NITROGEN EXCRETION IN TWO PULMONATE LAND SNAILS

By
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# TABLE OF CONTENTS (Continued)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) QUALITATIVE RESULTS</td>
<td>26</td>
</tr>
<tr>
<td>b) QUANTITATIVE RESULTS</td>
<td>41</td>
</tr>
<tr>
<td>TNPN</td>
<td>41</td>
</tr>
<tr>
<td>PROTEIN IN EUGLANDINA EXCRETA</td>
<td>41</td>
</tr>
<tr>
<td>TOTAL NITROGEN EXCRETION BY EUGLANDINA</td>
<td>41</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>43</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>50</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>52</td>
</tr>
<tr>
<td>BIOGRAPHICAL SKETCH</td>
<td>56</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table | Page
-----|------
1. NON-PROTEIN NITROGEN CONTENTS OF KIDNEY AND EXCRETA | 23
2. BALANCE SHEET OF NON-PROTEIN NITROGEN CONTENTS OF KIDNEY AND EXCRETA | 42
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>DIFFUSION CHAMBER FOR DETECTION AND MEASUREMENT OF GASEOUS AMMONIA</td>
<td>12</td>
</tr>
<tr>
<td>2.</td>
<td>CONWAY DIFFUSION DISH</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN URIC ACID AND UNKNOWN COMPOUND OF MESOMPHIX KIDNEY</td>
<td>28</td>
</tr>
<tr>
<td>4.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN GUANINE AND UNKNOWN COMPOUND OF M. KIDNEY</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN URIC ACID AND UNKNOWN COMPOUND OF EUGLANDINA KIDNEY</td>
<td>32</td>
</tr>
<tr>
<td>6.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN GUANINE AND UNKNOWN COMPOUND OF E. KIDNEY</td>
<td>34</td>
</tr>
<tr>
<td>7.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN URIC ACID AND UNKNOWN COMPOUND OF E. EXCRETA</td>
<td>36</td>
</tr>
<tr>
<td>8.</td>
<td>ULTRA-VIOLET SPECTRA OF KNOWN GUANINE AND UNKNOWN COMPOUND OF E. EXCRETA</td>
<td>38</td>
</tr>
<tr>
<td>9.</td>
<td>TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAMS SHOWING PATTERNS OF AMINO ACIDS</td>
<td>40</td>
</tr>
</tbody>
</table>
INTRODUCTION

Of the major classes of biological materials, sugars, fats, and proteins, only proteins cannot be stored. Ingested proteins, or protein from breakdown and turnover of body cell components, must be degraded and the products used or discarded. Products of this degradation are amino acids, which are transaminated or deaminated. If an animal is a carnivore, very large amounts of protein nitrogen are ingested, and much of this nitrogen must be eliminated. Another source of waste nitrogen is catabolism of nucleic acids; the major degradation products of these are purines and pyrimidines.

Several nitrogen-containing compounds have been found commonly in animal tissue, body fluids, and excretions. Free amino acids are found in invertebrates (Awapara, 1962), with fresh-water and land invertebrates having a lower concentration of amino acids than marine invertebrates (Potts, 1967). Molluscs have been reported to excrete large amounts of amino acids. *Lymnaea stagnalis* excretes 26% of its nitrogen as amino acids (Spitzer, 1937), and *Helix pomatia*, 5 - 8% (DeLaunay, 1931).

Ammonia is produced by transamination and deamination of amino acids. Because of its toxicity, it must be excreted
et once, or converted to another, less toxic, substance. Some aquatic molluscs are known to excrete large amounts of ammonia. Delsman (1925) found that ammonia represents 65% of the non-protein nitrogen of the urine of Sepia officinalis. Land pulmonates have been reported to have larger amounts of ammonia in their blood than do fresh-water pulmonates (Delsman, 1931), but excrete less ammonia (Spitzer, 1937).

Urea is found in large amounts in urine of vertebrates. Man excretes 85 – 90% of his waste nitrogen as urea (Folin, 1905). Invertebrates produce much smaller amounts of urea. Arginine is a major amino acid in plants, forming about 14% of the total amino acids (Baldwin and Needham, 1934). Since the notion of arginine on arginine results in urea formation, and land molluscs are known to contain a great deal of arginine activity (Baldwin, 1935; Garton and Campbell, 1966), presumably they could excrete urea as a result of dietary arginine. Early authors, notably Delsman (1931), Needham (1935), and Baldwin (1947), believed urea was an important excretory product of land snails, but more recently it has become apparent that urea represents only a very small percentage of waste nitrogen (Jazewska, Gorzkowski and Heller, 1963a; Campbell and Speeg, 1966).

