The Journal of Experimental Biology 200, 2347–2354 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JEB0952

URATE DEPOSITS IN THE GECARCINID LAND CRAB *GECARCOIDEA NATALIS* ARE SYNTHESISED *DE NOVO* FROM EXCESS DIETARY NITROGEN

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Accepted 18 June 1997

Summary

The urate content of the gecarcinid land crab *Gecarcoidea natalis* was correlated to the amount of nitrogen assimilated. Crabs fed a high-nitrogen diet (*ad libitum* amounts of soy beans and fig leaves, *Ficus macrophylla*) for 6 weeks assimilated approximately 23 times more nitrogen (33.9±5.6 mmol kg^{-1} day^{-1}) than animals fed a diet low in nitrogen (fig leaves alone) (1.5±0.7 mmol kg^{-1} day^{-1}). Animals maintained on a high-nitrogen diet accumulated urate (67.1±29.4 mmol kg^{-1} dry mass), while animals fed the low-nitrogen diet did not accumulate significant amounts of urate compared with the control animals killed at the beginning of the dietary period. The urate deposits clearly originate from the excess dietary nitrogen ingested on the high-nitrogen diet. The intake of preformed dietary purine was low (0.028±0.005 mmol kg^{-1} dry mass) and at most could only account for 0.04 % of the urate accumulated by crabs fed the high-nitrogen diet. This indicates that the urate was synthesised *de novo*. When crabs were fed a high-nitrogen diet supplemented with [^{15}N]glycine, the ^{15}N heavy isotope was incorporated into urate. This provided direct evidence that the urate was synthesised *de novo*.

Key words: urate, land crab, *Gecarcoidea natalis*, dietary nitrogen, purine synthesis, Crustacea.

Introduction

A number of invertebrate taxa store large amounts of solid purine within their bodies, the best known being the insects and crustaceans. Cockroaches, termites and lepidopterans all synthesise urate *de novo* and store it intracellularly in the fat body (cockroaches, Cochran, 1985; termites, Potrikus and Breznak, 1980, 1981; lepidopterans, Buckner and Caldwell, 1980; Buckner *et al.* 1985; Levenbrook *et al.* 1971). In cockroaches, urate accumulation is a mechanism for the storage of excess dietary nitrogen (for a review, see Cochran 1985). Cockroaches and termites use their urate stores as a nitrogen reserve, which can be degraded and the released nitrogen recycled into tissue protein (for a review, see Cochran, 1985; Mullins *et al.* 1992). In Lepidoptera, however, solid urate acts as a temporary store of accumulated excretory nitrogen (e.g. Levenbrook *et al*. 1971; Buckner and Caldwell, 1980). Terrestrial crustaceans, such as decapods and isopods, also store solid purine within their bodies, often in very substantial amounts (for reviews, see Greenaway, 1991; O'Donnell and Wright, 1995). In the land crab *Gecarcoidea natalis*, 85 % of the stored purine is urate and the remaining 15 % consists of hypoxanthine, guanine and xanthine (Linton and Greenaway, 1997). Urate is also the dominant purine stored by other crustaceans (for reviews, see Greenaway, 1991; O'Donnell and Wright 1995). The urate stores in the Crustacea are intracellular and are located in connective tissue cells in, for example, the crabs *Gecarcoidea natalis* and *Callinectes sapidus*, the amphipod *Niphargus schellenbergi* and the isopod *Asellus aquaticus* (Linton and Greenaway, 1997; Johnson, 1980; Graf and Michaut, 1975; Wägele, 1992).

The purines stored by crustaceans may be derived in two ways; from the diet as preformed dietary purine and by *de novo* synthesis from non-purine precursors. Preformed purine in the diet would largely be present as nucleotides (AMP, GMP), nucleosides (adenosine and guanosine) and purine bases (adenine and guanine), which can be readily degraded to urate (Stryer, 1988). In the *de novo* synthetic pathway, urate is synthesised from a range of precursors which contribute carbon and nitrogen atoms as follows: 1 glycine (2 carbons and 1 nitrogen), 2 glutamines (1 nitrogen each), 1 aspartate (1 nitrogen), 2 N^{10}-formyl tetrahydrofolate derivatives (1 carbon each) and carbon dioxide (1 carbon) (Stryer, 1988). The Crustacea were believed to lack the ability to synthesise purines *de novo* (Claybrook, 1983), but evidence for *de novo* synthetic capability has been presented for the brine shrimp *Artemia salina* and the robber crab *Birgus latro* (Liras *et al.* 1992; Greenaway and Morris, 1989).

