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URINARY BLADDER VOLUME AND THE REABSORPTION OF WATER FROM THE URINE OF CRABS

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INTRODUCTION

In recent years some disagreement has arisen with regard to the ability of marine decapod crustaceans to withdraw water from their urine. The concentration of the presumably non-metabolized and non-reabsorbed substance inulin may be elevated in the urine relative to its concentration in the haemolymph. This is true of almost all decapods whose ability to excrete inulin has been tested (Riegel, 1972). Riegel & Lockwood (1961) showed that the ratio of the concentrations of inulin in urine and haemolymph (U/H) approached an average value of three when specimens of *Carcinus maenas* were exposed to moist air for 4 days. Further, the inulin U/H of crabs in 100% seawater may approach two. These observations suggest that a proportion of the water in the primary urine is reabsorbed before release of the definitive urine from the bladder. Similarly, Bryan & Ward (1962) have suggested that inulin U/H of the order of 1·5 to 2·0 which they observed in the prawn, *Palaemonetes varians*, could indicate water reabsorption. Green, Harsch, Barr & Prosser (1959) have suggested tentatively that water reabsorption from the urine could account for discrepancies in the concentrations of potassium and calcium between haemolymph and urine in the fiddler crab, *Uca*.

Binns (1969) has challenged the contention that elevated inulin U/H, at least in *Carcinus maenas*, indicates water withdrawal from the urine of animals immersed in sea water. He has argued that U/H in excess of one could result from a lack of equilibrium between the haemolymph and urine in the bladder with respect to inulin concentrations. The urinary bladder of *Carcinus* and other brachyuran decapods is very extensive, ramifying throughout much of the thoracic cavity between other organs. If this extended shape necessarily reflects a large bladder volume, then Binns's criticism could be justified to some extent; the U/H would be influenced by the fact that the concentration of inulin in the urinary bladder at the time of sampling would be partially representative of the concentration of inulin in the haemolymph some hours earlier.

The present paper presents the results of experimental and theoretical analyses designed to investigate the relationship between bladder volume, loss of marker substances to the urine and other extrahaemolymph sites, and rate of urine formation.

These analyses lead to the conclusion that, although a lag effect such as that postulated by Binns can indeed account for some of the discrepancy between U and H, water is, nevertheless, reabsorbed by the excretory organ/bladder complex. Such a conclusion is of importance both to analyses of the degree to which ions are secreted into and out of the primary urine and to calculations of the actual volume of urine produced by these crustaceans.

MATERIALS AND METHODS

Specimens of two species of crabs were studied: *Carcinus maenas* (L.), an euryhaline osmoregulator, which lives on the bottom throughout the intertidal and subtidal regions of rocky open shores and estuaries, and *Macropipus* (formerly *Portunus*) *depurator* (L.), a stenohaline crab which does not osmoregulate.

Initial experiments (bladder and haemolymph volume estimations) were carried out at the Marine Biological Laboratory, Plymouth. The crabs were maintained at a temperature of 17·5 to 18 °C and were not fed during the experiments. All other experiments were conducted at Southampton under similar conditions, except as specified.

Estimations of haemolymph and urinary bladder volumes

Estimates of haemolymph and urinary bladder volumes were made as follows. Twelve crabs of each species were allowed to drain and were then weighed. Each was injected with 25 μl (*Carcinus*) or 15 μl (*Macropipus*) of a ^{131}I-sodium diatrizoate solution (7·5 mg per ml) using a 250 μl Hamilton syringe. If overt bleeding occurred during injection, the fact was noted and such crabs were not used for volume estimations. At intervals of 20 (or 30) min and 60 min after injection, haemolymph samples (20 μl) were taken. The crabs were placed in tanks of natural sea water. After 2 days urine and haemolymph samples were taken, the crabs were then individually killed and homogenized in a MSE blender; the homogenate was made up to a volume of one litre (*Carcinus*) or 500 ml (*Macropipus*). Homogenization was sufficiently effective to ensure that soft tissues were liquified and the exoskeleton was broken up into fragments no larger than 1–2 mm diameter. The homogenate was filtered and duplicate 5 ml samples of the filtrate were taken for radioactivity analysis.

Radioactivity was measured with a Packard 'Tricarb' automatic scintillation counter fitted with a NaI crystal detector. Triplicate one microlitre volumes of the original diatrizoate injection solution were placed in 5 ml of distilled water in a plastic test tube. These served as standards both for making decay corrections and for detecting changes in the counting efficiency of the apparatus.

The results of the forgoing analyses are shown in Table 1. Haemolymph volumes were calculated from the dilution of diatrizoate injected into each crab. These calculations were based on the averaged count rates of haemolymph samples taken 20–30 and 60 min after injection. The count rate of haemolymph at the two times was comparable in all cases. The average haemolymph volume of specimens of *Carcinus* calculated by diatrizoate dilution is identical with the haemolymph volume of specimens of *Carcinus* which has been calculated from inulin dilution (Binns, 1969*b*). Furthermore, the haemolymph volumes of specimens of *Carcinus* and *Macropipus* are very similar to inulin spaces observed in other crabs (e.g. *Gecarcinus lateralis*, *Ocypode albicans* and *Goniopsis cruentatus*; Flemister, 1958).

