J. exp. Biol. 143, 333–346 (1989)
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ADAPTATIONS TO A TERRESTRIAL EXISTENCE BY THE ROBBER CRAB, *BIRGUS LATRO* L.

III. NITROGENOUS EXCRETION

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Accepted 14 December 1988

Summary

Nitrogenous excretion by the terrestrial anomuran crab *Birgus latro* L. was examined. The main excretory product was uric acid, representing 79·5 % of total excretory nitrogen. It was eliminated as white faeces separate from undigested food material and made up 82·6 % of excretory faecal nitrogen. The faeces were the principal route of nitrogenous excretion, accounting for 96·2 % of total excretion. Loss of nitrogen in the urine and as gaseous ammonia was negligible. The midgut gland had substantial activity of xanthine oxidase and was considered to be the site of production of uric acid and its point of entry into the gut.

Introduction

Habitat has long been recognized to exert a major selective influence on the form of waste nitrogen excreted by animals. The most efficient excretory product from the energetic viewpoint is ammonia but, owing to its high diffusiveness and toxicity, it is useful primarily to aquatic animals. In most terrestrial species, lack of abundant water and the problems of concentrating a highly diffusive and toxic material have led to selection for different nitrogenous end products such as urea and purines. These are energetically more costly to produce but are relatively non-toxic and can be concentrated to a high degree. Purines are sparingly soluble and precipitate readily at *in vivo* pH whereas urea is highly soluble. Consequently urea is characteristically excreted in solution in the urine and precipitated purines are excreted as a paste with the faeces.

The main taxa of terrestrial arthropods are the Insecta and Chelicerata, both of which characteristically excrete waste nitrogen as purines in the faeces. Insects

Key words: *Birgus*, land crabs, nitrogenous excretion, uric acid, xanthine oxidase.

generally excrete uric acid or its degradation products allantoin and allantoic acid (Corrigan, 1970). Most spiders and scorpions excrete guanine (Hartenstein, 1970), although the scorpion *Palamnaeus* is reported to produce hypoxanthine, adenine and uric acid (Kanungo *et al.* 1962).

Crustaceans are represented on land by certain amphipods, isopods and brachyurans. Of these, only the Isopoda have been examined in any detail and they excrete waste nitrogen principally as gaseous ammonia volatilized across the body surface (Dresel & Moyle, 1950; Hartenstein, 1968; Wieser & Schweizer, 1970). Although practicable for these small animals with a large surface area: volume ratio, this mechanism may be less suitable for large animals. Little is known of nitrogenous excretion in terrestrial amphipods but a supralittoral species of *Orchestia* is reportedly ammonotelic (Dresel & Moyle, 1950). Amongst the land crabs nitrogenous excretion has been examined only in *Cardisoma* spp. Unfortunately, although *Cardisoma* are active on land, they are amphibious and have ready access to free water in their burrows or surroundings (Herreid & Gifford, 1963; Hicks *et al.* 1984; Wood & Boutilier, 1985). In consequence, excretion of nitrogen by these animals may not be tailored to the terrestrial environment and this may account for the somewhat confusing reports of nitrogen excretion in the genus. *Cardisoma guanhumi*, for example, is reported to produce both ammonia and urea whilst storing large amounts of uric acid in the haemocoel (Horne, 1968; Gifford, 1968). In *Cardisoma carnifex* also, ammonia and urea have been identified as excretory products, although uric acid has been detected in burrow water (Wood & Boutilier, 1985; Wood *et al.* 1986). No ammonia appears to be lost by volatilization (Wood & Boutilier, 1985). No studies of nitrogenous excretion in truly terrestrial crabs have been published.

Synthesis of urea from the ammonia produced during transamination and deamination reactions requires a functional urea cycle. Crustaceans generally are reported to lack these enzymes and urea cannot be a major excretory product in the group (Claybrook, 1983). Some urea may be formed from dietary arginine but arginine is an essential amino acid in crustaceans (Claybrook, 1983) and is unlikely to contribute more than a small fraction of total excreted nitrogen unless very large amounts of arginine are present in the diet.