In both isopods and gecarcinid crabs, it has been suggested that urate accumulation performs a storage excretion role (Dresel and Moyle, 1950; Horne, 1968; Gifford, 1968; Wolcott and Wolcott, 1984, 1987; Henry and Cameron, 1981; Wolcott,

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1991). Terrestrial crustaceans, however, have efficient nitrogen excretory mechanisms and are well able to excrete waste nitrogen as ammonia under normal conditions (reviewed in Greenaway and Nakamura, 1991; O'Donnell and Wright, 1995). Given this situation, it is unlikely that storage excretion is necessary in Crustacea under normal environmental conditions.

An alternative function for the urate deposits is the storage of excess dietary nitrogen for later use, as reported for cockroaches. In support of this hypothesis, the urate content of the gecarcinid crabs *Gecarcinus lateralis* and *Cardisoma guanhumi* is positively correlated with their intake of dietary nitrogen (Wolcott and Wolcott, 1984, 1987). Similarly, in *Gecarcoidea natalis*, the urate cells in animals fed a high-nitrogen diet were larger, had a higher urate concentration, and were present in greater numbers and at more connective tissue sites than the urate cells in animals maintained on a low-nitrogen diet (Linton and Greenaway, 1997).

This study utilised the herbivorous land crab *Gecarcoidea natalis* to determine whether stored purine originated from preformed dietary purine or was synthesised *de novo* from excess dietary amino acid nitrogen. Two approaches were used. The first utilised nitrogen balance experiments which assessed the intake of purine and non-purine nitrogen and related this to the measured urate accumulation over the experimental period. In this way, the amounts of nitrogen assimilated and urate accumulated could be correlated, and the maximum contribution that preformed dietary purines could make to the accumulated purine could be determined. By correlating urate levels of animals on diets containing different nitrogen levels with the amount of nitrogen assimilated, it was possible to elucidate whether urate stores represent storage of excess dietary nitrogen. In the second approach, crabs were fed a diet containing ^{15}N-enriched glycine and the appearance of ^{15}N in stored purines was measured. As the purine ring is synthesised *de novo* from amino acids, the incorporation of ^{15}N into purine is direct evidence of *de novo* purine synthesis from excess dietary nitrogen.

Materials and methods

Collection and maintenance of animals

Gecarcoidea natalis (Pocock) of both sexes and weighing 100–200 g were collected from rainforest on Christmas Island and air-freighted to Sydney. In the laboratory, they were maintained at 25 °C and 80 % humidity with a 12 h:12 h light:dark cycle. Crabs were kept in large communal containers and supplied with tap water and fallen leaves. Occasionally their diet was supplemented with fruit (apples and figs). Recently collected animals were used in the experiments.

Nitrogen balance experiment

Three groups of ten animals which had been fed on fig leaves were selected for the experiment. The first group was killed at time zero and the concentration of urate in their bodies determined to establish the level in the population at the beginning of the experiment. Animals in the remaining two groups were housed individually in fish boxes (645 mm×413 mm×276 mm) and supplied with tap water. One group was supplied *ad libitum* with a high-nitrogen diet which consisted of fallen fig leaves (*Ficus macrophylla*) (600 μmol N g^{-1} dry mass; 68.84 nmol total purine g^{-1} dry mass) and soy beans (4622 μmol N g^{-1} dry mass; 338.1 nmol total purine g^{-1} dry mass) for 6 weeks (42 days). The other group was fed a low-nitrogen diet (fallen fig leaves only) over the same period. Each day, excess fresh food was given (dry mass known) and faeces and uneaten food were collected and dried at 60 °C. Dry matter intake and assimilation were then assessed gravimetrically. Assimilation coefficients for dry mass and nitrogen were calculated from the following general equation:

$$\text{assimilation coefficient} = 100 \times [1 - (\text{faecal output/food intake})] .$$

After this feeding period, the animals were killed by chilling, dried to a constant mass at 60 °C and pulverised to a homogeneous powder.