Purines, especially uric acid, have been found in a wide variety of molluscs. Strohl (1914) suggested that
uric acid is typically an excretory product of adult gastropods. Xanthine and guanine have also been found in the kidney and excreta of gastropods (Jezewska, et al., 1963; Lee and Campbell, 1965).

The usual method for disposing of waste nitrogen is elimination in the urine, but gastropods have the additional capacity to store materials in their kidneys. Jacobson (1820) showed that the kidney of *H. pomatia* contains largely uric acid, and Marchal (1889) purified and measured this uric acid. He reported that *H. pomatia* kidney contains an average of 7 mg of uric acid. Delsunay attempted measurements of nitrogenous materials from both kidney and urine of *H. pomatia*. In an often-quoted study, Delsunay (1931) reported that urea made up 20% of the excretory products. He also found amino acids and small amounts of uric acid in water extracts of excreta and much uric acid in the kidney.

However, Delsunay recognized that his collection procedures did not recover all the uric acid. His water extract of kidneys left about 50% of the purines un-extracted (Jezewska, et al., 1963a). He collected "liquid excreta" by keeping the snails partially immersed in water, and analysing the water for nitrogenous materials, again leaving most of the purines behind.

The uricotelic nature of *H. pomatia* was determined by Baldwin and Needham in 1934. Identification of molluscan excretory products was largely neglected until 1963, when
Jezowska, et al. (1963a) analysed *H. pomatia* kidneys and excreta. They found that purines accounted for 91 - 93% of the nitrogen in the kidney and 95 - 102% of the nitrogen in voided excreta. Three purines were found: uric acid, guanina, and xanthine, the latter two often being in excess of uric acid. The same three purines were found in *Otaia lactea* (Lee and Campbell, 1965).

The pattern of nitrogenous excretory products of mollusca has been reported to be determined by several factors. The most widely known theory is that of Needham (1935) which holds that the major nitrogenous excretory product of the adult can be correlated with the environment of the early embryo. Aquatic embryos, having access to unlimited water, are able to excrete toxic ammonia. Land snails, however, spend their embryonic life in cleidoic eggs. The adults should excrete mainly uric acid, which is easily stored or excreted in a solid form, conserving water. This theory, holding well for vertebrates, also seems to be valid for mollusca. Sloan (1958), however, studied two closely related fresh-water snails, *Caratodes cernuae* and *Pomacea paludosa*. *C. cernuae* embryos develop in fresh-water; *P. paludosa* eggs are cleidoic, deposited above the water level. The cleidoic egg of *P. paludosa* accumulates uric acid, the egg of *C. cernuae* excretes ammonia. Adults of both species excrete mainly ammonia. Thus it is apparent that the excretory product of the embryo
is not necessarily that of the adult. Duerr (1957) also questions Needham's theory, suggesting instead that uricatably is a phylogenetic trait among the gastropods, and is not affected by environment.

Jezewska, et al., (1963b) have found that the pattern of purine metabolism is affected by activity. During estivation, large amounts of uric acid accumulate in the kidney of *Helix pomatia*. Active snails store and void proportionately more xanthines and guanines. Type and amount of food ingested may possibly affect the pattern of nitrogen waste materials. Dzemal and Moyle (1950) found that land isopods and amphipods excrete a large percent of their nitrogen waste as ammonia, and some fresh-water forms have large amounts of uric acid in their tissues. They suggested some sort of "suppression" of nitrogen metabolism in land forms. Recently Sloan (personal communication) confirmed ammonotelism in amphipods, and indicated that gaseous ammonia is a metabolic end-product of amino acid metabolism. The quantity of ammonia excreted seems a function of diet, but there is no indication of suppression of nitrogen metabolism. *O. lecane* is reported to excrete gaseous ammonia, and the amount is apparently greater in estivating snails than active ones (Speeg and Campbell, 1966).