Excreted purines may come from two sources. First, degradation of endogenous and dietary nucleic acids liberates purine nucleotides which are then degraded to any of the other commonly excreted purines. These processes may result in small amounts of excreted purine. Second, nitrogen derived from catabolism of protein can be incorporated into the purine ring structure using *de novo* synthetic pathways, and in purinotelic animals most waste nitrogen is excreted *via* this route. Crustaceans, however, are not believed to have the ability to synthesize the purine ring *de novo* (Claybrook, 1983) and purine excretion must, therefore, be a minor constituent of total excreted nitrogen derived from degradation of endogenous and dietary purine.

To summarize, the expectation is that land crabs, lacking the ability to synthesize either urea or purines, must be ammonotelic. This conclusion does not

however, fit well with the highly terrestrial nature and large body size of many species of land crabs.

In this study, the nitrogenous excretory products of one of the most terrestrial and also the largest crabs, *Birgus latro*, were examined with the intention of solving this apparent paradox. To achieve this, the contributions of ammonia, urea, purines and free amino acids to total nitrogen excretion in the faeces and urine were determined and the possibility of elimination of gaseous ammonia was tested.

Materials and methods

Birgus latro (L.) were collected from Christmas Island (Indian Ocean) and air-freighted to Sydney where they were housed in a constant-temperature room at 25°C and 80% relative humidity (RH) on a 12h: 12h light: dark cycle. They were fed fruit, dry dog food and occasionally prawns and provided with tapwater for drinking.

Experimental protocols

A series of experiments was carried out to determine the nitrogenous excretory products in the faeces. Crabs were housed individually in plastic fishboxes and supplied *ad libitum* with food and tapwater. The boxes were cleaned and food and water renewed on a daily basis.

Three different experimental diets were used. The first was a breakfast cereal 'Sustain' (Kellog Australia) (10% protein, 5% fat, 6·6% fibre and 48% carbohydrate) which was mixed with water and then dried to form small cakes. The second diet consisted of slices of fresh corn cob (10% protein, 4·5% fat, 80% carbohydrate, 3·5% fibre as percentage dry mass) and sunflower seeds (10·6% protein, 51·5% fat, 5% carbohydrate, 5·2% fibre as percentage dry kernels) supplied *ad libitum*. Both were eaten readily by most crabs. In a third experiment, crabs were starved. As the precise dietary requirements for nitrogen were not known, both a high-nitrogen (Sustain) and a low-nitrogen diet (corn + sunflower seeds) were used. The low-nitrogen diet was aimed at mimicking a natural diet whereas the high-nitrogen diet ensured measurable excretion of nitrogen. Starved animals were included in the study to provide information on the nature of the substrate metabolized during inanition and the possibility of continued excretion of stored uric acid. Each experiment lasted 2 weeks. During the first week the crabs were acclimated to the experimental diet and in the second week faeces were collected daily from each individual and frozen until analysed.

Blood samples were taken from crabs on each of the three experimental diets and analysed for nitrogenous excretory products as described below. Haemolymph was taken from the venous sinus at the base of the walking legs in 1 ml glass syringes with 22 gauge needles. The syringes were iced to prevent clotting and samples were kept on ice until analysed.

A second experimental series was designed to measure nitrogen excreted in the

urine and lost as gaseous ammonia. Crabs on a diet of Sustain were placed individually in large glass desiccators. The crab sat on a plastic mesh platform and was provided with tapwater and food (Sustain). Urine released from the animal was collected under mineral oil in the base of the desiccator whilst faeces remained on the platform. The desiccator was sealed and air was pumped through it with a small aerator pump. Ammonia was removed from the incoming air by bubbling it through a gas-washing bottle containing $0{\cdot}1\,\mathrm{mol\,l^{-1}}$ HCl, and a glass-wool trap prevented carry-over of acid. Ammonia in the air leaving the desiccator was trapped in a similar fashion. These experiments were run for 2–3 days. It was considered possible that ammonia might be generated by decomposition of the faeces during the experiment so control experiments were run over similar periods to test this hypothesis. These desiccators contained a small amount of faecal material from crabs on a diet of Sustain, but no animal.