Evidence for de novo *synthesis*

Twenty animals were placed in fish boxes as described above and acclimated to an artificial diet high in nitrogen for 1 week. The diet was prepared in batches by mixing 10 g of powdered fig leaves, 10 g of powdered soya beans and 5 g of agar into 100 ml of boiling deionised water and allowing it to set in shallow trays. Each day, excess food (% dry mass known) was supplied to each crab, and uneaten food and faeces were removed and dried at 60 °C. Dry matter assimilation was determined gravimetrically as described above.

After the acclimation period, the crabs were split into an experimental and a control group (ten crabs in each) and the experiment was continued for a further 2 weeks. The diet of the control group differed from the artificial diet described above by the inclusion of 2 % (dry mass) commercial glycine (Sigma Chemical Company, St Louis, MO, USA). The diet of the experimental group was the basic artificial diet supplemented with 1.2 % (dry mass) ^{15}N-labelled glycine ([^{15}N]glycine, 99.8 atom %, Sigma Chemical Company, St Louis, MO, USA). At the end of the experiment, all animals were killed by chilling, dried at 60 °C and pulverised.

Analyses

Food, faeces and animal carcases were ground to a fine powder in a Fritsch 'Pulverisette 14' rotor speed mill using a 1 mm screen prior to analyses.

Nitrogen

Nitrogen concentrations were determined in duplicate feed, faecal and animal samples using a micro-Kjeldahl system (Gerhardt Kjeldatherm and Vapodest 3,C. Gerhardt, Bonn) as described previously (Greenaway and Linton, 1995).

Urate

Triplicate samples of milled animal carcases (approximately 0.15 g) were accurately weighed and extracted for 30 min with

5 ml of 68 mmol l^{-1} $LiCO_3$ at 60 °C with frequent vortex mixing. The suspension was then centrifuged at 10 000 ***g*** for 10 min, and the supernatant decanted off and retained. To ensure complete solubilisation, the extraction procedure was repeated and the two supernatants were pooled. Subsamples of this supernatant were neutralised with 0.1 mol l^{-1} HCl and diluted with 4 mmol l^{-1} KH_2PO_4 buffer (pH 5.8). This solution was then deproteinised by centrifugation at 5000 ***g*** for 20 min in a ultrafiltration device with a 30×10^3 M_r cut-off limit (Ultrafree-MC with regenerated cellulose membrane; Millipore Corporation, Bedford MA, USA). Before injection onto the HPLC column, the deproteinised samples were diluted further by a factor of either 5, 10 or 20 times with KH_2PO_4 buffer, pH 5.8. This dilution bought the purine concentrations in the samples into the calibrated range of the column.

The urate concentrations in samples were determined by reverse-phase HPLC using a Waters 600E system, a μBondapak C18 column and a Waters 486 ultraviolet absorption detector (Waters Millipore, Milford, MA, USA) (Linton and Greenaway, 1995). In the present study, the flow rate of the eluent buffer (4 mmol l^{-1} KH_2PO_4, pH 5.8) was 1 ml min^{-1}. A mixed purine standard containing urate, guanine, hypoxanthine and xanthine at 10 μmol l^{-1}, 20 μmol l^{-1} or 50 μmol l^{-1} was used to calibrate the column.

Purification of urate for mass spectroscopy

Urate extracted from milled animal samples as described above was purified as follows. A sample of the extract was neutralised with 0.1 mol l^{-1} HCl and diluted with 0.1 mol l^{-1} ammonium acetate buffer, pH 3.89. This solution was loaded onto a C18 Sep-pak cartridge (Waters, Milford, MA, USA) which had been prewetted with methanol and equilibrated with 0.1 mol l^{-1} ammonium acetate buffer, pH 4. The Sep-pak column was then washed with 0.1 mol l^{-1} ammonium acetate buffer (pH 4) and the purines eluted with 50 % methanol. The eluent was evaporated to dryness at 60 °C and the dry samples reconstituted in 2 ml of 50 mmol l^{-1} ammonium acetate buffer (pH 5.8) at 60 °C for 30 min and then injected onto the HPLC column. Urate (50–90 μg) was purified by semi-preparative HPLC using the equipment described above. The column was perfused with 50 mmol l^{-1} (pH 5.8) ammonium acetate buffer at a rate of 1 ml min^{-1}. The urate fraction was detected by its absorbance at 265 nm and the middle 1 ml of the urate fraction was collected by a Waters fraction collector (Waters, Milford, MA, USA). The volatile ammonium acetate buffer was removed from the collected urate fractions by drying them *in vacuo* at 60 °C.

