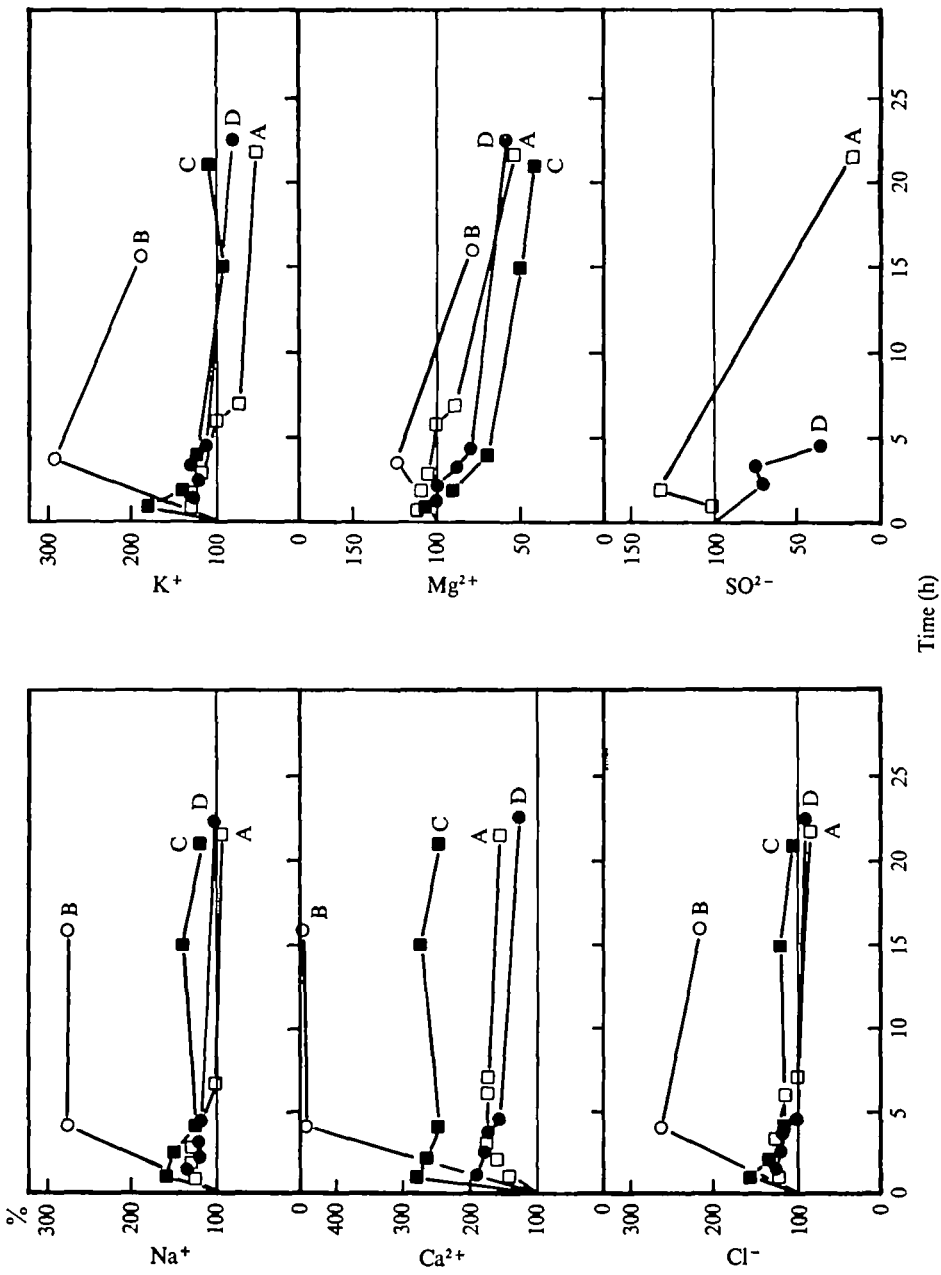

Fig. 3. Changes with time in concentrations (molar) of six ions in proventricular fluid as % of the changes in PEG concentration (mg/ml). Values of each ion at t_0 set at 100 %. A, B, C and D are the four lobsters shown in Fig. 2.