Table 1. *Calculated haemolymph and urinary-bladder volumes of crabs injected with* ^{131}I*-labelled sodium diatrizoate. Also shown are the diatrizoate urine:haemolymph ratios (U/H)*

Crab no.	Sex	Weight (g)	Haemolymph vol. (% body wt.)	Bladder vol. (% haemolymph vol.)	Diatrizoate U/H
			Carcinus maenas		
2	M	75·5	16·0	16·8	1·37
3	M	50·5	17·2	15·0	1·36
4	M	48·5	20·1	5·23	2·30
5	M	71·0	24·2	9·89	1·90
7	M	73·5	20·7	4·07	1·35
8	M	50·5	20·5	6·71	1·67
10	M	65·6	20·2	3·56	1·96
11	M	75·0	14·9	26·60	2·07
			Average 19·2 ± 3·0	10·98 ± 8·0	1·75 ± 0·36
			Macropipus depurator		
1	M	33·7	22·7	9·11	2·24
2	M	22·6	28·9	7·52	2·55
3	M	26·1	18·1	10·2	3·00
5	M	34·5	16·7	20·7	2·41
6	M	24·2	30·5	15·8	2·36
7	M	32·0	17·2	16·9	2·85
9	M	33·0	22·1	8·22	2·73
10	M	27·4	22·2	8·42	2·66
			Average 21·0 ± 4·0	12·1 ± 5·0	2·60 ± 0·26

The average volume of the haemolymph shown in Table 1 was used to calculate the proportion of the total count (in each crab at the time of homogenization) which was assignable to the haemolymph. The remaining counts represent the extrahaemolymph diatrizoate space. It was assumed for the purposes of calculation that the volume of the haemolymph did not change over the 2 days. It was further assumed that the space occupied by extra-haemolymph diatrizoate is equivalent to the volume of the urinary bladder after correction had been made for the diatrizoate U/H. However, if diatrizoate enters the cells or is bound to them, the volume of the bladder calculated in this way will be overestimated. The values for bladder volumes shown in Table 1 are therefore the maximum volumes possible in the crabs concerned. Naturally, the bladder volume of crabs may be expected to vary, perhaps widely, but it may be noted in Table 1 that in only one specimen of each species did the bladder volume exceed 20% of the haemolymph volume.

^{131}I*-activity in haemolymph and urine*

Table 2 shows the concentrations of sodium diatrizoate (cpm/μl) in the haemolymph 1 h after the initial injection, and in the urine 2 days later. If the presumption is correct that urine is formed by a process comparable to filtration, then the diatrizoate U/H in the primary (just formed) urine should approximate to unity. Therefore, if the diatrizoate U/H grossly exceeds unity, two mechanisms may be involved: (1) diatrizoate may be secreted, or (2) water may be reabsorbed during the process of urine elaboration. Listed in Table 2 are five specimens of *Carcinus* and one specimen of

Table 2. *Initial haemolymph and final urine concentrations of* ^{131}I*-labelled sodium diatrizoate and diatrizoate clearances of crabs kept in normal sea water for 2 days. Clearances were calculated from the relationship:* $c_t = c_0 e^{kt}$

		Carcinus			*Macropipus*		
Crab no.	M	Initial haemolymph count (cpm/μl)	Final urine count (cpm/μl)	Diatrizoate clearance (% haem./day)	Initial haemolymph count (cpm/μl)	Final urine count (cpm/μl)	Diatrizoate clearance (% haem./day)
1	M	460	113	23·7	483	451	43·0
2	M	554	449	28·6	569	373	66·7
3	M	769	652	25·9	787	513	74·8
4	M	684	968	26·6	—	—	—
5	M	435	566	20·6	645	498	55·7
6	M	701	1369	34·4	751	373	76·4
7	M	438	450	14·8	675	562	60·4
8	M	779	700	33·7	381	333	26·5
9	M	377	329	28·6	508	451	55·1
10	M	526	509	29·2	612	637	46·1
11	M	600	689	32·1	487	398	61·1
12	M	—	—	—	463	283	51·2
			Average	27·1 ± 5·8			56·1 ± 14·5

Macropipus in which the final urine diatrizoate concentration exceeds the initial haemolymph concentration of that substance despite a 2-day period intervening.

In an experiment comparable to the foregoing, specimens of *Carcinus* were injected with diatrizoate and the haemolymph and urine were sampled the same day and then periodically for the next 11 days. The time course of the experiment is shown in Fig. 1, which also illustrates that, on average, the urine count on the second and fourth days after injection of the tracer exceeded the haemolymph count on the day of injection. It is difficult to explain this result unless water reabsorption occurs or there is a change in haemolymph volume.

Effect of ethacrynic acid on diatrizoate U/H

Proverbio, Robinson & Whittembury (1969) found that ethacrynic acid interferes with the reabsorption of water in the proximal tubule of the mammalian nephron. If this substance acts in a similar manner in crustaceans, then it should effect a reduction in the diatrizoate U/H if water reabsorption normally occurs.

In an experiment designed to test this hypothesis specimens of *Carcinus* were injected with *ca.* 7 μCi of ^{131}I-sodium diatrizoate and placed in normal sea water. Three days later the crabs were injected with 0·1 mg ethacrynic acid in 100 μl of sea water. Injections of this dosage of ethacrynic acid were repeated every 24 h for 4 days. A control series was run in which crabs were injected with 100 μl of sea water at the same times as the experimental crabs were receiving pharmaceutical injections. The results of this experiment are summarized in Fig. 2.