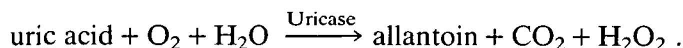
A second series of control experiments to monitor possible production of gaseous ammonia was run continuously for several weeks as a check against episodic release of ammonia. Animals were treated as before but to maximize excretion of nitrogen they were supplied with cat biscuits containing 26 % protein (at 2-day intervals). Faeces were removed twice daily and transferred to a second desiccator chamber so that evolution of ammonia could be measured (as far as possible) separately for each animal and its faeces.

Analyses

Faecal samples, bulked for each individual, were thawed and homogenized in a known volume of distilled water. Samples of the stirred suspension were taken for analyses. The faeces were dried at 80 °C and nitrogenous components were related to their dry mass.

Ammonia trapped in the acid as NH_4Cl was measured using an Orion (95-10) ammonia electrode and an Orion model 940 specific ion meter after alkalinizing the samples with $10\,\mathrm{mol\,l^{-1}}$ NaOH. Ammonia and urea in samples of haemolymph, urine and faeces were measured using a urea test combination (Boehringer Mannheim GmbH, catalogue no. 124788). This kit measures ammonium using the Solorzano colour reaction after conversion of urea to ammonium with urease.

Uric acid and urate were measured using Sigma 685 test kits. This method uses the following reaction:



$$\text{uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{allantoin} + CO_2 + H_2O_2\,.$$

The H_2O_2 reacts with other chemicals in the kit to produce a quinoneimine dye, the absorption of which is measured with a spectrophotometer. Checks indicated that all uric acid in the faecal suspensions was utilized by the enzyme within the incubation period. As the test did not distinguish between urate ions and uric acid, the actual form present was not identified and the terms uric acid and urate are subsequently used synonymously. Xanthine was determined with the same kit

after pretreatment of the samples with xanthine oxidase (Sigma) to reduce xanthine to uric acid.

Free amino acids were measured relative to a $2{\cdot}5\,\mathrm{mmol\,l^{-1}}$ glycine standard using the ninhydrin method described by Lee & Takahashi (1966). Protein in the samples was precipitated with methanol and the analysis was performed on the supernatant after centrifugation.

The concentrations of Na, K, Ca and Mg were measured using a Varian Atomic absorption spectrophotometer as described previously (Sparkes & Greenaway, 1984). Chloride concentrations were measured with a CMT 10 chloride titrator (Radiometer Copenhagen) and osmolality with a Wescor 5100C vapour pressure osmometer (Wescor, Logan, Utah).

All experiments were carried out at 25 °C.

Tissue preparation for enzyme assay

Tissues were taken from eight crabs and tested for activity of xanthine oxidase and xanthine dehydrogenase. Animals were chilled until torpid and then the heart, lung, gill and leg muscle tissue were removed rapidly. In addition, samples of tissue were taken from the posterior lobes of the midgut gland in the abdomen and from the anterior and median lobes in the thorax. The hindgut samples were taken from the abdomen and gut contents were carefully flushed out with buffer before homogenization. Tissues were homogenized in 4 volumes of ice-cold buffer ($10\,\mathrm{mmol\,l^{-1}}$ sodium phosphate buffer + $1\,\mathrm{mmol\,l^{-1}}$ 2-mercaptoethanol, pH 7·5) and stored at 4 °C until assayed.

Xanthine oxidase activity

The activity of xanthine oxidase in the homogenates was tested by measuring the rate of disappearance of added xanthine, as follows. $20\,\mu\mathrm{l}$ of homogenate was mixed with $525\,\mu\mathrm{l}$ of uricase solution (Boehringer Mannheim GmbH or Sigma) and diluted to 1 ml with assay buffer ($100\,\mathrm{mmol\,l^{-1}}$ sodium phosphate + $1\,\mathrm{mmol\,l^{-1}}$ dithioerythritol, Boehringer Mannheim, pH 7·5). The mixture was incubated at 25 °C for 15 min to remove all uric acid present in the homogenate. $100\,\mu\mathrm{l}$ of $0{\cdot}2\,\mathrm{mmol\,l^{-1}}$ xanthine solution was then added and the solution mixed. The reaction (xanthine to uric acid) was followed by monitoring the decrease in absorbance of the solution due to xanthine at 270 nm in a Varian spectrophotometer calibrated with xanthine standards of 0·02 and $0{\cdot}1\,\mathrm{mmol\,l^{-1}}$. The uricase further degraded uric acid to allantoin, preventing product inhibition of xanthine oxidase.