Mass spectroscopy

A tetra-trimethylsilourate (TMS) derivative was prepared from the purified urate. The relative abundance of the molecules of this derivative with relative molecular masses of M and M+1 was determined by gas chromatography (HP 5890 Series II gas chromatograph with an SGE 12QC2/BP1 0.25 column) and mass spectroscopy (HP 5971A mass-selective detector). After integration, these intensities were expressed as percentage amounts relative to the amount of the molecule of molecular mass M.

Measurement of total purines in foodstuffs

Nucleic acids were extracted from powdered soya beans and fig leaves using the method of Tyner *et al.* (1953). Purine bases were then liberated by formic acid hydrolysis (Wyatt and Cohen, 1953), and the formic acid was removed by drying the samples in a vacuum. The residue was reconstituted in 4 mmol l^{-1} KH_2PO_4 buffer (pH 5.8) at 60 °C for 30 min and further diluted with the same buffer. The diluted samples were filtered by centrifugation at 5000 ***g*** for 10 min in microcentrifuge filtration devices (0.45 μm pore size) (Lido Manufacturing Corp., Kenosha, WI, USA). The total amounts of purine bases in these filtered solutions were then analysed by HPLC as described above. The HPLC column was calibrated with a mixed purine standard containing urate, guanine, xanthine, hypoxanthine and adenine at 5 μmol l^{-1}, 10 μmol l^{-1}, 20 μmol l^{-1} and 50 μmol l^{-1}.

Statistics

All data are expressed as means ± S.D. Means were compared using one-way analysis of variance (ANOVA) and *a posteriori* comparisons were performed using the LSD method (SPSS for Windows 6.0).

Results

Nitrogen balance experiment

Crabs on both diets consumed similar amounts of dry matter, but animals fed the low-nitrogen diet had a lower assimilation coefficient for dry matter (Table 1). Animals maintained on the high-nitrogen diet consumed 4.5 times, and assimilated 23 times, more nitrogen than crabs fed the low-nitrogen diet (Table 1). The assimilation coefficient for nitrogen was 4.7 times larger for crabs maintained on the high-nitrogen diet (Table 1). Preformed purine constituted a very small percentage of the total nitrogen intake; 0.008 % of the total nitrogen consumed by crabs fed the low-nitrogen diet and 0.011 % on the high-nitrogen diets (Table 1).

Crabs maintained on the low-nitrogen diet for 6 weeks had similar concentrations of urate nitrogen, non-urate nitrogen (NUN) and total nitrogen in their bodies to those measured in the control group killed at the start of the experiment (Table 2). In contrast, the crabs fed a high-nitrogen diet for the same period had substantially higher urate nitrogen, NUN and total nitrogen concentrations than either the control group or the crabs maintained on the low-nitrogen diet (Table 2). Thus, crabs fed the high-nitrogen diet for 6 weeks accumulated 67.1±29.4 mmol urate kg^{-1} dry mass (equivalent to 268±118 mmol nitrogen kg^{-1} dry mass), 363±146 mmol NUN kg^{-1} dry mass and 631±175 mmol total N kg^{-1} dry mass (Table 2).

Accumulated urate and non-urate nitrogen must have originated from the assimilated nitrogen. Of the nitrogen assimilated by animals fed a high-nitrogen diet, 18.5±12 % was

Table 1. *Dietary intake and nitrogen assimilation in* Gecarcoidea natalis *maintained on a low-nitrogen diet (*ad libitum *amounts of fallen leaves of* Ficus macrophylla*) or a high-nitrogen diet (*ad libitum Ficus *leaves plus soya beans)*

	Low-N diet	High-N diet
Dry matter consumed ($g\,kg^{-1}\,day^{-1}$)		
Fig leaf	15.0±4.6	8.2±1.4
Soya bean	–	7.8±1.4
Total	15.0±4.6[a]	16.0±2.5[a]
Dry matter assimilated ($g\,kg^{-1}\,day^{-1}$)	4.4±1.4[a]	10.0±1.3[b]
Assimilation coefficient for dry matter	30.2±9.8[a]	63.0±5.8[b]
Nitrogen intake ($mmol\,N\,kg^{-1}\,day^{-1}$)	9.1±2.9[a]	41.1±7.2[b]
Nitrogen assimilated ($mmol\,N\,kg^{-1}\,day^{-1}$)	1.5±0.7[a]	33.9±5.6[b]
Assimilation coefficient for nitrogen	17.7±10.6[a]	82.7±3.5[b]
Purine intake ($\mu mol\,kg^{-1}\,day^{-1}$)	0.206±0.063[a]	0.642±0.110[b]
Purine intake as a percentage of nitrogen intake	0.008	0.011

All values (means ± S.D.) are expressed per kilogram dry animal mass.