Few attempts have been made to draw up a complete balance sheet of nitrogenous waste products of a molluscan species. Deleanay was successful in a study of *S. officinalis*
(1925), but not as successful with \textit{H. pomatia} (1931). Albritton (1954) gives tables containing data gathered from various sources. The species of land snails for which data are given are \textit{Arion eburacorum}, \textit{Lumax agristta}, and \textit{H. pomatia}. The data must be carefully interpreted, however, since they were obtained by several different authors, using different techniques, and under a variety of conditions, creating inconsistencies. Baldwin (1935) was able to account for 50\% of the nitrogen excretion of \textit{H. pomatia} as:

\begin{center}
\begin{tabular}{l l}
Ammonia & 13.7\% \\
Urea & 20.0\% \\
Uric acid & 10.0\% \\
\end{tabular}
\end{center}

Amino acids, creatinine, and other compounds 6\%.

Jezowska, \textit{et al.} (1963a) found that in \textit{H. pomatia}, over 90\% of total nitrogen in the kidney and voided excreta consists of uric acid, xanthine, and guanine. Ammonia, urea, and amino acids were not detected in kidney or excreta.

Due to the paucity of this type of information on land snails, it was felt that there was a need for a systematic identification and measurement of the major nitrogenous components of land snail excreta and kidney, with the goal of drawing up a balance sheet of the components. It is necessary to have a complete picture of the spectrum of nitrogenous wastes of these animals before attempting to draw any conclusions about their adaptations.
The species chosen for this analysis were *Euglandina rossae* (Ferussac) and *Mesomphix vulgaris* Baker. *Euglandina* is a large carnivorous pulmonate. *Mesomphix*, also a pulmonate, is a small scavenger and is the natural prey of *Euglandina*. These two species give an opportunity to compare the nitrogenous excretory products of a predator and its prey, and also those of a carnivore and a scavenger.
Most of the specimens of *Englundina* used in this study were collected in a hillside hardwood hammock on the University of Florida campus in Gainesville, Florida. The main trees in the area are oaks, with a few magnolias. The snails were found under decaying logs and leaves on the ground. Other specimens were collected near or on the sides of houses in the city of Gainesville, itself. In two instances, clutches of eggs were found and hatched in the laboratory, and the snails raised to useable size. The *Mesomphix* were collected exclusively from the campus hammock, under leaves or partially buried in the humus.

The *Englundina* were kept in individual glass fingerbowls on damp filter paper. They were fed 3 - 4 times a week. Food consisted of terrestrial and aquatic pulmonates, with aquatic pulmonates predominating since they were easier to obtain in quantity.

*Mesomphix* were used as soon after collection as possible since they did not feed or survive well in the laboratory. When held for a few days, they were kept in large fingerbowls on damp filter paper with about 20 snails to a bowl. Both species were kept in a Labline Environette at 23°C and 50% relative humidity. The snails remained
active under these conditions, usually extended partway out of their shells when not actually moving about.

_Euglandina_ excreta emerge from a slit just anterior to and continuous with the pneumostome as a cylindrical ribbon of white pasty material, physically separate and easily distinguishable from the jet black fecal material. The excreta dry rapidly when exposed to air. Only fresh moist excreta were collected for this study in order to minimize bacterial degradation of waste products. Excreta were either used fresh, or frozen at -20 C for future analysis.

Mesomphix were held for 2 - 3 days after collection in small finger bowls to collect all waste products, which were removed periodically and frozen at -20 C for future analysis.

Kidneys of both species were carefully separated from surrounding tissue, removed, and frozen at -20 C.
METHODS

Ammonia

e) Gaseous Ammonia

Two methods were used to determine whether the two species studied release any gaseous ammonia into the atmosphere. In the first method, a modified Conway diffusion technique (Conway, 1933), a snail was placed in a small glass cylinder (Figure 1). Near one end was cemented a piece of plastic screen to keep the snail restricted from that end of the cylinder. The open end of the cylinder was closed with a glass slide, sealed onto the cylinder with a paraffin-vaseline mixture. The screened end was closed with a paraffin-coated slide on which a hanging drop of HCl with known normality was placed. Any ammonia given off by the snail would be collected in the HCl, which could be titrated and the ammonia determined. Snails were left in the cell for 2 hours. Recovery of ammonia standards averaged 96.8%.