As shown in Fig. 2, 3 days after the injection of diatrizoate there was no significant difference in the average U/H between experimental and control groups of crabs. Following treatment with ethacrynic acid, the average diatrizoate U/H of experimental crabs gradually diverged from the average diatrizoate U/H of the control crabs (Fig. 2), so that after 4 days the difference was statistically significant ($P < 0{\cdot}01$). These results

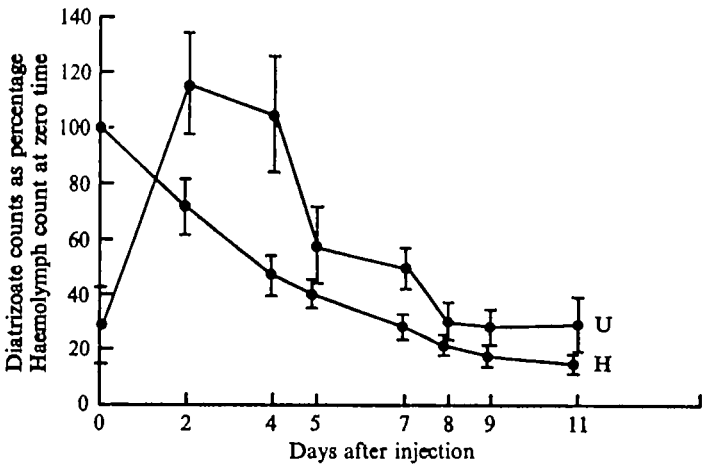

Fig. 1. The relationship between the concentration of ^{131}I-sodium diatrizoate in the haemolymph and urine of specimens of *Carcinus maenas* (from Bulleid, 1969). All values are expressed as per cent count of unit volume of haemolymph at zero time. U = urine; H = haemolymph.

indicate that ethacrynic acid reduces the diatrizoate U/H. This finding suggests that the reduction in the U/H was underlain by a reduction of water reabsorption since there was no appreciable variation in diatrizoate clearance as between the experimental and control groups of crabs.

Limits of extra-renal loss of diatrizoate

Loss of diatrizoate from the haemolymph by routes other than the urine will influence the U/H if the bladder volume is finite. The magnitude of the effect will depend upon both bladder size and the relative rate of loss of marker to the urine or other extrahaemolymph sites. In order to establish limits for such losses, a study was made of the relative losses of diatrizoate from the haemolymph and from the whole animal.

A known amount of sodium diatrizoate was injected into each of seven specimens of *Carcinus maenas*, and between one-half and 1 h later samples of the haemolymph were taken for radioassay. Subsequently, a sample of medium was taken daily and haemolymph samples were taken daily commencing with the third day after injection. On the sixth day post-injection, the animals were homogenized and their total radioactivity, together with the accumulated count in the medium, was compared with that of the initial injection. The observed count recovered was $95 \pm 3{\cdot}7$ % of the count of the original injection. (Part of the missing 5 % could be accounted for in the haemolymph samples taken during the course of the experiment.) On this basis, it is assumed that measurements of the count appearing in the medium give a reasonably accurate assessment of loss from the animals. In Table 3 and the figures described below, the count in the medium substracted from the count of the initial injection is used to calculate the proportion of the initial injection remaining in the animals.

Three days after injection of diatrizoate the count calculated to be remaining in the seven animals was $76{\cdot}1 \pm 18{\cdot}4$ % of the count injected initially. The count cal-

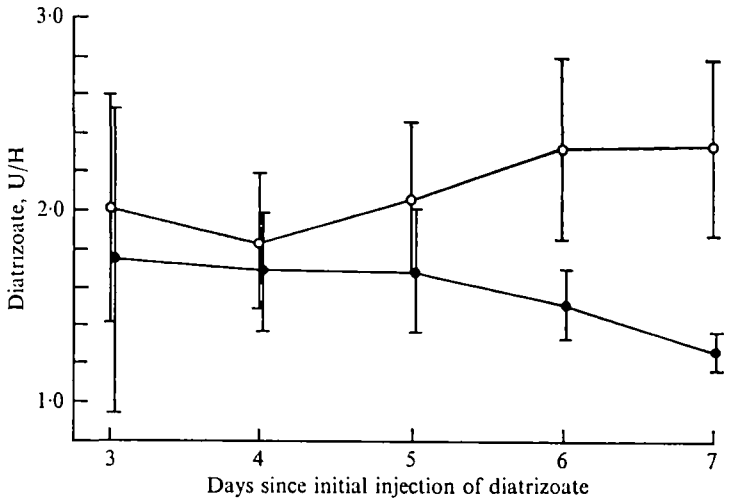

Fig. 2. The effect of ethacrynic acid on the sodium diatrizoate U/H in specimens of *Carcinus maenas*. O, Controls injected with 100 μl of sea water; ●, experimental animals injected with ethacrynic acid three days following initial injection of diatrizoate.

Table 3. *Loss of sodium diatrizoate from the blood and from the body as a whole*

Days since injection	Percentage of initial count remaining	
	Haemolymph	Whole body
0	100 (7)	100 (7)
3	46·4 ± 14·7 (7)	76·1 ± 18·4 (7)
4	37·8 ± 14·8 (7)	65·1 ± 17·1 (7)
5	29·8 ± 13·6 (7)	58·4 ± 16·7 (7)
6	24·3 ± 12·7 (6)	54·2 ± 15·5 (7)

culated to be present in the haemolymph was 46·4 ± 14·7% of the count injected initially. If it is assumed that the average diatrizoate U/H is the same in these animals as it is in other animals (i.e. 1·75, Table 1), then the urinary bladder volume will be equal to (76·1 − 46·4)/1·75 or 17% of the haemolymph volume. This volume would account for all of the activity not in the haemolymph.