Xanthine dehydrogenase converts xanthine to uric acid in the presence of NAD^+ and a test for this activity was made by measuring the appearance of NADH in the solution. $20\,\mu\mathrm{l}$ of homogenate was added to $525\,\mu\mathrm{l}$ of uricase solution, $100\,\mu\mathrm{l}$ of NAD^+ and the solution made up to 1·1 ml with assay buffer. The mixture was incubated at 25 °C for 15 min to remove any uric acid present in the homogenate. $100\,\mu\mathrm{l}$ of $0{\cdot}1\,\mathrm{mmol\,l^{-1}}$ xanthine was then added and the change in absorbance at 340 nm with time was followed.

Table 1. *The rate of production of excretory nitrogen in the faeces (μmol nitrogen kg^{-1} body mass h^{-1} ± s.d., N = 10)*

Diet	Ammonia	Urea	Amino acids	Uric acid	Total
Sustain	1·91 ±1·695 (27·0)	0·65^a ±0·436 (9·25)	0·63^a ±1·26 (8·9)	3·87 ±2·998 (54·8)	7·068 ±3·745
Corn + sunflower seed	1·55 ±2·299 (11·2)	0·017^b ±0·028 (0·12)	0·010^b ±0·029 (0·07)	12·273 ±14·41 (88·64)	13·846 ±16·44
Starved	1·640 ±1·414 (7·19)	0·419^a ±0·536 (1·84)	0·651^a ±0·729 (2·85)	20·109 ±25·8 (88·12)	22·819 ±26·920

Values in brackets represent the percentage contribution of each product to total faecal excretion.

Different superscripts within a column indicate significant differences ($P<0{\cdot}05$) between rates for different diets.

Results

Nitrogenous excretion in faeces

The rates of output of ammonia, urea, free amino acids and uric acid in the faeces were determined for *B. latro* maintained on three different dietary regimens (Table 1). On both diets and in starved crabs uric acid was the major excretory product. Xanthine was either absent or detectable only in trace amounts in the faeces. Ammonia was the next most abundant component, making up 7·19–27 % of total excretory nitrogen but urea and free amino acids were minor constituents. Ammonia was shown (see below) to be generated by decomposition of faeces and much of the measured ammonia in the faeces may have originated in this way.

Faeces produced by *B. latro* were of two distinct types; brown faeces containing no uric acid, and presumably representing undigested food material, and white faeces containing up to 2·04 mmol uric acid g^{-1} dry faeces. Both faecal types were encased in peritrophic membrane. No correlation was found between the dry mass of faeces produced and total excretory nitrogen in crabs on diets of Sustain ($r=0{\cdot}474$, difference not significant at 10 % probability level) and corn/sunflower seeds ($r=0{\cdot}436$, not significant at the 10 % level) but a significant correlation was found in starved crabs ($r=0{\cdot}741$, $P<0{\cdot}05$), where the amount of brown faeces was small. This suggested that the production of white faeces was largely independent of feeding.

No significant difference in the total nitrogenous excretion in the faeces was found when the crabs were subjected to different diets. Excretion of urea and amino acids were significantly lower on the corn and sunflower seed diet than in starved crabs or those fed on Sustain.