Different superscripts within a row indicate that means differed significantly (P<0.05); N=10.

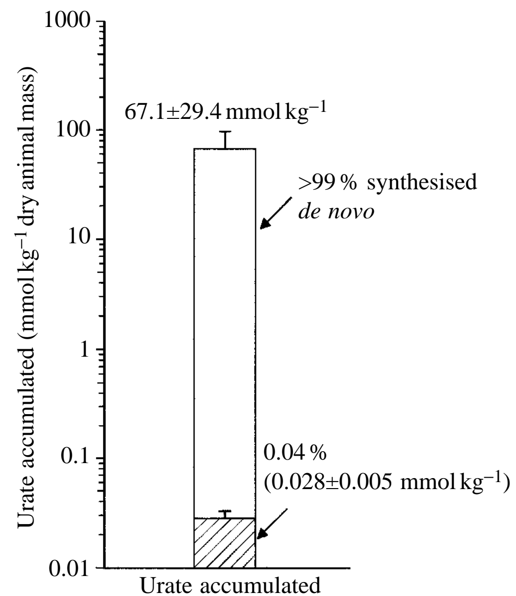

Fig. 1. Urate accumulation by *Gecarcoidea natalis* maintained on a high-nitrogen diet for 6 weeks (mean ± 95 % confidence limits). The hatched bar represents the maximum contribution from intake of dietary purine (mean ± S.D.). All values are expressed per kilogram dry mass; N=10.

channelled into urate stores and 27.0±21.7 % was converted into non-urate nitrogen. Thus, these animals retained 45.5±18.5 % of their assimilated nitrogen. Approximately half (54.5±18.5 %) of the assimilated nitrogen was unaccounted for and was probably excreted. The total amount of preformed purine consumed ($0.028\pm0.005\,mmol\,kg^{-1}$ dry mass) by animals on the high-nitrogen diet was equivalent to only 0.04 % of the urate accumulated (Fig. 1).

[^{15}N]glycine incorporation

In this experiment, crabs were fed an artificial high-nitrogen diet supplemented with either commercial glycine (controls) or ^{15}N-enriched glycine (experimental group). Intake of glycine and dry matter and dry matter assimilation were statistically similar in the two groups (Table 3). Dry matter assimilation and consumption rates were also similar to the corresponding values for animals fed a high-nitrogen diet in the nitrogen balance experiment (Tables 1, 3).

Urate extracted from crabs that had been fed ^{15}N-enriched glycine (experimental group) had more urate containing one (M+1) heavy isotope than urate extracted from crabs in the control group (fed non-enriched glycine) (P<0.001) (Fig. 2). The heavy isotopes in a M+1 molecule could be ^{13}C, ^{2}H ^{18}O or ^{15}N. As the only difference between the two groups was ^{15}N enrichment of the glycine in the diet fed to experimental crabs, the additional heavy isotope must have been ^{15}N. Thus,

Table 2. *Nitrogen concentrations in* Gecarcoidea natalis *maintained on high- or low-nitrogen diets*

	Time 0	Low-N diet	High-N diet	Nitrogenous compounds accumulated on high-N diet
Wet body mass (kg)	0.174±0.025[a]	0.184±0.027[a]	0.161±0.023[a]	
Dry body mass (kg)	0.068±0.008[a]	0.069±0.0012[a]	0.068±0.0010[a]	
Urate ($mmol\,kg^{-1}$)	48.5±50.0[a]	72.4±40.4[a]	115.6±45.1[b]	67.1±29.4
Urate nitrogen ($mmol\,N\,kg^{-1}$)	194±200[a]	290±161[a]	462±181[b]	268±118
Non-urate nitrogen ($mmol\,N\,kg^{-1}$)	3658±216[a]	3716±200[a]	4021±255[b]	363±146
Total nitrogen ($mmol\,N\,kg^{-1}$)	3852±295[a]	4006±287[a]	4483±219[b]	631±175

Levels in the control group (time 0) were measured at the start of the experiment and other values after 6 weeks on an high- or low-nitrogen diets. Amounts of nitrogenous compounds (mean ± 95% confidence limits) accumulated over 6 weeks by *G. natalis* fed a high-nitrogen diet were calculated.