Alternatively, some of the extra-haemolymph count may have been in the tissues. However, even if the bladder were of zero capacity, the total rate of loss of count to the tissues could not have been greater than (76·1 − 46·4)/(100 − 76·1) = 1·24 times the loss of counts by primary urine formation. Limits may therefore be set not only on the maximum size of the urinary bladder but also on the extra-renal loss of marker over a given time.

Studies of the possibility that diatrizoate is secreted

Studies based on the excretion of single compounds, such as diatrizoate, which supposedly are metabolically inert, are open to the criticism that a U/H in excess of unity could arise either because of active secretion or because water is reabsorbed from the urine. Sodium diatrizoate can be regarded as an organic acid. According to Mudge

Berndt, Saunders & Beattie (1971), in the rabbit diatrizoate is actively, but slowly, secreted. Secretion is inhibited by the usual inhibitors of organic acid secretion.

In order to explore the possibility that active secretion could explain the diatrizoate U/H values observed in specimens of *Carcinus*, the excretory characteristics of two other substances, ^{14}C-inulin and ^{51}Cr-EDTA (ethylene diamine tetraacetic acid) have been examined and compared to the excretion pattern of sodium diatrizoate. Specimens of *Carcinus* were injected with a solution containing ^{14}C-inulin and ^{51}Cr-EDTA. They were then maintained without food in normal sea water at 16 ± 2 °C. Haemolymph and urine samples were taken at 24-hour intervals for 4 days after injection and then once again 7 days after injection. Each urine and haemolymph sample was counted twice, once in a conventional crystal scintillation counter (which counts only the γ emission from ^{51}Cr) and once in a Panax liquid scintillation counter which counts the β emission from the ^{14}C-inulin as well as the γ emission from ^{51}Cr.

The rise of the U/H with time was also measured in a separate group of animals into which only sodium diatrizoate was injected.

The results of these experiments, shown in Table 4, permit four main conclusions to be drawn. First, there is a general similarity between the excretion of inulin and the excretion of EDTA, although there is some indication that a few individuals excrete EDTA more rapidly than inulin. Secondly, compared with the experiments of Binns (1969) carried out at a lower temperature (i.e. 9 °C), the inulin U/H in the present experiments rose more rapidly and reached a higher final value. In a number of crabs the U/H exceeded two within a week. Third, there was a tendency for the U/H to reach a plateau, implying that loss of inulin or other tracer from the haemolymph via routes other than the urine is quantitatively small. Fourth, the plateau of U/H is similar for all three marker substances.

Theoretical studies

The foregoing observations and experiments have permitted the estimation of values for the volumes of the haemolymph and urinary bladder and the limits of loss of injected marker both by renal clearance and loss through extrarenal routes. It is now possible to examine these values within a framework of a theoretical model to discover whether further light can be thrown on the problem of whether or not crabs reabsorb water from their urine.

Consider an 'ideal' excretory system: Primary urine is produced at a rate R from a space (haemolymph) of constant volume V_1 and passes into a bladder of constant volume V_2. If an inert, filterable* substance (marker) is introduced into V_1 at time $t = 0$ then its concentration in the haemolymph C_1 will decline at a rate governed by the clearance of the primary urine and loss through other routes, R. This proposition may be expressed mathematically as follows:

$$\frac{d}{dt}(C_1 V_1) = -(R+\alpha R)\, C_1, \qquad (1)$$

* It is here assumed for convenience that primary urine is produced by filtration of the haemolymph. However, the expression derived here will still apply if primary urine is formed by some other mechanism which also results in metabolically inert materials entering the excretory system at the same concentration as in the haemolymph.

Table 4. *Comparison of U/H rise with time after injection of* ^{14}*C-inulin,* ^{51}*Cr-EDTA or* ^{131}*I-diatrizoate into* Carcinus

Compound	Temp (° C)	Sex	Days since initial injection: 1	2	3	4	5	6	7	8
^{14}C-In	16	F	0·86 ±0·24 (5)	1·35 ±0·37 (5)	1·45 ±0·29 (5)	1·41 ±0·15 (5)	—	—	1·84 ±0·29 (5)	1·84 ±0·36 (5)
^{51}Cr-EDTA	16	F	0·85 ±0·26 (5)	1·22 ±0·31 (5)	1·46 ±0·30 (5)	1·53 ±0·18 (5)	—	—	2·04 ±0·44 (5)	1·98 ±0·58 (5)
^{14}C-In	16	M	1·31 ±0·28 (5)	1·46 ±0·16 (5)	1·64 ±0·23 (5)	1·48 ±0·13 (5)	—	—	1·80 ±0·21 (5)	1·72 ±0·17 (5)
^{51}Cr-EDTA	16	M	1·44 ±0·29 (5)	1·64 ±0·26 (5)	1·82 ±0·33 (5)	1·70 ±0·36 (5)	—	—	2·49 ±1·24 (5)	2·24 ±0·75 (5)
^{131}I-DAZ	15	—	—	—	1·70 ±0·34 (11)	1·60 ±0·30 (11)	1·79 ±0·26 (11)	1·89 ±0·40 (11)	—	—
^{131}I-DAZ	15	—	—	—	2·03 ±0·59 (9)	1·84 ±0·36 (9)	2·07 ±0·39 (9)	2·33 ±0·48 (9)	2·32 ±0·48 (9)	—
^{131}I-DAZ	15	—	1·15 ±0·62 (7)	1·51 ±0·64 (9)	1·73 ±0·065 (9)	1·83 ±0·61 (9)	—	—	—	—
^{131}I-DAZ	18	M	—	1·75 ±0·36 (8)	—	—	—	—	—	—

