THE REGULATION OF WATER AND SALT BY THE FIDDLER CRABS, UCA PUGNAX AND UCA PUGILATOR

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Ionic and osmotic regulation in decapod Crustacea are the result of selective ionic absorption and excretion through several routes (Prosser *et al.*, 1950; Robertson, 1953). The gills have been implicated as the primary site of absorption (Huf, 1936; Krogh, 1938; Webb, 1940; Koch, 1954; Gross, 1957) but the alimentary tract may also be important (Burger, 1957). The antennary glands are considered the chief organs of selective excretion (Nagel, 1934; Webb, 1940; Robertson, 1949; Prosser *et al.*, 1955; Burger, 1957). The cellular mechanisms of ionic absorption and excretion in crustaceans are poorly understood and hypo-osmotic regulation has been less extensively studied than hyper-osmotic regulation.

Recently (Prosser *et al.*, 1955) it has been shown that *Pachygrapsus crassipes,* when maintained in 170% sea water (S.W.) excretes a urine higher in Mg but significantly lower in Na than animals in normal sea water. The Na which is not excreted in the urine may be stored in tissues for short periods (Gross, 1958) or may be excreted by extra-antennary gland routes, as suggested by the finding (Gross, 1957) that salt exchanges, as measured by electroconductivity methods, occur in the gill chamber of *Pachygrapsus.*

Since Jones (1941) had shown that *Uca crenulata* is a stronger hypo-osmotic regulator than *P. crassipes,* studies were undertaken on several species of Atlantic coast *Uca* to determine their ability to excrete Na by extra-antennary gland routes. In a preliminary survey it was found that *Uca minax, U. pugilator* and U. *pugnax* all show hypo-osmotic regulation and reduced urine Na in concentrated sea water. These properties were not found in *Callinectes* and *Carcinus.* The object of the present paper is to report a detailed study of the response of the body fluids and tissues of *Uca pugnax* and *U. pugilator* to prolonged exposure to concentrated sea water.

Materials and Methods

Uca pugnax and *U. pugilator* were acclimated to 175% sea water during three-day periods by increasing the concentration of the sea water 25% per day. The crabs were held at 175% sea water in large finger bowls containing a small amount of the bathing medium for 2 to 4 days after reaching this concentration. They were not fed in the laboratory but the sea water in the bowls was changed daily. Usually crabs were used within 7 to 14 days after collection. Some of the variability in the experiments may be attributed to the starvation of the animals and their varied nutritional states upon collection. Greater experimental variability is found between different batches of crabs than between the sexes of the two species.

Urine was collected from single animals by mounting the crab, caudal end down, on a microscope stage, attaching one wire from a Harvard inductorium to the mouth and stimulating the opercular region at the base of the antenna with the other. A small capillary drawn out at the end was simultaneously placed near the opercular covering. Usually moderate shocks resulted in the expulsion of urine, as much as 10–20 microliters from a single crab. Urine from three crabs was generally pooled on a piece of Parafilm which was kept in a high humidity chamber.

Blood was collected in the manner described for *Pachygrapsus* (Prosser *et al.*, 1955).

Gill fluid was collected through small openings made in the gill plate prior to the experiment. Care was taken to prevent bleeding at the time the openings were made. After exposing crabs to isotopic solutions for a 12-hour period, the animals were exposed to non-isotopic sea water for 15 minutes and transferred to dry finger bowls for 30 minutes before removing gill fluid by fine capillaries through the gill plate openings.

Stomach fluid was collected in capillaries from excised stomachs.

Some studies were performed with isotopic Na^{24}. This ion was obtained with Na_2CO_3 as the carrier and was initially made up in a small amount of distilled water. Ten-ml. aliquots of this highly active sample, containing 0.1–0.2 mc./mg., were placed in finger bowls containing 490 ml. of the appropriate sea water. Crabs were exposed to these isotope solutions for 12–18 hours before sampling. Exploratory experiments had shown that the relative specific activity of the serum of crabs remained nearly constant after 12 hours during the period of sampling.

Routinely, samples of blood, urine, gill fluid and stomach fluid were pooled from three animals for analysis. Twenty-five microliters of such a sample were added to 10 ml. of glass-distilled water in small Pyrex tubes. From this solution Na^{24} counts were made with a well counter and scintillation tube. Sodium, K, Ca and Mg were analyzed by flame photometry in a conventional manner using the Beckman flame attachment with a photomultiplier tube. Chloride was analyzed by the method of Schales and Schales (1941), SO_4 by the method of Nalefski and Takano (1950) and NH_4 by the method of Russell (1944).