The second method was modified from Speeg and Campbell (1968). Air was bubbled through three flasks containing 1N H$_2$SO$_4$ to absorb any ammonia in the incoming air. The air then passed through a small glass cylinder containing a snail, and finally was bubbled through a fritted-glass
Figure 1. Diffusion chamber for detection and measurement of gaseous ammonia.
Paraffined glass slide with hanging acid drop

Screen

Snail chamber

Bottom slide
bubblier into 0.05 N HCl. After 12 hours, the HCl was titrated to determine the ammonia. Recovery of known amounts of ammonia placed in the small chamber was 100%.

**b) Dissolved Ammonia**

Excretory and kidney ammonia content was determined by the method of Shew and Beadle (1949) and, specifically, as modified by Gregg (1950). Half of the material to be analysed was dried to determine the water content. The remainder was analysed while wet to avoid driving off ammonia in the drying process.

The method of analysis involved the use of a small (0.5 ml) glass chamber, which was to be sealed with a paraffin-coated glass slide. The slide was prepared by cutting microscope slides in halves, and costing them with melted paraffin. When cool, the paraffin provided a hydrophobic surface to which could be applied a 6.7 μl drop of 0.05 N HCl to absorb ammonia during the distillation process to follow. The slide was sealed to the glass chamber during distillation by a grease made by mixing melted paraffin and vaseline to a thick consistency.

The wet excreta were extracted with 0.01 N phosphate buffer, pH 6.8. A 10.6 μl aliquot of the buffer extract was delivered into the glass diffusion chamber, and 20 μl of 20% NaOH was added to distill off any ammonia present. Immediately upon addition of the NaOH, the paraffined glass slide with the drop of acid was inverted and sealed onto the
top of the chamber. After 3 hours the drop of acid was titrated to determine the ammonia content. Recovery of known amounts of ammonia averaged 90.0%.

**Urea**

Urea was analysed by first degrading it to ammonia with urease (glycerol extract [Koch], from W. H. Curtin and Co.), then measuring ammonia as before. Recovery was 90.0%.

**Purines**

Material to be analysed for purines was homogenized in 0.01 N NaOH in a ground-glass homogenizer. Samples of the homogenate were placed on glass thin-layer chromatographic plates spread with Aluminum Oxide G obtained from Research Specialties Company, Richmond, Calif. The plates were developed in isopropanol-H₂O (10:3). Spots were visualized using a Mineralight short-wave ultra-violet lamp producing a wavelength of 254 μm. The spots were identified tentatively by Rf, and were then eluted with four different solvents: a) 0.06 M Borate buffer, pH 8.9, b) 0.06 N NaOH, c) 0.1 N HCl, and d) 0.1 N phosphate buffer, pH 6.8. Ultraviolet spectra of unknown spots were compared with those of known purines. Ultraviolet spectra were obtained with a Bausch and Lomb Model 505 Recording Spectrophotometer.

After spots were identified, paper chromatography was used for separation of the purines for measurement.
Aliquots of homogenates were placed on Whatman No. 1 filter paper and developed in isopropanol:H₂O (10:3). After development, the spots were located by viewing under ultraviolet light as before and were eluted with distilled water. The optical densities at the spectral maxima were obtained and compared with standard curves of known purines. Recovery of 80 - 85% was routinely obtained. Results were adjusted to correct for recovery.

Amino Acids

a) Identification

Amino acids were identified according to the method of Shkolipour, Harris, and West (1969).

Material to be analysed for amino acids was homogenized in a ground glass homogenizer with 95% ethanol. Samples of the ethanol extract were spotted on Eastman Chromagram Prespried Chromatography Sheets (Sheet 606 X, Cellulose Without Fluorescent Indicator). Components of the extract were separated two dimensionally. Solvents used were:

first dimension - isopropanol:formic acid:distilled water (80:4:20)


The chromatogram was allowed to develop in the first solvent for 16 hr, then was removed and allowed to dry for 1 hr at
room temp, heated at 65 °C for 15 min, and cooled for 15 min. The chromatogram then was placed in the second solvent and allowed to develop. After 6 hr it was removed and dried in air overnight.

Before location of the spots, the chromatogram was heated at 65 °C for 15 min and then cooled. The spots were located by spraying with 0.5% ninhydrin in acetone and heating for 30 min at 65 °C.

b) Estimation of Total Amino Acids

Although the amino acids were separated for qualitative identification, no attempt was made to determine the amount of each individual amino acid present. Rather, samples were analysed for total amino acid content.