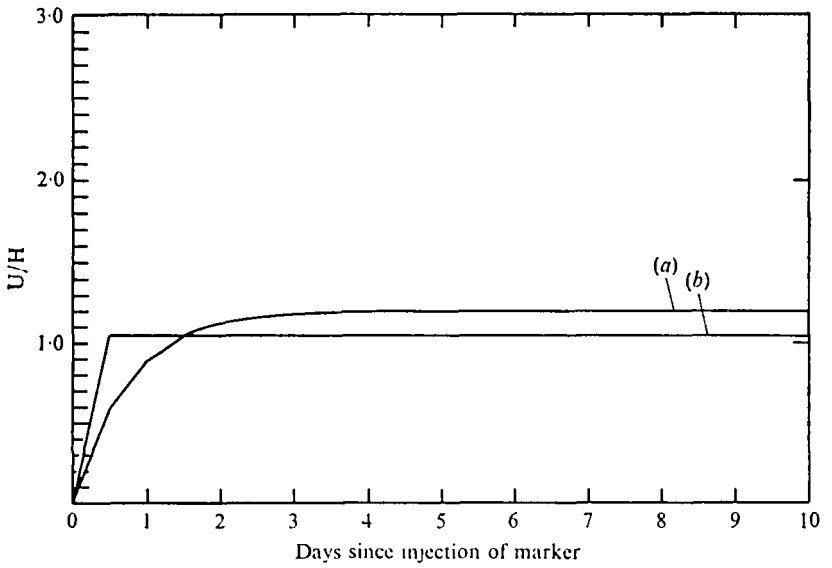

Fig. 3. Theoretical plot of the change of U/H with time after the injection of marker, assuming that there is no water reabsorption from the urine. In (*a*) clearance is 27% of the haemolymph volume per day, the bladder volume is 17% of the haemolymph volume, and the loss of marker to the tissues (extra-renal loss) is zero. In (*b*) clearance is 27% of the haemolymph volume per day, the bladder volume is 2% of the haemolymph volume, and loss of marker to the tissues is 1·24 times the loss from the haemolymph by clearance to the excretory organ.

where αRC_1 is the extra-renal loss. The rate of change of the concentration of the marker in the urinary bladder will be described as follows:

$$\frac{d}{dt}(C_2V_2) = R(C_1 - C_2), \tag{2}$$

where C_2 is the concentration of the marker in the bladder. Combining equations (1) and (2) and solving (see appendix) provides a description of the effect on the ratio of the concentration of marker in the haemolymph and bladder (C_2/C_1) at $t = 1$:

$$\frac{C_2}{C_1} = \frac{1}{1 - \frac{V_2}{V_1}(1+\alpha)}\left[1 - \exp - \left(1 - \frac{V_2}{V_1}1 + \alpha\right)\frac{Rt}{V_2}\right]. \tag{3}$$

Equation (3) will apply provided $1 - V_2/V_1(1+\alpha) > 0$. (It is highly unlikely that this condition will fail to be true.)

As
$$t \to \infty, \quad \frac{C_2}{C_2} \to \frac{1}{1 - V_2/V_1(1+\alpha)},$$

and does so monotonically from below. Therefore, utilizing estimated volumes of the haemolymph and urinary bladder, and the rates of renal clearance and limits of extrarenal loss of marker, it is possible, using equation (3), to calculate theoretical limits to the marker U/H (i.e. C_2/C_1) which can occur in the absence of water reabsorption.

Fig. 3 illustrates the effect on marker U/H, calculated using equation (3), of two factors which could give rise to a misassessment of the distribution of injected tracer.