Different superscripts within a row indicate that the means differed significantly (P<0.05).

All values (means ± S.D.) are expressed per kilogram dry weight animal mass; N=10.

Table 3. *Dietary intake by* Gecarcoidea natalis *maintained on an artificial high-nitrogen diet*

	Control	Experimental
Glycine eaten ($g\,kg^{-1}$)	4.8±2.2	2.84±3.09
Assimilation coefficient for dry matter	68.3±9.4*	66.2±8.9*
Dry matter consumed ($g\,kg^{-1}\,day^{-1}$)	14.18±6.56*	19.17±3.83*

This diet consisted of powdered fig leaf, soya bean and glycine in an agar gel. In experimental animals, the glycine was ^{15}N-enriched.

All values (means ± S.D.) are expressed per kilogram dry animal mass.

Control and experimental means did not differ significantly (P>0.05).

*Indicates that the means did not differ from the corresponding means for animals fed *ad libitum* with fig leaves and soya beans for 6 weeks (high-nitrogen diet) (see Table 1); (P>0.05); N=10.

urate purified from the experimental group of animals contained amino ^{15}N from [^{15}N]glycine. The calculated probability of a TMS–urate derivative containing one, naturally occurring, heavy isotope of ^{13}C, ^{2}H, ^{18}O or ^{15}N is 40.3. This was very close to the M+1 relative percentage measured in urate extracted from crabs in the control treatment (Fig. 2).

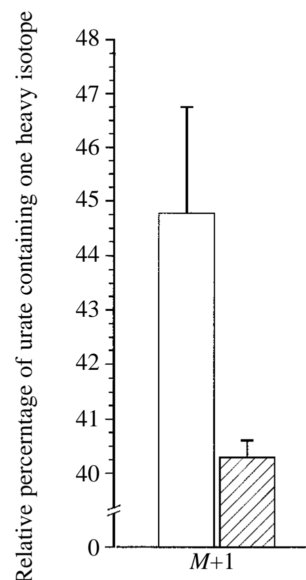

Fig. 2. Incorporation of heavy nitrogen (^{15}N) into urate by *Gecarcoidea natalis*. The relative amount of urate containing one heavy isotope (M+1) is measured. The urate was isolated from crabs fed either [^{15}N]glycine (experimental treatment, open bar) or commercial glycine (control treatment, hatched bar). The experimental treatment mean was significantly higher than the control mean because the urate from the experimental animals contained ^{15}N from [^{15}N]glycine (P<0.001). Values are means ± S.D.; N=10.

Discussion

Nitrogen balance

In the present study, the urate nitrogen and non-urate nitrogen (NUN) contents of *G. natalis* were closely correlated with the amount of nitrogen assimilated. Crabs fed a low-nitrogen diet of leaves for 6 weeks contained similar amounts of NUN, urate and total nitrogen to animals killed at the beginning of the dietary period (Table 2) so that, in the short term, they were in approximate nitrogen balance. A similar situation has been reported for the gecarcinid crabs *G. lateralis* and *C. guanhumi* (Wolcott and Wolcott, 1987). Crabs maintained on a high-nitrogen diet for 6 weeks had a higher nitrogen intake and assimilated 23 times more nitrogen than animals maintained on a low-nitrogen diet (Table 1). These animals substantially increased both their urate and NUN contents (Table 2).

De novo *synthesis*

The amount of preformed dietary purine consumed by animals fed the high-nitrogen diet could at most represent 0.04 % of the urate accumulated over the experimental period (Fig. 1). Assuming that all of the dietary purine was added to the stores, more than 99 % of the urate must have been synthesised *de novo* (Fig. 1). This provides very strong circumstantial evidence that *Gecarcoidea natalis* possesses the ability to synthesise urate *de novo* from dietary amino acids.