Material to be analysed was homogenized in a ground glass homogenizer with 95% ethanol. Aliquots of the ethanol extract were spotted on thin-layer chromatography plates, using Silica Gel G as the absorbant. Plates were developed in n-butanol-acetic acid:water (60:20:20). Individual amino acids were identified by comparison of $R_f$ with that of known amino acids. Measurement was done by elution from the thin-layer chromatography plates with distilled water and reaction with ninhydrin (Moor and Stein, 1954). The reaction was carried out as follows: 2 g of ninhydrin and 0.3 g of hydridendentin were dissolved in 75 ml of methyl celloseolve without incorporating bubbles. Twenty-five ml of 4 M sodium acetate buffer (pH 5.5) were added and the
resulting reddish reagent solution was immediately transferred to a dark-glass reservoir bottle and stored in the refrigerator. The pH 5.5 buffer was made by adding 200 ml of water to 272 g of sodium acetate·3 H₂O and stirring the mixture on a hot-water bath. The solution was cooled to room temp, 50 ml glacial acetic acid added, and the volume made up to 500 ml. It was stored at 4°C.

One ml of sample containing amino acids to be measured was added to 1 ml of the reagent, the capped tube shaken ( <10 sec.) and heated for 15 min in a boiling water bath. Five ml of diluent (50% ethanol) were added to each tube, cooled to 30°C, and the tubes were shaken in a rack for <30 sec. Absorbance was read at 570 nm against a blank of water. The unknowns were compared with a standard curve made with leucine (0.05 - 0.2 mM), and results reported as "leucine equivalents". Recovery of known amounts of amino acids averaged 90.0%.

**Total Non-Protein Nitrogen (TNPN)**

It was found that the usual methods for deproteination were not applicable for this study, since most call for addition of acid, such as trichloracetic acid. Upon acidification of a solution, any purines present were precipitated and lost along with the protein. Therefore it was decided to separate protein from the non-protein components, measure the protein nitrogen, and subtract the protein
nitrogen from the total nitrogen to give TNPN. Separation of the protein from other components was accomplished by Sephadex column chromatography. Sephadex G-25 (medium), obtained from Pharmacie Fine Chemicals Inc., Piscataway, N. J., was used in a column of 12 mm diameter and 200 mm height. A test solution containing bovine serum albumin and uric acid was found to be separated completely by this column. Mucous from the snail studied gave a characteristic ultra-violet spectrum and could therefore be detected in the fractions collected from the column.

The material to be analysed was homogenized in 0.01 N NaOH and centrifuged at 12,000 RPM in a Servall Type SS-1 Superspeed Angle Centrifuge. A 0.05 ml aliquot of the supernate was placed on the Sephadex column for fractionation. Fractions containing only protein were collected and the nitrogen content was determined by a Kjeldahl method, as was the nitrogen content of aliquots of the unfractionated supernate.

The material to be analysed for nitrogen was placed in a 10 ml volumetric flask with a small glass bead to prevent bumping of the fluid in the intense heat used. One ml of 20% H₂SO₄ and a small amount of selenium metal (Koch and McMeekin, 1924) were added. The flask was placed in an angled hole in a large aluminum block on a hot plate. The fluid was heated to a temperature in excess of 260 °C for 6 hours. It was then allowed to cool to room
temperature, 4 drops of 30% H₂O₂ were added to oxidize all remaining material, and the flask was heated again for 20 min. It was then cooled, and the contents diluted to 10 ml. The Conway diffusion method was used to determine the ammonia content of the flasks. One-ml aliquots from each flask were pipetted into the reaction chamber of a Coleman Microdiffusion dish (Figure 2), 1 ml 0.05 N HCl containing methyl red was added to the collector chamber, 1.5 ml 20% NaOH pipetted into the sealing chamber and 1.0 ml of 20% NaOH pipetted into the reaction chamber. The dish cover was quickly placed over the dish and the dish was allowed to stand for 12 hours. The dish cover was then removed and the HCl titrated with a Coleman Microtractor to determine the ammonia content. Recovery of known ammonia was 98.8%. Recovery of known uric acid averaged 89.6%. Results of determinations were adjusted for recovery. Protein nitrogen was subtracted from total nitrogen to give TNPN.
Figure 2. Conway D Diffusion Dish. A = top view
1) Collector Chamber 2) Reactor Chamber
3) Sealing Chamber B = side view
RESULTS

Results of analyses on kidney and excretory material are summarized in Table 2. No material identifiable as excretory in origin was found for Mesomphix vulgatus.