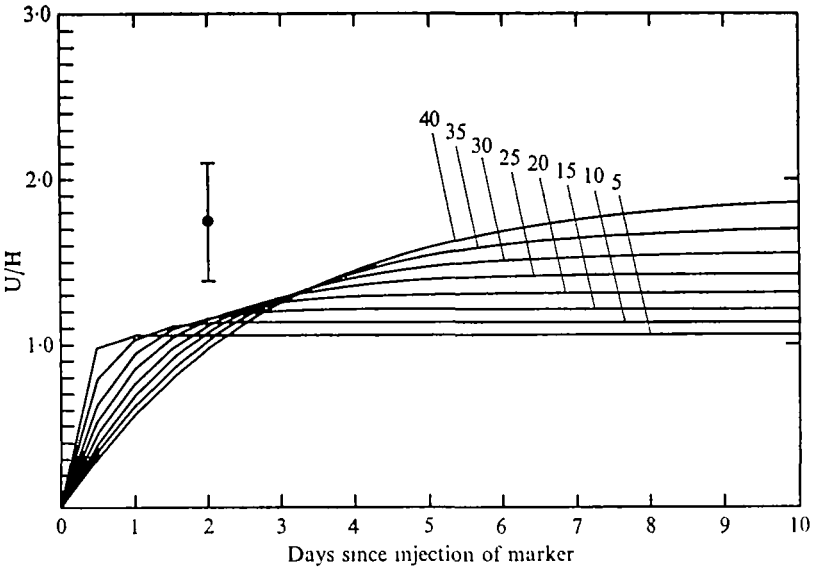

Fig. 4. Theoretical plots illustrating the influence of bladder volume on marker U/H. For all plots the values are: *clearance*, 27% of the haemolymph volume per day (the mean value for specimens of *Carcinus* shown in Table 2); *extra-renal loss*, 0·2 times the clearance; *bladder volume*, variable between 5 and 40% of the haemolymph volume. Note that the observed diatrizoate U/H (●) two days after injection lies outside the range of any of the predicted levels of U/H even though the theoretical plots include bladder volume values in excess of those possible.

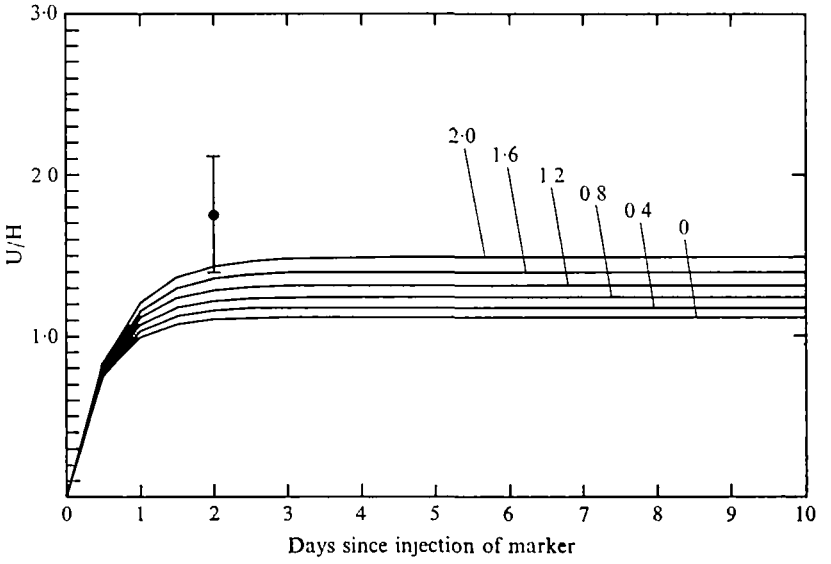

Fig. 5. Theoretical plots illustrating the influence of various rates of extra-renal loss of marker. Parameters are: *clearance*, 27% of the haemolymph volume per day; *bladder volume*, 11% of the haemolymph volume (mean value for specimens of *Carcinus* shown in Table 1); *extra-renal loss*, varies from zero to twice the loss of marker due to clearance to the renal organ. Note that the observed U/H (●) two days after injection of diatrizoate exceeds the range of predicted levels.

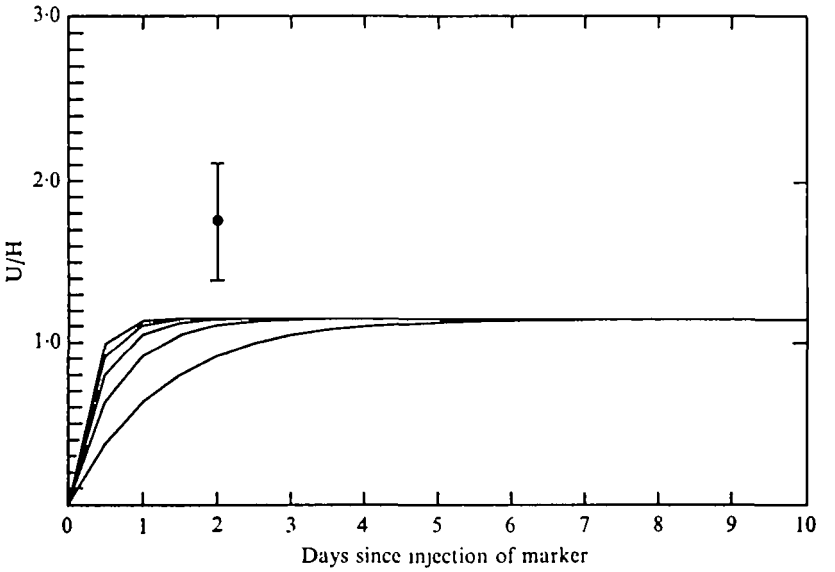

Fig. 6. Theoretical plots illustrating the influence of clearance rate on the marker U/H. Parameters are: *bladder volume*, 11 % of the haemolymph volume; *extra-renal loss*, 0·2 times the renal clearance; *renal clearance*, variable rate in the range from 10 to 50 % of the haemolymph volume per day. Note that the observed diatrizoate U/H two days after injection (●) exceeds the range of predicted values.