Direct evidence for such *de novo* synthesis was provided using ^{15}N-enriched glycine. This amino acid, in the *de novo* synthetic pathway, contributes one of the nitrogen atoms in the purine (Stryer, 1988). In the crabs fed ^{15}N-enriched glycine, the heavy isotope was clearly incorporated into urate and appeared in the urate stores. Hence, the urate was synthesised *de novo* from dietary amino acids (Fig. 2).

These two data sets provide unequivocal evidence that *Gecarcoidea natalis* can and does synthesise urate *de novo*. From this evidence, and the positive correlation between urate content and nitrogen retention (Table 1, 2), it is clear that urate accumulation is a vehicle for storage of excess dietary nitrogen. The synthesised urate is stored as a solid in spongy connective tissue cells throughout the body (Linton and Greenaway, 1997).

The increase in NUN content of *Gecarcoidea natalis* fed a high-nitrogen diet is consistent with increased protein levels and tissue growth on the high-nitrogen diet. This response to increased dietary nitrogen intake suggests that, in the usual diet of fallen leaves, nitrogen is a limiting nutrient (Matterson, 1980). A similar situation exists in other gecarcinid land crabs (Wolcott and Wolcott, 1984, 1987).

Of the nitrogen assimilated by animals maintained on the high-nitrogen diet, 18.5±12 % was channelled into urate stores and 27.0±21.7 % was converted into non-urate nitrogen. Approximately half (54.5±18.5 %) of the assimilated nitrogen was unaccounted for and was presumably excreted. The rationale for nitrogen excretion by a nitrogen-limited animal should be explained. Excretion may be unavoidable if the

dietary amino acids are transaminated and deaminated faster than they can be incorporated into urate or non-urate nitrogen. The ammonia produced may have to be excreted to prevent toxicity.

Strong correlations between nitrogen assimilation and urate content of the body have been reported for two other gecarcinid crabs, *Cardisoma guanhumi* and *Gecarcinus lateralis* (Wolcott and Wolcott, 1984, 1987), and it is probable that urate accumulated in these species also represents storage of excess dietary nitrogen. Indeed, storage of excess dietary nitrogen as urate may be common to the numerous other species of crustaceans that store solid urate (for reviews, see Greenaway, 1991; O'Donnell and Wright, 1995). Synthesis of urate and subsequent intracellular storage is certainly common in cockroaches (McEnroe and Forgash, 1957; Cochran, 1985).

The present study has provided a direct demonstration that a terrestrial brachyuran crustacaean can synthesise purines *de novo* from amino acid nitrogen. There is also direct evidence that the branchiopod crustacean *Artemia salina* synthesises purines *de novo* from amino acids (Liras *et al.* 1992). In the terrestrial anomuran *Birgus latro*, there is strong indirect evidence for *de novo* synthesis, since the main nitrogenous excretory product of this species is urate and its midgut gland has high xanthine oxidase activities, a key enzyme in the production of urate from other purines (Greenaway and Morris, 1989). The magnitude of the purine deposits contained in many terrestrial crustaceans (Henry and Cameron, 1981; Greenaway, 1991; Linton and Greenaway, 1997) also suggests *de novo* synthesis. The capability to synthesise purine *de novo* in such evolutionarily diverse species of Crustacea indicates that, contrary to previous ideas (Claybrook, 1983), this ability may be common to the Phylum.

The enzymatic steps involved in purine synthesis in the Crustacea may well be similar to those described for insects and birds (Barret and Friend, 1970; McEnroe and Forgash, 1957; Stryer, 1988). The first enzyme in the pathway, 5-phosphoribosylpyrophosphate amidotransferase, has been demonstrated in *Artemia salina* (Liras *et al.* 1990), while enzymes for the last step in urate formation, xanthine dehydrogenase or xanthine oxidase, are reported from a number of species of crabs (Greenaway and Morris, 1989; Dykens, 1991; Lallier and Walsh, 1991). Crustaceans also incorporate carbon and nitrogen from glycine into the purine ring (Liras *et al.* 1992; present study).

The paucity of organelles in the urate storage cells of *Gecarcoidea natalis* suggests that purine synthesis may occur at a different site (Linton and Greenaway, 1997). The probable site is the midgut gland as this organ shows significant xanthine dehydrogenase/oxidase activities in those terrestrial decapods that have been studied (Greenaway and Morris, 1989; Dykens, 1991; Lallier and Walsh, 1991).