Osmotic determinations were made on all fluid samples, using the Jones method (1941) as modified by Gross (1954).

In those studies where Na^{24} counts of tissue were made, tissues were removed to Parafilm, weighed, placed in test tubes with the Parafilm and counted in the same manner as were the fluids. Counts were expressed per 25 mg. of wet tissue.

Results

Several preliminary experiments were performed to test our methods and to establish optimum levels for Na^{24} use. Table I presents the results from our two most extensive experiments for osmotic and ionic analyses of several fluids from crabs in 100% and 175% sea water. The measure of variability is the standard error. Significant differences between 100% and 175% groups were found for all components of serum except Mg, K, Ca and NH_4; for urine components except Ca, NH_4 and Cl; for gill fluid components except NH_4, and for components of stomach fluid except K and osmotic concentration.

A statistical evaluation of the difference between the analytical values (from

TABLE I

Osmotic and electrolyte concentrations in Uca expressed as mM/L

Fluid	No. crabs	Osmotic conc.*	Na	Mg	K	Ca	NH_4	Total mEq.$^+$	Cl	SO_4	Total mEq.$^-$
For Crabs in 100% S.W.											
Serum	28	.497 ±.012	328 ±4.40	46 ±2.55	11 ±0.32	16 ±1.35	20 ±1.28	483	537 ±7.75	42 ±1.26	621
Urine	23	.583 ±.014	276 ±17.4	108 ±11.2	16 ±1.10	17 ±0.89	75 ±7.2	617	622 ±25.8	47 ±1.90	716
Gill fluid	28	.506 ±.011	314 ±9.73	64 ±4.63	10 ±0.50	12 ±0.41	18 ±2.04	494	569 ±6.99	36 ±1.96	641
Stomach fluid	13	.758 ±.036	335 ±21.1	101** ±21.2	17 ±0.88	31 ±3.19	63 ±3.84	679	542 ±17.7	143 ±8.52	828
For Crabs in 175% S.W.											
Serum	33	.587 ±.011	375 ±9.1	55 ±3.64	15 ±0.48	14 ±0.61	21 ±1.88	549	574 ±6.96	49 ±1.11	672
Urine	33	.683 ±.012	218 ±18.18	255 ±12.9	20 ±0.71	20 ±1.77	116 ±7.7	904	704 ±14.1	120 ±4.89	944
Gill fluid	33	.860 ±.023	503 ±6.56	123 ±6.69	15 ±0.39	19 ±0.31	18 ±2.19	820	855 ±9.40	60 ±3.13	975
Stomach fluid	18	.828 ±.015	393 ±7.87	167 ±12.6	16 ±0.48	22 ±0.65	43 ±3.34	830	704 ±37.0	111 ±5.33	926
Composition of S.W. used for experiments											
100% S.W.	—	.560	397	88	9	12	0	606	576	22	620
	—	.750	579	139	17	20	0	914	941	29	999

* Equivalent moles of NaCl; stomach average of 10 crabs.
** Average of 8 crabs.

Table I) of each of the fluids from crabs within the same medium is given in Table IV. The different fluids from animals within the same medium are as quantitatively distinct as are the same kinds of fluids from crabs in the two different media. For example, serum and urine from crabs in 100% sea water are as different as sera from

TABLE II

Analysis of Na^{24} counts in Uca tissues. Counts per 25 mg. wet weight

	100% S.W. Aver. cts.	175% S.W. Aver. cts.	% change 175%/100%	P values 100 vs. 175	P values 100 vs. sera	P values 175 vs. sera
Serum	3891.9	4365.1	112	<.01		
Muscle	1243.43	2035.06	166	<.005	<.005	<.005
Mid-gut gland	2020.95	1507.44	75	<.005	<.005	<.005
Stomach	2663.85	3508.3	132	<.005	>.050	<.005
Gill	5000.96	3576.9	72	<.005	<.01	<.005
Heart	1819.1	2122.36	117	>.100	<.005	<.005
Intestine	948.62	1132.15	119	>.050	<.005	<.005

crabs in normal and concentrated sea water. This finding emphasizes the existence of homeostatic mechanisms in this group of crabs. The ability of these crabs to maintain their sera hypo-osmotic to the medium in both normal and concentrated sea water as shown by the osmotic concentration, appears to be shared with other members of the grapsoid group (Robertson, 1953). More striking is the finding that crabs in both types of media produce a urine which is hypertonic to the serum. The data from Tables I and IV show that the crabs regulate all serum ions in concentrated sea water and all but Ca in normal sea water. With the exception of Na all other electrolytes occur in higher concentrations in urine than in serum. Since the degrees to which these ions are concentrated in the urine varies in crabs from the same medium and between the two media and also varies for the different ions, it is probable that their concentration is a result of secretion or selective ion reabsorption.