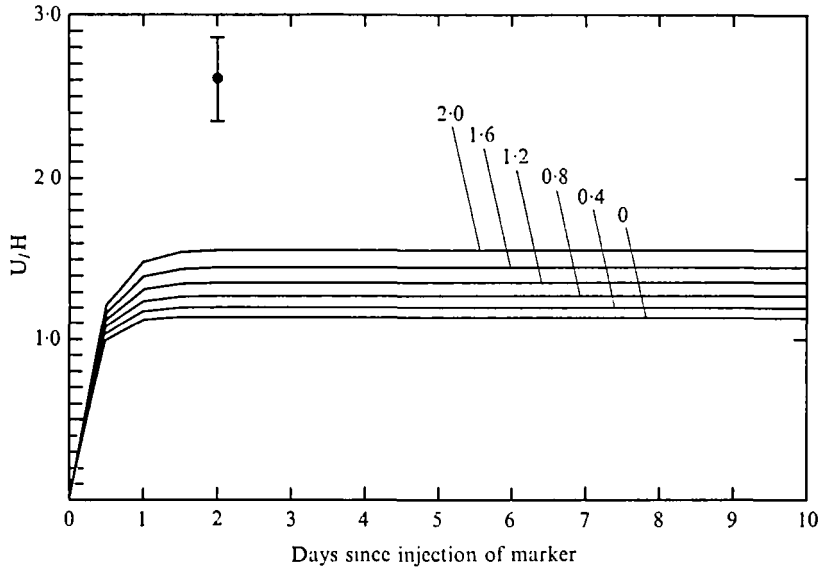

Fig. 7. Theoretical plots illustrating the effect of extrarenal loss when renal clearance is 56 % of the haemolymph volume per day (mean for specimens of *Macropipus* taken from Table 2). The bladder volume is equal to 12 % of the haemolymph volume (Table 1), and the extra-renal loss varies within a range of zero to twice the renal clearance. Note that the diatrizoate U/H observed in specimens of *Macropipus* (●) exceeds the range of the predicted values.

These are urinary bladder volume and extrarenal loss of marker. In both examples shown in the figure, the clearance of marker from the haemolymph is the same, 27% of the haemolymph per day. In crab (*a*) the extrarenal loss of marker is nil and the bladder volume is relatively large (17% of the haemolymph volume). The marker U/H rises gradually over 3 to 4 days to a maximum value of about 1·2. In crab (*b*) the bladder volume is small (2% of the haemolymph volume), but the extrarenal loss of tracer is large (1·24 times renal clearance). In this case the marker U/H reaches a maximum value very rapidly, but the maximum is only slightly in excess of one. Reference to Table 4 shows that the situation in specimens of *Carcinus* studied by us is intermediate between cases (*a*) and (*b*) shown in Fig. 3.

Families of curves may be constructed using equation (3) and a variety of values of renal clearance, bladder volume and loss of marker by extrarenal routes. These curves are illustrated in Figs. 4, 5, 6 and 7. As shown in Figs. 4 and 5, the larger the size of the bladder, the greater will be the steady-state value of U/H. Furthermore, for any given bladder size the magnitude of the U/H will be increased further by any loss of marker from the haemolymph via extrarenal routes. As shown in Fig. 6, increased renal clearance rates shorten the time taken for the U/H to reach a maximum, but they do not alter the final value.

DISCUSSION

As shown above mathematically, purely mechanical factors can give rise to differences in concentration of marker in haemolymph and bladder. The resulting concentration ratios (U/H) may then be interpreted as being due to water reabsorption if the marker is known to be an inert, filterable substance. For this reason, Binns (1969*a*) was quite correct in assuming that a time lag effect could produce inulin U/H in excess of unity in crabs where the bladder volume is large relative to the haemolymph volume. However, it is not feasible to account for the magnitude of the U/H of various markers seen in present experiments by purely mechanical factors. As shown in Figs. 4 to 7, when acceptable limits are used for bladder volume, renal clearance rate and possible loss of marker to the tissues, the resulting U/H which can be attributed to a lag effect falls short of that observed. It is necessary, therefore, to postulate either that active secretion of marker occurs or that water reabsorption from the urine contributes to the rise in U/H.

Two additional factors also suggest that either water reabsorption or secretion of marker contribute to the rise in U/H. First, urine diatrizoate concentrations rise in a number of individual crabs to a level exceeding the concentration present initially in the haemolymph (Fig. 1). Secondly, the diatrizoate U/H in specimens of *Carcinus maenas* treated with ethacrynic acid are significantly lower than the diatrizoate U/H of control crabs. Furthermore, the clearance of diatrizoate from the haemolymph is similar in the two groups (Fig. 2).

The question as to whether a U/H in excess of that predicted from purely physical effects arises from water reabsorption or from active secretion of marker is difficult to resolve unequivocally. In most experiments sodium diatrizoate was used as a marker. In man, the clearance of that substance is reported to be similar to that of inulin (Tauxe, Burbank, Maher & Hunt, 1964). However, Mudge, Berndt, Saunders

Beattie (1971) have recently found that diatrizoate may be secreted into the urine by the rabbit kidney. The rate of secretion is very low, even compared with slowly secreted organic acids, such as PAH. Secretion was detectable in kidney slices and in stop-flow preparations, but under normal conditions of urine flow the clearance ratio (diatrizoate U/H ÷ inulin U/H) was somewhat less than unity. This latter result indicates that secretory activity may form but a small part in the total clearance of diatrizoate. No secretion of diatrizoate could be detected in the dog (Mudge, Berndt, Saunders & Beattie, 1971). No experiments comparable to those performed on the rabbit or dog have been performed on crabs, but it seems likely that secretion of diatrizoate by specimens of *Carcinus*, if it occurs, must be of insufficient magnitude to exert any gross influence on the U/H, since the ratios for both inulin and EDTA do not differ to any appreciable extent from those for diatrizoate.