Ammonia

Dissolved ammonia was found in all material studied, also in very small amounts. Ammonia nitrogen represents a larger percentage of the TNPN in Euglandina kidney than does urea ($p < 0.05$, t-test), but in Euglandina excreta and Mesomphix kidney there is no significant difference ($p < 0.20$). No gaseous ammonia was detected in either species by any method used. Recovery of known added ammonia was 100.0%.

Urea

Urea was present in all material studied, but in very small amounts. Results are given in Table 2.

Purines

Fresh Euglandina excreta consist of a white paste, immediately suggesting the presence of purines. Kidneys of Mesomphix and Euglandina are creamy in color. Thin-layer chromatography of alkaline extracts of Euglandina kidney and excreta and Mesomphix kidney revealed only two spots under ultraviolet light. The materials in these two spots
TABLE 1

<table>
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<tr>
<th>Species</th>
<th>Void</th>
<th>1/3 dry wt.</th>
<th>% TNNK</th>
<th>15/3 dry wt.</th>
<th>% TNNK</th>
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<tr>
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<td>SE (N)</td>
<td>SE (N)</td>
<td>SE (N)</td>
<td>SE (N)</td>
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<tr>
<td>N. vulgatus</td>
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<tr>
<td>kidney</td>
<td>38.7 ± 6.1 (9)</td>
<td>42.9 ± 3.5 (9)</td>
<td>18.3 ± 1.8 (9)</td>
<td>23.0 ± 2.2 (9)</td>
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<td>2.4-10.2</td>
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<td>excrete</td>
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<td>82.9 ± 4.6 (7)</td>
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<td>3.4 ± 0.7 (3)</td>
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<td>0.3-2.6</td>
<td>0.7-6.4</td>
<td>0.7-4.3</td>
</tr>
<tr>
<td>excreta</td>
<td>range</td>
<td>3.0 ± 0.8 (6)</td>
<td>1.1 ± 0.3 (6)</td>
<td>1.4 ± 0.7 (6)</td>
<td>0.5 ± 0.3 (6)</td>
</tr>
<tr>
<td></td>
<td>range</td>
<td>0.7-5.5</td>
<td>0.3-2.0</td>
<td>0.8-4.8</td>
<td>0.0-1.8</td>
</tr>
<tr>
<td>Species</td>
<td>AMINO ACIDS (leucine equivalents)</td>
<td>TNPN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
<td>------</td>
<td></td>
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<tr>
<td></td>
<td>mg. N/kg dry wt.</td>
<td>% TNPN</td>
<td>mg. N/kg dry wt.</td>
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<td></td>
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<tr>
<td></td>
<td>X  SE (N)</td>
<td>X  SE (N)</td>
<td>X  SE (N)</td>
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</tr>
</tbody>
</table>

**Kennebunkia tulpa**

- Kidney: 21.5 ± 4.9 (3) 24.7 ± 3.4 (3) 59.7 ± 12.7 (1)
  - Range: 5-36.0 6.0-62.4 26.6-13.7

**Eulecanium coca**

- Kidney: 30.5 ± 5.4 (10) 19.3 ± 3.4 (10) 157.8 ± 12.7 (7)
  - Range: 12.3-62.6 7.6-39.7 109.0-220.0

- Excreta: 1.7 ± 0.3 (3) 0.6 ± 0.1 (3) 270.1 ± 5.5 (7)
  - Range: 0.8-3.5 0.3-1.3 245.1-286.2
chromatographed identically with guanine and uric acid, respectively. Ultra-violet spectra of eluates of the spots using four different solvents are shown in Figures 3 - 8, confirming the presence of guanine and uric acid. No other purines were detected in extracts of kidneys or excreta of either species studied. Uric acid represents the major portion of TNDN: 43% in Mesomphix kidney, 56% in Euglandina kidney, and 83% in Euglandina excreta. Guanine represents a significant but much lesser plentiful component.