Actual urate accumulation by *Gecarcoidea natalis* maintained on a high-nitrogen diet was 1.6 ± 1.1 mmol kg^{-1} dry mass day^{-1}. Xanthine oxidase/xanthine dehydrogenase activities indicate that urate production for *Cardisoma guanhumi* (11.48 mmol urate kg^{-1} dry mass day^{-1}), *Birgus latro* (18.35 mmol urate kg^{-1} dry mass day^{-1}) and *Gecarcinus lateralis* (2.03 mmol urate kg^{-1} dry mass day^{-1}) could potentially be higher than for *Gecarcoidea natalis* (Lallier and Walsh, 1991).

Physiological role of urate deposits

In view of the substantial capacity of *Gecarcoidea natalis* to synthesise and store urate, the physiological function of the reserves should be considered. As outlined in the Introduction, the reserves may possibly represent stored nitrogenous wastes, a cation store or a nitrogen reserve. Storage of nitrogenous wastes seems unlikely since terrestrial crustaceans have efficient excretory mechanisms for nitrogenous waste, either as ammonium ions in excreted fluid or as ammonia gas (for reviews, see Greenaway, 1991; O'Donnell and Wright, 1995). Under dry conditions, when urine production and hence ammonia excretion are curtailed, urate storage may, however, offer a temporary solution to excretory problems (Wolcott, 1991; Greenaway, 1991; Linton and Greenaway, 1995). The robber crab *Birgus latro* already excretes its waste nitrogen as uric acid and it is unnecessary to utilise the same product in storage excretion (Greenaway and Morris, 1989). This indicates that the urate deposits must have some other function.

Internal urate stores potentially exist either as uric acid or as urates combined with body cations (Na^+, K^+, Ca^{2+}, NH_4^+). The latter form could act as an ion reserve. *Gecarcoidea natalis* collected from the field have a mean urate content of 1.73 mmol urate 100 g^{-1} dry mass (S. M. Linton, unpublished data) which, in the form of sodium urate, could bind only 6 % of the total body sodium. Binding of such a small amount of sodium would be insignificant in controlling any perturbations in haemolymph osmolarity caused during dehydration or moulting. Indeed, there is no indication that ion binding plays a significant role in osmoregulation as the osmolality of the haemolymph of terrestrial crabs rises in proportion to the amount of water lost during desiccation (Gross, 1963; Harris, 1977; Harris and Kormanik, 1981; Wood *et al.* 1986; Taylor and Greenaway, 1994). Although the isopods *Armadillidium vulgare* and *Porcellio scaber* maintain their haemolymph osmolarity within a narrow range during dehydration, the level of urate in these animals is too low to contribute significantly to osmoregulation by storage of cations (O'Donnell and Wright, 1995). In contrast, cockroaches, aided by the storage of cations as urate salts in fat body cells, regulate the osmolarity of their haemolymph precisely during dehydration (Wall, 1970; Edney, 1968; Hyatt and Marshall, 1977, 1980, 1985*a*,*b*).

The urate deposits are most likely to function as a nitrogen reserve. The diet of gecarcinid crabs consists principally of leaves and fruit and is low in nitrogen. *Gecarcoidea natalis* shows a number of adaptations characteristic of species with low nitrogen intakes, notably a large body size (up to 500 g), slow growth, long life span (>13 years), a low metabolic rate and infrequent breeding (once a year) (Hicks *et al.* 1990; Adamczewska and Morris, 1994; Greenaway and Linton, 1995). Although this species supplements its diet by occasional

predation or scavenging, the prevalent pattern is one of low nitrogen intake. Whilst this intake is adequate for normal intermoult growth, it may not meet the acute nitrogen requirements associated with moulting and oogenesis. Accumulation throughout the year of small daily nitrogen surpluses and occasional windfalls from animal material as urate could overcome any shortfall. These stores could then be remobilised for use in oogenesis and moulting. The advantage of this system would be that urate could be slowly accumulated throughout the year. The ability of *Gecarcoidea natalis* to remobilise urate is currently being investigated.

We are indebted to Professor M. Duncan and K. Mason of the Biomedical Mass Spectroscopy Unit for preparation and measurement of urate by gas chromatography and mass spectroscopy. This work was supported by an Australian Research Council grant (942611) to P.G. and an Australian Postgraduate Award to S.L.

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