Table I indicates that the gill fluid from crabs in 175% sea water is hyper-osmotic to the medium, the serum and the urine. That this hypertonicity results from water and solute absorption as well as solute secretion will be apparent later. Directly related to the gill fluid hypertonicity is the urine Na concentration which is signif-

TABLE III

Relative specific activities for crabs in normal and hypertonic sea water

Fluid	100% S.W.				175% S.W.				P Values
	No. crabs	Na mEq./L.	Counts per min.	RSA CPM/ Na^{23}	No. crabs	Na mEq./L.	Counts per min.	RSA CPM/ Na^{23}	RSA_{100} vs. RSA_{175}
Serum	13	349	2335	6.7	18	403	2955	7.8	0.01
Urine	13	258	2144	6.6	18	210	1599	7.6	0.05
Gill fluid	13	320	1099	3.7	18	486	2487	4.8	0.01
Stomach fluid	13	346	1816	5.8	18	393	2111	5.3	0.10

icantly lower (Table IV) in crabs in concentrated than in normal sea water. And while this result is not unexpected (Prosser *et al.*, 1955) it indicates the extra-antennary gland excretion of this ion, possibly through the gills, and hence its association with gill fluid hypertonicity.

The stomach fluid of crabs in 100% sea water (Table I) is marked by its significant hypertonicity to serum, urine and gill fluid. Its ion content is different from serum except for Na and Cl; from urine except for Na, Mg and K and from gill fluid except for Na, Mg and Cl. The stomach fluid from crabs in 175% sea water is hypertonic to serum and urine but not to gill fluid. Its ion content is greater than that of serum for all ions except Na and K; stomach fluid is more concentrated than urine except for Ca, Cl and SO_4 and more concentrated than gill fluid except for K. Both water and solute absorption probably occur from the stomach and the distribution of electrolytes in the stomach fluid makes some secretion into the gut probable.

Fluid/serum ratios have been summarized in Figure 1 for osmotic concentration and the electrolyte values. The extent to which the urine/serum ratio (U/S) departs from unity has been used as a measure of antennary gland regulation (Prosser

TABLE IV

Probability values of analyses

Fluid	Osmotic conc.	Na	Mg	K	Ca	NH_4	Cl	SO_4
	A. Comparison of fluids of crabs in 100% and 175% S.W.							
Serum	<.01	<.01	=.05	>.10	>.10	>.50	<.01	<.01
Urine	<.01	<.01	<.01	<.01	>.05	>.10	>.50	<.01
Gill fluid	<.01	<.01	<.01	<.01	<.01	>.50	<.01	<.01
Stomach fluid	>.10	<.02	<.02	>.10	<.01	<.01	<.01	<.01
100% S.W.	B. Comparison of fluids from crabs in the same medium							
Serum vs. urine	<.01	<.01	<.01	<.01	>.50	<.01	<.01	<.02
Serum vs. gill fluid	>.50	>.10	<.01	>.10	<.01	>.10	<.01	>.02
Serum vs. stomach fluid	<.01	>.50	<.02	<.01	<.01	<.01	>.50	<.01
Urine vs. gill fluid	<.01	>.05	<.01	<.01	<.01	<.02	>.05	<.01
Stomach fluid vs. urine	<.01	=.05	>.50	>.10	<.01	>.10	<.02	<.01
Gill fluid vs. stomach fluid	<.01	>.10	>.05	<.01	<.01	<.01	>.10	<.01
175% S.W.								
Serum vs. urine	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
Serum vs. gill fluid	<.01	<.01	<.01	>.10	<.01	>.10	<.01	<.01
Serum vs. stomach fluid	<.01	>.10	<.01	>.02	<.01	<.01	<.01	<.01
Urine vs. gill fluid	<.01	<.01	<.01	<.01	>.10	<.01	<.01	<.01
Stomach fluid vs. urine	<.01	<.01	<.01	<.01	>.50	<.01	>.50	>.10
Gill fluid vs. stomach fluid	>.10	<.01	<.01	>.10	<.01	<.01	<.01	<.01
100% S.W.	C. *Comparisons of ratios of fluids from crabs in the same medium							
U/S vs. one	<.01	<.02	<.01	<.01	>.10	<.01	=.01	<.01
SW/S vs. GF/S	<.01	<.01	<.01	>.02	>.50	<.01	>.10	<.01
SW/S vs. SF/S	<.02	<.01	>.10	<.01	<.01	<.01	>.10	<.01
175% S.W.								
U/S vs. one	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01
SW/S vs. GF/S	<.01	<.01	>.10	<.02	>.50	<.01	>.5	<.01
SW/S vs. SF/S	<.01	<.01	>.05	=.01	>.05	<.01	>.1	<.01