Table 4. *Differences in osmoconcentration and in concentrations (molar) of ions between luminal and bathing fluid of isolated hind gut preparations from* Panulirus argus, *expressed as % of t_0 values per hour of incubation*

Ion or osmoconcentration	N	% change/h ($\bar{X} \pm$ S.E.)	P
Na^+	9	$-0{\cdot}68 \pm 0{\cdot}15$	$< 0{\cdot}01$
K^+	9	$0{\cdot}97 \pm 0{\cdot}56$	$> 0{\cdot}5$
Ca^{2+}	9	$6{\cdot}31 \pm 1{\cdot}56$	$< 0{\cdot}01$
Mg^{2+}	9	$2{\cdot}15 \pm 0{\cdot}64$	$< 0{\cdot}02$
Cl^-	9	$-0{\cdot}09 \pm 0{\cdot}13$	$> 0{\cdot}5$
SO_4^{2-}	8	$0{\cdot}66 \pm 4{\cdot}02$	$> 0{\cdot}5$
Osmoconcentration	5	$4{\cdot}47 \pm 1{\cdot}34$	$< 0{\cdot}05$

N = number of samplings, each at the end of an incubation period, from five hind gut preparations. P = probability that the mean % change/h equals zero using Student's t test.

Movements across the isolated hind gut

Ion transport by the hind gut was studied in *in vitro* preparations from five lobsters including both sexes obtained from Florida (carapace length, 9·8–13·0 cm) and from Bermuda (carapace length, 24·0 and 27·7 cm; weight 596 and 843 g).

At time 0, luminal and bathing solutions were identical physiological saline. The preparations were sampled at the end of one or two consecutive incubation intervals of 2·25–4·0 h for a total of nine samplings. Mean concentration changes per hour were calculated for each ion from the difference between luminal and bathing fluids at the end of the incubation period and were expressed as % of t_0 concentrations per hour of incubation. Sodium concentration in the luminal fluid decreased, relative to that in the bathing medium, in a statistically significant manner with time; concentrations of Ca^{2+} and Mg^{2+} increased significantly and K^+, Cl^-, and SO_4^{2-} concentrations did not change (Table 4). The changes in Na^+ concentration, although statistically significant, may be the result of slight evaporation of the bathing medium rather than a biologically significant process. Changes in Cl^- concentration tend to parallel those in Na^+. Osmoconcentration of the luminal fluid tended to increase during the incubation period (Table 4).

In the third and fourth incubation periods of one preparation, PEG concentration in the luminal saline decreased 2·24 and 3·66%/h, respectively. The PEG-free bathing medium contained no detectable PEG at the end of each incubation period. This hind gut, however, showed no increase in Ca^{2+} concentration in the lumen during the second incubation period, so ion transport may have ceased in this preparation. In a second preparation, PEG was added to both luminal and bathing fluids at the same concentration during the first two incubation periods. In the luminal fluid concentrations of PEG increased 3·98 and 0·84%/h, and of Ca^{2+}, 7·28 and 5·23%/h, respectively, during the two periods.

Therefore, *in vitro* preparations of hind gut show an increase with time of Ca^{2+}, Mg^{2+} and osmoconcentration, and a small decrease in Na^+ and Cl^- in the luminal fluid. There is the suggestion also that the luminal fluid decreases in volume with time but this observation requires further confirmation.

DISCUSSION

Water balance

Since *Panulirus* is essentially isosmotic with sea water, fluid lost as urine must be replaced by the production of metabolic water or by the uptake of environmental water in the absence of a favourable osmotic gradient. Although the rate of urine production has apparently not been reported for *Panulirus* (Sims, 1966), it is assumed to be within the range of other stenohaline, isosmotic, marine decapods, i.e. 25–100 ml/kg body weight 24 h (Potts & Parry, 1963). *Panulirus argus* was found to drink at the rate of 1·5 ml/kg body weight.24 h. An approximate calculation of the rate of production of metabolic water is based on a Q_{O_2} of 1556 ml O_2/kg body weight.24 h (Jolyet & Regnard, 1877, in Wolvekamp & Waterman, 1960) for the related *Palinurus elephas* and the relationship that 1 ml O_2 consumed results in the production of 0·67 mg H_2O (derived from figures in Hoar, 1966, p. 365, and Prosser & Brown, 1961, p. 160, and assuming a nutritionally mixed diet). This estimate is 1 g water/kg body weight.24 h. Clearly, drinking and the production of metabolic water which provide about 2·5 ml/kg body weight.24 h cannot supply sufficient water to balance estimated urine flow even if all imbibed sea water is absorbed.