Table 2. *The concentrations of nitrogenous excretory products and salts in the final excretory product of* Birgus latro *on a diet of Sustain*

	Nitrogenous constituents				Salt concentrations					
	NH_3	Urea	Amino acids	Uric acid	Na	K	Ca	Mg	Cl	Osmotic pressure
Mean	0·78	0·37	0·19	0·06	59·3	38·1	3·0	5·7	74·4	185·4
±S.D.	1·31	0·72	0·26	0·08	89·0	13·8	2·4	2·7	82·1	157·1
Range	0–3·6	0–2·3	0–0·6	0–0·2	9–294	19–66	1–8·8	2·4–10·3	26·5–290	97–598
N	10	10	9	10	8	8	8	8	8	8

Values are $mmol\,l^{-1}$ (osmolality as $mosmol\,kg^{-1}$ water).

Table 3. *Mean levels of nitrogenous excretory products in the haemolymph of* Birgus latro *(*$mmol\,l^{-1}$ *± S.D.)*

Diet	Ammonia*	Urea	Amino acids	Uric acid
Sustain $N=10$	$1·28 \pm 0·086^a$	$0·78 \pm 0·118$	ND	$0·053 \pm 0·017$
Corn/sunflower $N=10$	$1·701 \pm 0·116^b$	0	$0·30 \pm 0·465^a$	$0·347 \pm 0·481$
Starved $N=9$	$1·671 \pm 0·347^b$	0	$1·86 \pm 0·750^b$	$0·677 \pm 1·178$

Different superscripts within a column indicate significant differences between values ($P<0·05$).

*cf. values for humans (47–65 $\mu mol\,l^{-1}$, Ganong, 1979).

ND, not done.

Urinary excretion

Fluid released by crabs in the second series of experiments was collected under mineral oil and analysed for osmolality, salt content and nitrogenous compounds (Table 2). With the exception of potassium, salt contents were low, averaging only about 20 % of those in the haemolymph (Morris *et al.* 1988). Potassium level was elevated fourfold compared with that in the haemolymph. The concentrations of all nitrogenous materials were low and not significantly different from those in the haemolymph of animals on the same diet (Table 3).

Excretion of gaseous ammonia

Thirteen *B. latro* were individually monitored for production of gaseous ammonia as described above. The experiments covered a total of 640 h of monitoring. The mean rate of production was $0·565 \pm 1·420\,\mu mol$ nitrogen kg^{-1} body mass h^{-1} [range 0 (3 animals) to $5·4\,\mu mol$ nitrogen kg^{-1} body mass h^{-1}). Most

animals produced faeces during the period of monitoring and a series of control experiments ($N = 8$) showed that these faeces released substantial amounts of ammonia at a mean rate of $9{\cdot}21 \pm 20{\cdot}0\,\mu$mol nitrogen g^{-1} dry faeces h^{-1}. No correlation was found between uric acid content of the faeces and the rate of ammonia evolution ($r = 0{\cdot}27$, not significant at the 10 % level), indeed the highest production rate came from the smallest faecal sample which contained no detectable uric acid. Clearly, the faeces produced by the crabs may have contributed substantially or entirely to production of gaseous ammonia which presumably originated from microbial action on undigested material and/or uric acid in the faeces.

Production of gaseous ammonia from animals fed a high-nitrogen diet (cat biscuits) and from their faeces was monitored for periods of up to 3 weeks. Total ammonia evolved from the faeces was 10·3 times that from the animal. Subsequent analysis of the faeces revealed high levels of uric acid ($0{\cdot}69 \pm 0{\cdot}581$ mmol g^{-1} faeces), a very high ammonia content ($2{\cdot}04 \pm 4{\cdot}4$ mmol g^{-1} dry faeces) and an appreciable concentration of urea ($165 \pm 350\,\mu$mol g^{-1}), suggesting activity by microorganisms.

Excretory products in the haemolymph

The concentrations of nitrogenous excretory products in venous haemolymph of *B. latro* were measured (Table 3). The level of ammonia in the haemolymph of crabs fed Sustain was significantly lower than that measured in starved crabs or animals fed with corn and sunflower seeds. Urea was not detectable in the haemolymph of starved crabs or those fed a corn/sunflower seed diet but was present at low levels in crabs fed Sustain. The concentration of free amino acids in the haemolymph was not measured for crabs on Sustain but was significantly lower in animals on the corn/sunflower diet than in starved animals. The levels of uric acid were very variable among individuals, including several very high values which suggested that particles of solid uric acid inadvertently may have been included in some of the haemolymph samples causing artificially high values. Alternatively, the haemolymph of these individuals may have been supersaturated with uric acid (McNabb & McNabb, 1980).