Amino Acids

a) Qualitative Results

Several amino acids were found in the kidneys and excreta (Figure 9). Fewer ninhydrin-positive spots were found on thin-layer chromatograms of Euglandina excreta than those of kidneys of the two species analysed. Amino acids present in Euglandina kidney were, in decreasing order of intensity of ninhydrin staining: tyrosine, histidine, erginine and lysine, homoerine, phenylalanine, norleucine, isoleucine, glutamine, asparte, glycine, and valine. Mesomphix kidney contained, in decreasing order: tyrosine, phenylalanine, isoleucine, leucine, asparte, glutamic acid, glycine, arginine and lysine, norleucine, histidine, and valine. Euglandina excreta contained tyrosine, histidine, lysine and arginine, isoleucine, leucine, glutamine, and serine.
Figure 3. Ultra-violet spectra of known uric acid and unknown compound from complex kidney.
Solvents: A = 0.05 M KH₂PO₄; M = 0.06 M borate buffer, pH 8.3; C = 0.1 M phosphate buffer, pH 6.8; D = 0.1 M HCl.
--- Known uric acid;
--- --- Unknown compound.
Figure 4. Ultra-violet spectra of known guanine and unknown compound of 
Promophex kidney.
SOLVENTS: A = 0.06 M NaOH, B = 0.06 M borate buffer, pH 8.9; C = 0.1 M
phosphate buffer, pH 6.8; D = 0.1 N HCl.

Known guanine;

Unknown compound.
Figure 5. Ultraviolet spectra of normal and unknown compound of L. luna kidney.

Solvent: A = 0.06 M Ol; B = 0.06 M borate buffer, pH 8.9; C = 0.1 M phosphate buffer, pH 6.8; D = 0.1 N HCl.

———Known uroic acid;
———Unknown compound.
Figure 6. Ultra-violet spectra of known guanine and unknown compound of Euglandina's kidney.
Solvents: A = 0.06 N NaOH; B = 0.06 N borate buffer, pH 8.9; C = 0.1 M phosphate buffer, pH 6.8; D = 0.1 N HCl.
— Known guanine;
- - - - Unknown compound.
Figure 7. Ultra-violet spectrum of known uric acid and unknown compound of Euglandina excreta.

Solvents: \( A = 0.06 \text{ N NaOH} \); \( B = 0.06 \text{ M borate buffer, pH 8.9} \); \( C = 0.1 \text{ M phosphate buffer, pH 6.8} \); \( D = 0.1 \text{ N HCl} \).

--- Known uric acid;
--- --- Unknown compound.
Figure 8: Urea, guan, and guai late in urine. Known guanine; unknown compound.
Figure 9. Two-dimensional thin-layer chromatograms showing patterns of amino acids. 
A = Mesomphyr kidney; B = Euglandina kidney; C = Euglandina excreta.
b) Quantitative Results

Amino acid of *Euglandina* and *Mesomphix* were represented 19% and 25% of the TNPN, respectively. Less than 1% of the TNPN of *Euglandina* excreta was amino acid nitrogen (Table 1).

**TNPN**

*Mesomphix* kidney contained widely varying amounts of TNPN, but averaged 90 mg/g dry wt. *Euglandina* kidney contained nearly twice as much TNPN, 155 mg/g dry wt, and was somewhat less variable. *Euglandina* excreta contained 270 mg TNPN/g dry wt. The range of this fraction was small, and indicates a fairly consistent composition of the excreta.

**Protein in Euglandina Excreta**

The protein content of *Euglandina* excreta averaged 7.3 mg N/g dry excreta (SE = 1.2, N = 18).

**Total Nitrogen Excretion by Euglandina**

An average of 4.1 mg of non-protein nitrogen was found for each excretion of *Euglandina* (N = 8, SE = 1.0, range = 1.6 - 10.2 mg N).

A summary of all quantitative results is given in Table 2.
<table>
<thead>
<tr>
<th>Species</th>
<th>Material</th>
<th>Uric Acid</th>
<th>Guanine</th>
<th>Urea</th>
<th>Ammonia</th>
<th>Amino Acids</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xeroscincus</td>
<td>kidney</td>
<td>42.9</td>
<td>22.0</td>
<td>2.5</td>
<td>1.2</td>
<td>24.7</td>
<td>93.3</td>
</tr>
<tr>
<td>Euphractus</td>
<td>kidney</td>
<td>55.2</td>
<td>5.0</td>
<td>1.1</td>
<td>2.3</td>
<td>19.3</td>
<td>86.6</td>
</tr>
<tr>
<td>Euphractus</td>
<td>excreta</td>
<td>82.3</td>
<td>6.9</td>
<td>1.1</td>
<td>0.5</td>
<td>0.6</td>
<td>92.0</td>
</tr>
</tbody>
</table>
DISCUSSION

It is interesting that *Mesomphix* produced no detectable excretory material. This finding, while not expected, may be quite common among species of small pulmonates. Most small snails appear to have a life span of only 1 year. Boycott (1934) found that all small British snails he studied were annual, with a life span of 9 – 15 months. Larger species, such as *H. pomatia*, had a life span of more than 3 years.