The drinking rate of *P. argus* is lower than that of other decapods. Hypo-osmoregulating *Metapenaeus* drinks at a rate of 144–168 ml/kg body weight.24 h (Dall, 1967). The drinking rate in this shrimp is significantly reduced to about one-third of these values when it is in a solution isosmotic with haemolymph. Isosmotic *Homarus americanus* has a drinking rate 10 times that of *P. argus* (Malley, unpublished results).

Therefore, the gut in *P. argus* is a minor site of the uptake of water from the aquatic environment. It is reasonable to suggest that most of the water uptake which balances urine production occurs through the gills. This is the situation in non-moulting *Carcinus maenas* (Webb, 1940), but uptake of the large amount of water required for moulting in that species takes place through the foregut and hepatopancreas (Robertson, 1960). Water absorption by several terrestrial crabs occurs via the gills (Bliss, 1968) and in *Gecarcinus* the microanatomical basis of this water uptake has been described (Copeland, 1968). The low rate of drinking in non-moulting *P. argus* is correlated with a small degree of dependence upon the gut as a source of water during moulting. In this species water for moulting is taken up across the body surface. No fluid is present in the gut at moult, instead, expansion of the stomach at that time is brought about by gas (Travis, 1954).

Movements of water and ions across the gut wall

Sea water is potentially a net source of water and ions to *P. argus* by passage across the gut wall. Another source of fluid movement into the gut, presumably ultimately from the haemolymph, is digestive juice secreted by the hepatopancreas into the proventriculus. Results reported here suggest that the digestive juice is a NaCl solution hypo-ionic to sea water, with elevated Ca^{2+} levels and with K^+ less concentrated than in sea water. It contains little if any Mg^{2+} and SO_4^{2-}. Proventricular fluid is thus derived from imbibed sea water and the secreted digestive juice as modified by the movement of water and ions out of the gut lumen. The gut wall appears to be largely impermeable to the movement of Ca^{2+} out of the lumen, or

alternatively most of the Ca^{2+} is bound. The Ca^{2+} of the digestive juice and sea water are concentrated in the gut fluid by the withdrawal of water. These results do not indicate whether water and ions are moved into the gut by other means in addition to the secretion of digestive juice and drinking of the medium.

Although it is possible that part of the movement of water from the gut to the haemolymph may be osmotic, at times it is moving in the absence of a favourable osmotic gradient. The concentration of PEG in PVF of lobsters A and D at the end of the gut fluid replacement experiment exceeded, by 104 and 107%, respectively (Fig. 2), the t_0 PEG values calculated on a molal basis, assuming that this terminal PVF had the same water content as pre-experimental PVF. This indicates that there was a small net movement of water across the proventicular wall during the experiment. At t_0 the haemolymph and gut fluid replacement fluid are isosmotic, therefore this net movement of water must be non-osmotic. Water movement in the absence of a favourable osmotic gradient is invariably associated with active transport of solutes or with mechanical pumps (Gordon *et al.* 1968). It is assumed that in *P. argus* this non-osmotic movement of water is linked to the active transport of salt, but it is not clear which ions are responsible for the movement. It is probable that either Na^+ or Cl^- is actively transported and accompanied by the oppositely charged ion for electrical balance. Their movement out of the gut is correlated with that of water (period II, Fig. 2), and in lobster B, where the gut resorbs little or no water, there is no movement of Na^+ and Cl^-. But this may not be the entire explanation. One would expect that the molal NaCl concentration in PVF would be less than or equal to that in sea water, were isotonic water transport taking place from the imbibed sea water. Although Cl^- in PVF is hypo-ionic to sea water, Na^+ is hyperionic. Evidently, then, water and Na^+ are not transported out of the lumen isotonically, although possibly active Cl^- transport, if it is occurring, may be associated with the water uptake.