The site of production of uric acid

Uric acid was produced as quite separate and distinct white faecal strands encased in peritrophic membrane. As this membrane is secreted by a ring of cells at the posterior end of the midgut, uric acid must enter the gut more anteriorly. As the foregut is lined with cuticle, uric acid must enter the midgut and originate from the midgut gland (hepatopancreas) or midgut caeca. Dissection of several individuals indicated that neither anterior nor posterior midgut caeca were present in *B. latro* and uric acid must, therefore, have been produced by the midgut gland. This gland is very extensive and three distinct regions were distinguished for this study. The posterior lobes filled the large abdomen whereas much of the body cavity in the thorax was filled with the median lobes and the greenish-yellow

Table 4. *The activity of xanthine oxidase in homogenates of tissue from* Birgus latro

Tissue	1	2	3	4	Mean ± S.D.
Heart	0	0			0
Gill	0	0			0
Lung	0	0			0
Leg muscle	0	0			0
Hindgut	0	0			0
Posterior midgut gland	80·8	30·9	87·0	127·4	81·5 ± 34·3
Median midgut gland	80·0	50·3	80·8	49·7	65·2 ± 15·2
Anterior midgut gland	99·2	36·4	77·7	52·8	66·5 ± 23·9

Values represent the rate of formation of uric acid from xanthine in $nmol\,min^{-1}\,g^{-1}$ wet mass tissue.
Measurements were performed on eight tissues from four individuals (nos 1–4).

anterior lobes pushed forwards around the foregut. All the lobes open into the very short midgut in the thoracic region of the crab.

During dissection, extensive deposits of white material were observed throughout the haemocoel. This was readily broken down by uricase and gave a strong colour reaction with the uric acid test kit, indicating that it contained uric acid. Similar deposits of uric acid have been reported in other land crabs (Gifford, 1968; Wood & Randall, 1981).

Distribution of xanthine oxidase activity

Homogenates of eight different tissues were tested for activity of xanthine oxidase. No activity was detected in the homogenates of the heart, gills, lungs, hindgut or muscle from the second pereiopod (Table 4). Substantial activity, however, was present in homogenates of all three regions of the midgut gland from all individuals that were tested (Table 4). Activity was uniformly high in each of the three regions. No activity of xanthine dehydrogenase was detected in the tissues examined.

Discussion

The faeces were the principal route of nitrogenous excretion in *B. latro* contributing at least 96·2 % of the total excreted nitrogen (Table 5). Of the nitrogenous excretory products, uric acid was by far the most important, comprising 82·6 % of the faecal excretion and 79·5 % of total nitrogenous excretion. Urea and free amino acids were unimportant constituents making up only 5·3 % of total excretion. It was not possible to distinguish between amino acids of dietary or microbial origin and amino acids excreted into the faeces but this component was, in any case, very small. Ammonia contributed some 15 % of total excreted nitrogen but, as discussed above, this was almost certainly an

Table 5. *Summary of the rates of excretion of nitrogenous wastes (μmol nitrogen kg^{-1} body mass h^{-1} ± S.D.) and the importance of the different excretory routes in* Birgus latro

Excretory route	Uric acid	Urea	Ammonia	Amino acids	Total
Faeces ± S.D.	11·81 ±18·02 (82·6 %)	0·36 ±0·476 (2·5 %)	1·70 ±1·86 (11·9 %)	0·423 ±0·895 (3·0 %)	14·29 ±19·07 (96·2 %)
Urine ± S.D.	$2·6\times10^{-5}$ $\pm6·2\times10^{-5}$	$8·4\times10^{-5}$ $\pm2·65\times10^{-4}$	$6·1\times10^{-5}$ $\pm2·04\times10^{-4}$	$1·5\times10^{-5}$ $\pm3·7\times10^{-5}$	$1·87\times10^{-4}$ $\pm5·19\times10^{-4}$ (0·0012 %)
Gaseous ± S.D.			0·565 ±1·420		0·565 (3·8 %)
% Total nitrogen all routes	79·48	2·42	15·24	2·85	