* See Figure 1 for meaning of ratios.

et al., 1955). When the U/S ratios for crabs in 100% sea water are compared with those from 175% sea water only the ratios for osmotic concentration, K and Cl are found to be alike; the 175% sea water U/S ratio for Na is lower and all others higher than the corresponding 100% sea water values. The considerable regulation exhibited by the antennary glands of these crabs in normal sea water (Tables I and IV) is increased under the stress of concentrated sea water, particularly for Mg, NH_4 and SO_4.

Because both gills and stomach have a direct contact with the external medium and appear to be the most likely sites of exchange of water and salts with the medium, it is reasoned that the extent to which the gill fluid/serum (GF/S) and the stomach fluid/serum (SF/S) ratios deviate from the sea water/serum ratio should provide a measure of the absorptive and secretory capacities of gill and stomach tissues. These ratios are presented in Figure 1, and the statistical significances of a variety of internal comparisons (for example, GF/S with SF/S ratios from crabs in 100% sea water) are given in Table IV.

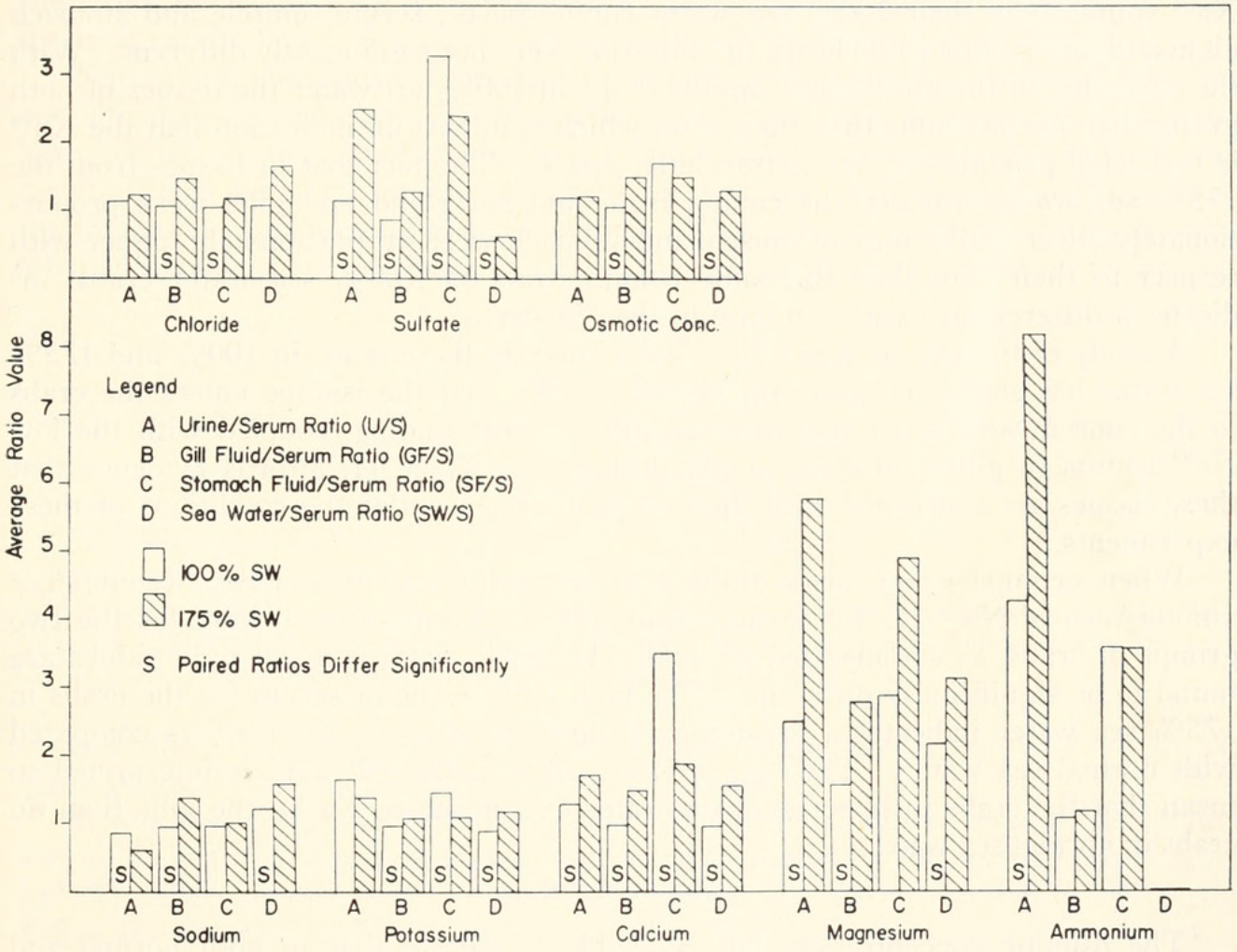

FIGURE 1. Ratios of the osmotic and electrolyte concentrations of fluids from crabs in 100 and 175% sea water. Statistical significance was attributed to P values of 0.02 or less.

In a few experiments crabs were exposed to sea water containing Na^{24}. The same quantity of the isotope was added to equal volumes of 100% and 175% sea water. Since the Na^{23} concentration of the 175% sea water was greater than that of the 100% sea water, a factor was used to correct the counts obtained from fluids and tissues of crabs in the concentrated sea water to make them comparable to those from normal sea water. This correction factor was obtained by dividing the Na^{24}/Na^{23} ratio in 100% sea water by the Na^{24}/Na^{23} ratio in 175% sea water. Multiplying the counts from the fluids and tissues from crabs in 175% sea water by this factor gave the corrected counts. Approximate isotopic equilibrium was attained in blood and urine in both groups of crabs after 12 hours. Isotopic analyses of