The preliminary experiments on active transport in isolated hind gut preparations are consistent with the pattern of water and ion movement seen in the gut fluid replacement experiment. While the simplest hypothesis to explain the results (Table 4) is active transport of Ca^{2+} and Mg^{2+} into the hind gut, it is more plausible that Na^+, K^+, Cl^- and possibly SO_4^{2-} move out of the hind gut, accompanied by water, and that Ca^{2+} and Mg^{2+} are passively concentrated within. Whether the movement of individual ions is concluded to be passive or active depends on knowledge of the electrical potential gradients across the hind gut. But it is clear that active transport of ion(s) is occurring to alter the initially identical conditions on the two sides. Magnesium ion may be less concentrated within the isolated hind gut than Ca^{2+} because it is diffusing out whereas the hind gut, like the proventriculus, may be relatively impermeable to Ca^{2+} resulting in less of an elevation of Mg^{2+} within the hind gut compared with Ca^{2+}. Polyethylene glycol became more concentrated within the hind gut in one experiment, apparently indicating movement of water out of the hind gut consistent with the above hypothesis.

The gut in salt balance

If the gut is a site of active ion uptake or secretion, ion levels in gut fluids should be below those in sea water for the ions hyperionically regulated in the haemolymph, and vice versa. Further, it must be demonstrated that these gradients have not arisen by

water movements without movement of the particular ions in question. Thirdly, the gut fluid must be shown to exchange with the outside medium. In no case do the data (Fig. 1, Tables 2, 4 and 5) suggest such a role in ion regulation for the proventriculus or the posterior hind gut, except possibly for Cl^- by the hind gut. The concentrations of Cl^- in PHGF above those in sea water and haemolymph may be due to the absorption of water by the rectum in faecal pellet formation rather than secretion of Cl^-.

It is concluded that in the water and salt economy of *P. argus*, the gut is a site of uptake against concentration gradients of water, Na^+, K^+, Cl^- and SO_4^{2-}, each species, except water, itself presumably actively transported or linked to the movement of another ion. The gut appears to be a site of diffusion of Mg^{2+} into the body. Because the rate of drinking is low, it is concluded that the gut is a minor source of water and salts to the non-feeding lobster. Rather, these activities of the gut may be primarily important in absorbing water and salts from food and resorbing water and salts from the secreted digestive juice. Calcium is apparently not taken up from the gut and therefore the gut represents a route of net loss of Ca^{2+} from the body, particularly from the digestive juice.

In a study of osmotic and ionic regulation in *Panulirus longipes* published subsequently to the time the present work on *P. argus* was carried out, Dall (1974) reaches the same conclusion that the gut in *P. longipes* is not a site of ionic regulation. Dall's analyses showed proventricular fluid composition in *P. longipes* to be comparable to that for *P. argus* reported here. His values for gastric fluid (= PVF) from lobsters in normal salinity (34·0–37·0‰) in mM concentrations are Na^+ 528·9; K^+ 11·6; Ca^{2+} 16·3; Mg^{2+} 33·6; and Cl^- 519·8. He did not measure SO_4^{2-}. Comparing the PVF in *P. longipes* with haemolymph values, both expressed as molar concentrations, gives PVF as % haemolymph: Na^+ 96, K^+ 95, Ca^{2+} 129, Mg^{2+} 249 and Cl^- 97. Although the values given here for *P. argus* are molal, the relation between PVF and haemolymphis remarkably constant between the two species. In experiments in which Dall salt-loaded *P. longipes* there was no evidence that excess ions, with the possible exception of Ca^{2+}, were secreted into the gut.

Salt and water balance in P. argus

The overall picture of salt and water balance in *P. argus* which emerges is that the regulation of Na^+ and Ca^{2+} must result from the active uptake of these ions by the gills, since the gut and antennal glands have been eliminated as sites of their regulation. Dall (1965), using $^{45}Ca^{2+}$ to follow influx and efflux of this ion in the shrimp *Metapenaeus*, obtained the similar results that the site of the net uptake of Ca^{2+} is the gills and also that a small net output of Ca^{2+} occurs via the anus. Magnesium, SO_4^{2-} and Cl^- are selectively excreted by the antennal glands in *P. argus*. Water which must be actively taken up, in the absence of a favourable osmotic gradient, to balance urine flow is to a small extent absorbed throughout the gut or formed as metabolic water. But largely it must be absorbed through the gills although other permeable sites may exist.

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