Values are means of data for all threee dietary regimens.
Percentage values indicate the contribution of each product or each route to total excretion.

overestimate due to microbial breakdown of undigested material in the faeces. Quite clearly *B. latro* is uricotelic, apparently uniquely so amongst the Crustacea, e.g. compare data for *Cardisoma guanhumi* which apparently excretes 71·8 % of its waste nitrogen as ammonia and only 4·5 % as urate (Horne, 1968).

It is probable that other land crabs may also excrete purines. The large deposits of uric acid and often high concentrations of uric acid in the haemolymph reported in a variety of land crabs (Gifford, 1968; Horne, 1968; Henry & Cameron, 1981) indicate an active purine metabolism. Elimination *via* the faeces is the normal method of excretion by animals producing purines and it is rather surprising that no systematic studies of this route have been reported for land crabs. In *B. latro*, faeces containing purines were produced at irregular intervals, often with several days between excretory bouts, whereas brown faeces were produced daily in feeding animals. Reliable measurement of faecal nitrogen excretion thus requires long-term experiments and in 24 h studies (Horne, 1968) purine excretion could pass undetected. Data for long-term excretion of faecal nitrogen are available for *Gecarcinus lateralis* and *Cardisoma guanhumi* (Wolcott & Wolcott, 1984, 1987) but the individual excretory components have not been analysed. In *Cardisoma guanhumi*, it has been suggested that uric acid deposits are eliminated at the moult with the exuviae. This seems unlikely, as it would necessitate transport through the epidermis and new cuticle in the premoult stage and storage in the moulting fluid. In any case, most terrestrial crustaceans eat the exuviae after the moult (Greenaway, 1985, 1988).

Given that *B. latro* excretes the bulk of its nitrogenous waste as uric acid, it must possess the metabolic pathways for *de novo* synthesis of the purine ring. As uric acid is accumulated, if not excreted, in terrestrial brachyurans (see below) it is probable that they too have *de novo* synthetic ability. The relationship between

Brachyura and Anomura is remote, dating to the Jurassic (Warner, 1977; Hartnoll, 1988) and, given the reported absence of synthetic pathways for purines in the crustaceans generally (Claybrook, 1983), it seems likely that ability in this direction has arisen independently in terrestrial anomurans and brachyurans. In birds the nitrogen atoms of the purine ring originate from the amino-N of glutamine, glycine and aspartate (Lehninger, 1982). There is no information on the presence of these substances in the haemolymph of *B. latro* but glycine is present at high concentrations in muscle whereas aspartate and glutamate are found as traces (Henry & Cameron, 1981).

Purine nucleotides are degraded *via* nucleosides to xanthine, which in turn is broken down to uric acid by the enzyme xanthine oxidase. The purine ring structure formed by *de novo* synthesis is inosinic acid which is then degraded to uric acid *via* xanthine. Thus, the presence of xanthine oxidase is a prerequisite for the formation of uric acid by both pathways; its presence in a tissue is indicative of this function. Of the eight tissues examined in *B. latro* only the midgut gland showed xanthine oxidase activity. The midgut gland, then, is indicated as the site of uric acid production in *B. latro* and it is perhaps significant that the midgut is also the only point at which solid material can be introduced into the lumen of the gut as the other areas are lined with cuticle. Uric acid was produced at intervals as white faeces, quite separate from the brown faeces which contained only undigested food material. Clearly, large amounts of uric acid were introduced into the gut lumen in a short period, which implies either storage within the midgut gland with periodic expulsion into the gut or episodic production of uric acid by the midgut gland.