tissues were made in a number of experiments and the assumption was made that these too had attained isotopic equilibrium. The results of the tissue studies are summarized in Table II. The high Na^{24} count in the gills from crabs in 100% sea water implies that these are the primary means of Na entrance or else that Na cannot be excreted as rapidly as it enters and dams up in this tissue. Since Na^{24} counts increased more in muscle (67%) and stomach tissues (32%) relative to the increase in sera in going from 100% sea water to 175% sea water, these tissues may serve for Na storage during the stress of high serum Na.

Only gill and mid-gut gland tissues from crabs in 175% sea water had lower Na^{24} counts than their 100% sea water counterparts; serum, muscle and stomach all had higher counts while heart and intestine were not significantly different. With the exception of the gill tissue from the crabs in 100% sea water the tissues of both groups had lower counts than their sera, which is in part an indication that the Na^{24} is restricted primarily to the extracellular space. The fact that in tissues from the 175% sea water animals, the counts from mid-gut gland and gills were proportionately lower while those of muscle and stomach were proportionately higher with respect to their sera than the same tissues from the 100% sea water crabs, indicates a differential tissue response to the Na stress.

A study of the rate at which Na^{24} can penetrate these crabs in 100% and 175% sea water has shown (Green and Harsch, 1958) that the isotope enters the crabs in the concentrated sea water more readily. This finding, coupled with the low Na^{24} counts in gills and mid-gut gland shown in Table II, affords evidence that these tissues are concerned with the excretion of Na under the conditions of these experiments.

When a comparison is made of the relative specific activities (RSA) (counts per minute/meq of Na^{23}/L) for serum, urine, gill fluid and stomach fluid for the two groups of crabs, as summarized in Table III, only serum and gill fluid values are found to be significantly different. The high RSA value of serum for the crabs in 175% sea water indicates a greater exchange rate of Na^{24} for Na^{23} as compared with normal sea water. The higher RSA value in the gill fluid is interpreted to mean that the crabs in hypertonic sea water excrete more Na by the gills than do crabs in normal sea water.