We conclude, therefore, that reabsorption of water is the prime cause of marker U/H values in excess of those predicted by lag effects. At the present time, it is not possible to speculate why our results should be so consistently at variance with the results of Binns's (1969) experiments.

Water reabsorption is not particularly well developed in *Carcinus*; for some reason, the stenohaline marine crab, *Macropipus* is more adept at withdrawing water from its urine. Such water reabsorption may perhaps assist in conserving water in the body, although it might be expected that variations in the rate of primary urine formation would be quantitatively more effective in regulating body water content. It must be confessed that there is at present no apparent reason why marine crabs should withdraw water from their urine.

It is probable that water conservation becomes of greater importance in crabs which adopt a more terrestrial mode of life than do *Carcinus* and *Macropipus*. In this respect it is of interest to note that Flemister (1957) found that the land crab, *Gecarcinus lateralis*, continues to clear inulin from its haemolymph at a rapid rate (60 hours to clear the equivalent of the haemolymph volume) when it is out of water, although it was not possible to collect urine. His findings suggest that these crabs reabsorb more water than does *Carcinus*, although actual inulin U/H are not available. Judging from the inulin U/H it appears likely that water is reabsorbed from the urine in some freshwater crustaceans also. Bryan & Ward (1962) observed that the mean inulin U/H in specimens of the British crayfish, *Austropotamobius pallipes pallipes* was 2·65 when the animals were in 0·1 % sea water, and the inulin U/H rose to a mean value of 9·0 in 50% sea water. Similarly, inulin U/H ratios in excess of two are normally observed in several species of crayfishes (Riegel & Kirschner, 1960). The occurrence of water withdrawal from the urine of freshwater forms would suggest that it is connected with some aspect of the function of the excretory organ and does not necessarily represent an adaptation to an environmental shortage of water.

SUMMARY

1. Measurements have been made to determine the blood volume, bladder volume, clearance of ^{131}I-sodium diatrizoate and U/H for diatrizoate in the crabs *Carcinus maenas* and *Macropipus* (*Portunus*) *depurator*.

2. Observed values of clearance blood volume and bladder volume in the two

species at 18 °C were: Clearance (as % blood volume per day), *Macropipus* 56·1 ± 14·5; *Carcinus* 27·1 ± 5·8; Blood volume (as % body weight), *Macropipus* 21·0 ± 4·0; *Carcinus* 19·2 ± 3·0; Bladder volume (as % blood volume), *Macropipus* 12·1 ± 5·0; *Carcinus* 11·0 ± 8·0.

3. It is shown that the measured U/H differs from that to be expected if no reabsorption of water or secretion of diatrizoate occurs.

4. ^{14}C-inulin and ^{51}Cr-EDTA are excreted in an essentially similar manner to ^{131}I-diatrizoate by *Carcinus*, implying that any active secretion of diatrizoate must be small in magnitude.

5. Injections of ethacrynic acid decrease the U/H ratio for diatrizoate relative to that in control *Carcinus* injected with sea water. In some *Carcinus* the concentration of diatrizoate in the urine comes to exceed that initially present in the blood. Both these points are taken, with 3, as support for the conclusion that water can be withdrawn from the primary urine of *Carcinus*.

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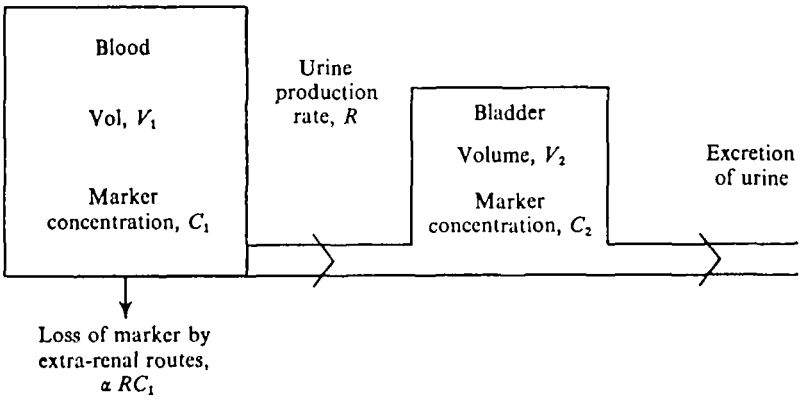

Fig. A 1. Schematic representation of idealized excretory system.

Appendix

The theoretical model

Our ideal excretory system is depicted schematically in Fig. A 1. We assume the marker concentrations to be uniform in the blood and separately in the bladder and assume that the urine is produced (and excreted) at a uniform rate R. With these hypotheses it is straightforward to write down the governing rate equations in the form (1) and (2) for C_1 and C_2 following an initial injection of marker into the blood. We assume the initial concentrations, at $t = 0$, to be C_0 in the blood and zero in the bladder. The solution to equation (1) satisfying $C_1 = C_0$ at $t = 0$ is

$$C_1 = C_0 e^{-(1+\alpha)Rt/V}. \tag{A 1}$$

Substituting this into equation (2) and solving the resulting linear, first-order equation subject to $C_2 = 0$ at $t = 0$, yields the solution

$$C_2 = \frac{C_0}{1 - V_2/V_1\,(1+\alpha)}\left(e^{-(1+\alpha)Rt/V_1} - e^{-Rt/V_2}\right). \tag{A 2}$$

Dividing (A 2) by (A 1) now gives equation (3).