Substantial deposits of uric acid were found in the haemocoel of *B. latro* and their role should be considered. Two possibilities exist; first, the deposits could represent waste nitrogen which cannot be excreted, and second, they could be a metabolic reserve of nitrogen which is useful to the animal under certain conditions. As *B. latro* can excrete uric acid, the second alternative seems more probable. In the cockroach *Periplaneta americana*, urate deposits in the fat body are utilized when the animals are fed a protein-free diet (McEnroe, 1966; Mullins & Cochran, 1975) and it is conceivable that urate deposits in land crabs may also provide a source of nitrogen under similar conditions. It has also been suggested that urate, by binding ions, might act as a store of salts (Mullins, 1979; Wolcott & Wolcott, 1984) which could be used to maintain haemolymph concentration in the postmoult period or after injury. Urate deposits might also play a minor role during dehydration, restricting the rise in haemolymph concentration. Deposits are reported from most of the land crabs studied (Gifford, 1968; Horne, 1968; Henry & Cameron, 1981; Wolcott & Wolcott, 1984, 1986) but their size is variable, with water availability and moult stage suggested as controlling factors (Gifford, 1968; Wood & Boutilier, 1985). A strong correlation was found between dietary intake of nitrogen and the size of uric acid deposits in *Gecarcinus lateralis* and *Cardisoma guanhumi* (Wolcott & Wolcott, 1984, 1986). It seems intuitively unlikely that such large stores of uric acid would be accumulated if they were not

metabolically useful but, at present, the ability of land crabs, other than *B. latro*, to excrete uric acid is unknown. In insects uric acid in the fat body is stored in urate cells. In crabs the material is assumed to be present as an extracellular deposit in the haemocoel but no histological or ultrastructural evidence is available to corroborate this.

The urine of *B. latro* is reported to be near isosmotic with the haemolymph (Gross, 1964) but the fluid released by crabs in the second series of experiments was generally of much lower concentration (Table 2). This fluid was not drinking water, which has a much lower concentration still, nor was it a simple dilution of urine with drinking water as potassium was elevated above haemolymph values whereas other ions were reduced in concentration. The likelihood is that it was the final excretory product 'P' described for several land crabs by Wolcott & Wolcott (1985), i.e. urine which was released into the branchial chambers where its composition was modified by ion-transporting cells of the gills and perhaps by evaporation. It is apparent, then, that *B. latro* reprocesses its urine, reabsorbing much of its salt content when given fresh drinking water. The carbonic anhydrase present in the cytoplasm of the epithelial cells of the gill could supply HCO_3^- as a counterion for uptake of Cl^- from the urine (S. Morris & P. Greenaway, unpublished data).

Although these experiments were designed to determine the form of excretory nitrogen rather than the rate of excretion it is, nevertheless, of interest to compare the values obtained for the rate of nitrogenous excretion with data for other land crabs. Ignoring the urinary contribution, the mean rate of excretion varied from 7·07 to 22·82 μmol kg^{-1} body mass h^{-1}, which is towards the bottom end of the range measured for *Cardisoma carnifex* (10–100 μmol kg^{-1} body mass h^{-1}) (Wood & Boutilier, 1985), *Cardisoma guanhumi* (Horne, 1968) and considerably less than the value for *Gecarcinus lateralis* (85·8 μmol kg^{-1} body mass h^{-1}) estimated from the data of Wolcott & Wolcott (1984). At first sight, then, nitrogenous excretion in *B. latro* appears to be rather low. If one looks at the data for individuals of *B. latro*, however, the range for all diets was 0–73·8 μmol kg^{-1} body mass h^{-1}. Comparisons of rates of nitrogenous excretion are subject to numerous difficulties and careful studies of nitrogen balance are necessary to determine the amount of food eaten, its nitrogen content and the retention of nitrogen as protein within the body. Added to these variables in land crabs is the retention of excretory nitrogen as uric acid deposits, in unknown proportion. Unless these factors are taken into consideration, it is impossible to gauge the rate of excretion with any accuracy. For *B. latro* we are only able to say that nitrogenous excretion is within the range for other land crabs.

This project was supported by ARGS grant no. A 18616299 to PG and NATO/NSERC UK and AHMFR grant 005299 to SM. We are grateful to D. Hair and Karen Brouwer for technical assistance and to the staff of ANPWS, Christmas Island for their support during collection of animals.

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