Discussion

The osmotic concentration data of Table I indicate that in both normal and concentrated sea water *Uca* is a hypo-osmotic regulator; sera of 100% sea water crabs were 12% lower in osmotic concentration and of 175% sea water crabs 22% lower than their respective media. Hypo-osmotic regulation occurs in crabs which spend much time out of water (Jones, 1941) and in shrimps and prawns (Parry, 1954). Ionic regulation in *Uca* is quantitatively different in the two media. In 100% sea water the serum concentrations as per cent of medium concentrations are: Na, 83; Mg, 52; K, 122; Ca, 133; Cl, 93; SO_4, 191; while in 175% sea water they are: Na, 65; Mg, 40; K, 88; Ca, 70; Cl, 61; SO_4, 169. In the concentrated sea water each ion is proportionately less concentrated in serum relative to the medium than in normal sea water; however, the extent to which the ions are regulated, as measured by the per cent increase in serum concentrations in 175% sea water relative to the sera concentrations in 100% sea water, is, in order of decreasing order of regulation: Cl, 7; Na, 14; Ca, 14; SO_4, 17; Mg, 20; K, 36. *Uca* differs from

Pachygrapsus crassipes (Prosser *et al.*, 1955) under similar conditions, especially in the greater ability of the fiddler crab to regulate Na. The osmotic concentration of the 100% sea water crabs approximates that of *Pachygrapsus marmoratus* as measured by Robertson (1953), as do the K, Ca, and SO_4 values relative to Cl, while Mg values in *Uca* are relatively higher.

A cation deficit of 12% exists in the serum of crabs in 100% sea water; a deficit of 10% in crabs in 175% sea water. The urine cation deficit is smaller in both groups. The serum deficits are attributed to organic cations. The lower cation deficit in urine than in serum is associated with the higher urine concentration of ammonia; however, if the NH_4 excreted by the antennary glands is subtracted from the total cation deficit, the cation deficit is still smaller than that in serum.

Tentative conclusions can be drawn concerning the formation of urine, gill fluid and stomach fluid in *Uca*. The urine in both normal and concentrated sea water has a higher osmotic concentration and a higher total electrolyte concentration than serum. This ability to produce a blood hyper-osmotic urine is one means of keeping the blood hypo-osmotic to the medium. *Pachygrapsus crassipes* failed to show a hyper-osmotic urine (Prosser *et al.*, 1955). *Uca* appears to spend more time out

TABLE V

pH of Uca urine

Treatment of crabs	Crabs in 100% S.W.	Crabs in 175% S.W.
Equilibrated to medium for 3 days	6.92±.15*	7.16±.10
Equilibrated to medium for 4 weeks	6.38±.11	6.42±.11

* Standard error. The pH was measured with the Beckman micro-glass electrode. Urine of crabs equilibrated at the same time was not significantly different. Differences in the urine pH of crabs in the same media for different lengths of time were real.

of water than *Pachygrapsus* and may be a better hypo-osmotic regulator, partly because of its ability to produce hyper-osmotic urine.

It was not feasible to obtain true urine volumes, and excretion of solutes which are unlikely to be transported actively was not studied. The urine/serum ratios (U/S) differ for different ions and are maximal for NH_4 (4 and 8 in 100% and 175% sea water). If NH_4 were excreted by simple filtration, marked reabsorption of all other ions would be required to give such high NH_4 values; hence it is probable that NH_4 is either secreted or its excretion is accelerated by acidification of the urine. This latter alternative appears unlikely from the pH data presented in Table V. The U/S ratio is next highest for Mg, increases proportionately in the concentrated sea water. The high U/S ratios for Mg and SO_4 in (175% sea water) could result from marked reabsorption of water and other ions (except NH_4); they could indicate secretion of Mg and, at least in 175% sea water, also of SO_4.

The treatment of Na by the antennary glands is unique. Its U/S ratio is less than one in normal sea water and is decreased in 175% sea water. This reduction in urine Na was found in *Pachygrapsus* (Prosser *et al.*, 1955) and has been seen in another semi-terrestrial genus, *Ocypode* (Gifford, unpublished data). Reduced urine Na in concentrated sea water is not necessary for hypo-osmotic regulation,

however, since it does not occur in hypo-osmotic shrimps and prawns (Parry, 1954). The decreased urine Na in 175% sea water could result from reduced secretion or increased reabsorption. In *Pachygrapsus* urine Na was not reduced in 175% sea water which lacked Mg (Prosser *et al.*, 1955); injection of extra Mg into land crabs in 100% sea water reduces urine Na (Gifford, unpublished data). In *Uca* in 175% sea water Mg excretion increases more than Na excretion decreases when both are compared with responses in 100% sea water. It seems probable from these observations that Mg secretion interferes in some way with Na secretion. Filtration and reabsorption of Na might serve a useful function in causing water absorption. However, one would expect such Na reabsorption to be associated with some Cl or SO_4 absorption; this does not appear to be the case. If the Na were reabsorbed by exchange with Mg one would expect two Na ions to be exchanged for one of Mg; the finding for 175% sea water was 1.3 ions of Mg for each ion of Na. Hence on a quantitative basis it is difficult to attribute an increased Na reabsorption to an increased Mg excretion. On an energetic basis Na reabsorption seems improbable. If the crab needs to remove Na in 175% sea water and is able to filter it in the kidney, why would this Na be reabsorbed against a Na gradient, using energy for this purpose, only to be secreted at another site using energy a second time? It is possible that Na is exchanged for NH_4 or for hydrogen ions, in which case its absorption would serve a useful function. By exclusion, active secretion of Na is indicated, a process which is reduced under a high Mg load.

The U/S ratios for K, Cl and Ca are slightly above one and increase slightly in 175% sea water. Since the ionic gradients of these elements are from urine to plasma (presumably because of water reabsorption) the differences among them could result from differences in back permeability among these ions. Rather than postulate secretion of all ions, it seems more reasonable that there is filtration coupled with reabsorption of Na and water and some secretion of NH_4 and Mg (possibly also SO_4).

The composition of fluid from the gill chamber indicates a combination of diffusion and secretion and a mixing with sea water. The osmotic concentration is intermediate between serum and 100% sea water; it may be as high or higher than 175% sea water. This could mean outward secretion of some ions or absorption of water. Ammonia in gill fluid is intermediate between serum and the medium, hence NH_4 appears to be lost from the gills only by diffusion.

Sodium concentration in gill fluid is similar to Na in serum in 100% sea water, but is much higher in 175% sea water. In both concentrations it is lower than in the medium. The gill fluid specific activity is significantly higher in 175% than 100% sea water while the gill tissue has significantly fewer Na^{24} counts in 175% than 100% sea water. It is concluded that active secretion of Na occurs in the gills, at least in 175% sea water. The diffusion gradient for SO_4 is outward in 100% sea water but in 175% sea water the SO_4 in gill fluid is higher than in either serum or medium; hence there might be some SO_4 secretion along with Na.

Magnesium and Cl in gill fluid resemble Na in being close to serum levels in 100% sea water and higher than serum but lower than 175% sea water. These gradients could result from diffusion, secretion (in 175% sea water) or from water absorption. Potassium is similar in serum, gill fluid and medium; Ca in gill fluid is similar to both media, lower than serum in 100% and slightly higher in 175%

sea water. It is difficult to see how these concentrations could be so similar if there were much absorption of water. The relative importance of differences in permeability, in secretion and of water uptake by the gills cannot be evaluated from the present data. However it appears that the gills are important in ionic regulation in *Uca* and that the univalent ions do not separate from the divalent ions in route of excretion as they do in marine teleosts.

It is probable that, like the lobster (Burger, 1957), *Uca* swallows some sea water, hence stomach fluid is a modification of sea water. Since NH_4 is absent from sea water and is higher in stomach fluid than in serum, gastric secretion of NH_4 is probable; however the concentration of NH_4 in stomach fluid is less than in urine, where more secretion is indicated, and greater than in gill fluid where NH_4 may be lost only by diffusion.

Sodium in stomach fluid is similar in concentration to Na in serum in both media but lower than sea water, especially 175% sea water, hence absorption of Na in the stomach is indicated. With the Na, water is also probably absorbed, as indicated by the higher osmotic concentration in stomach fluid than in sea water. Concentration of the other ions might then be established by different inward permeabilities. A less likely alternative would be absorption of sea water and then active secretion of the different ions. Sulfate in stomach fluid is so high that it may well be secreted. In any case there must be some absorption of ions other than Na along with water; presumably this is the source of the Mg which is excreted in such large amounts by the antennary glands (kidneys).

The significantly higher Na^{24} levels found in stomach and muscle tissues of crabs in concentrated sea water indicate that during Na stress these tissues may serve as repositories for Na, as indicated by Gross (1958). It is unlikely that this storage mechanism is confined to a single kind of ion or that it can account for ionic regulation in a concentrated medium for long periods of time.

In the absence of data on fluid volumes and kidney clearances, a tentative qualitative summary is as follows: Ammonia diffuses from the gills, is actively excreted in the stomach and very much concentrated in the urine. Sea water is swallowed, especially in 175% sea water, Na and water are absorbed, other ions to a less extent. Filtration occurs in the kidney although Mg and Na may be actively excreted; Na and water may be reabsorbed. In 175% sea water the heavy load of Mg excretion is coupled with decreased secretion or increased reabsorption of Na. Sodium (also probably SO_4) appears to be actively secreted by the gills, more in concentrated than in normal sea water.

The various fluids which have been measured represent steady-state concentrations resulting from diffusion and selective permeabilities combined with active transport, and fluxes can only be inferred.

Summary

1. Analyses were made of the serum, urine, gill and stomach fluids for total osmotic concentration and the electrolytes Na, Mg, K, Ca, NH_4, Cl and SO_4 in *Uca pugnax* and *U. pugilator* when these two species were kept in 100% and 175% sea water.

2. For crabs in 100% sea water the serum electrolyte values for Na, Mg and Cl are lower and those for K, Ca, NH_4 and SO_4 higher than in the medium; for crabs

in 175% sea water the serum electrolyte values of Na, Mg, K, Ca, NH_4 and Cl are lower and only SO_4 higher than the values in the medium. The sera of crabs from both media are hypotonic to their saline environment.

3. The electrolyte values of sera from crabs in normal sea water differ significantly from the gill fluid electrolytes for Mg, Ca and Cl only; while similar sera values from crabs in concentrated sea water differ significantly for Na, Mg, Ca, Cl and SO_4. In all cases except for Ca from crabs in normal sea water the significant gill fluid electrolyte concentrations are greater than the corresponding sera values.

4. Crabs in normal and concentrated sea water maintain their stomach fluids more concentrated than the external medium. Sera electrolyte concentrations from crabs in 100% sea water are significantly lower than stomach fluid concentration for Mg, K, Ca, NH_4 and SO_4. In crabs from 175% sea water corresponding serum electrolyte significance is found for Mg, Ca, NH_4, Cl and SO_4.

5. All electrolytes are regulated by the antennary gland by crabs in the high salinity medium and all except Ca in the normal sea water; Mg and NH_4 are especially controlled by the antennary gland. In concentrated media the antennary gland excretion of Na is significantly lower than in normal sea water while the Mg excretion is markedly elevated.

6. Ammonia appears to be secreted by both the antennary gland and the stomach but its appearance in the gill fluid is attributed to diffusion.

7. Urine osmotic and electrolyte concentrations are significantly higher than the corresponding serum concentrations for animals in both media.

8. For crabs in 100% sea water the average fluid osmotic concentrations are equivalent to the following moles of NaCl: serum, 0.497; urine, 0.583; gill fluid, 0.506 and stomach fluid, 0.758; for crabs in 175% sea water the corresponding values are: serum, 0.587; urine, 0.683; gill fluid, 0.860 and for stomach fluid, 0.828.

9. By the use of Na^{24}, the relative specific activities of serum and gill fluid from crabs in 175% sea water are shown to be significantly higher than the corresponding serum and gill fluid values from crabs in 100% sea water while the RSA values of the urines are not significantly different. Na^{24} counts in gill tissue from the 175% sea water crabs are significantly lower than in the 100% sea water crabs. Active excretion of Na by the gills is indicated.

10. The low isotopic concentration of the mid-gut gland from crabs in concentrated sea water, comparable to that of gill tissue, suggests a Na secretory mechanism for this organ. The high isotopic Na concentrations found in muscle and stomach tissues of crabs in 175% sea water indicate that these tissues may be serving as storage depots during periods of serum Na stress.

11. The data show that the chief sites of entrance of water and electrolytes into these fiddler crabs are the stomach and the gills. They show that the chief sites of regulation are the antennary glands and the gills with some regulation by the stomach and possibly the mid-gut gland.

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