A terrestrial species with a short life span of 1 year might conserve water and energy by excreting nothing. A material such as uric acid or guanine could be stored in the kidney and only released at death. Duerr (1966), in a study on *Lymnaea stagnalis*, found that these snails excrete very little, if anything. He suggested that they store uric acid in their body and excrete this infrequently. Needham (1935) reported 115 mg uric acid/g dry kidney wt for *L. stagnalis*, which has a life span of 14 months (Noland and Carriker, 1946).

An attempt was made in the present study to determine the life span of *Mesomphix* by weighing and measuring collected snails, but the results were inconclusive. It appeared, however, that by early spring almost all of the
large snails living through the winter had died. At about
the same time very small snails had begun to appear. Thus
there is a good possibility that *Mesomphix vulgatus* is
annual.

*Mesomphix* kidney contains a significantly higher
percentage of guanine nitrogen than does *Euglandina* kidney
($p < 0.01$, t test). Guanine is a more efficient storage
purine than uric acid, since it contains 25% more nitrogen
per molecule. Guanine is also less soluble than uric acid.
The solubility of uric acid is 0.0025 g/100 ml H$_2$O at
20°C, while that of guanine is 0.0005 g/100 ml H$_2$O at
20°C (Albert and Brown, 1954). By the storage of large
amounts of guanine, *Mesomphix* is able to store more nitro-
gen in its kidney, with less energy used in its deposition
than if it used uric acid alone.

Other aspects of *Mesomphix* bear on its nitrogen
storage. It is a scavenger, feeding on dead snails and
decaying vegetable matter. Therefore it ingests more
nitrogen per unit weight of food than does a strict
vegetarian. This may necessitate excretion of stored
purines on rare occasions. However, *Mesomphix* is not a
very active snail. It spends much of the time withdrawn
into its shell. Only after a rain was it possible to find
numerous active snails. While inactive, very little
metabolism is likely to occur, keeping nitrogen catabolism
to a minimum.
It would be desirable to determine whether there is an increase in the amount of TNPN stored in the kidney of *Mesomphix* over the period of a year.

*Euglandina*, a much larger snail and a rapacious carnivore, ingests appreciable amounts of protein nitrogen. However, large quantities of excreta are voided within 12 hours after feeding, thus eliminating excess nitrogen.

Uric acid is the major purine found in *Euglandina* kidney and excreta. Although there is an apparent difference in the percent of uric acid in the kidney and the excreta, the difference is mostly due to the amino acid nitrogen found in the kidney. If amino acids are disregarded, the percent of uric acid nitrogen of *Euglandina* kidney closely approximates that of excreta. Guanine is also a significant material in both kidney and excreta, but does not represent as high a percentage of the TNPN in *Euglandina* as it does in *Mesomphix*. Nucleic acid catabolism and ingested guanine from prey kidney can probably account for most guanine found in *Euglandina*.

Several previous studies have shown the presence of three purines in snail kidney and excreta: uric acid, guanine, and xanthine, in *H. pomatia* (Jezewski, *et al.*, 1963a,b) and in *G. lactea* (Lee and Campbell, 1965). Xanthine is approximately 25 times as soluble as uric acid (Dawson, *et al.*, 1959), thus requiring more energy for water resorption when this material is excreted, or
resulting in the loss of more water. These two restrictions do not affect Eulalandina and Mesomphix.

In snail uric acid biosynthesis, Eristeaux-Gregoire and Florkin (1962) conclude that the pathway elucidated by Buchanen, et al. (1948) is operative. The pathway contains the reaction sequence, guanase \[ \text{guanase} \rightarrow \text{xanthine} \]

\[ \text{xanthine oxidase} \rightarrow \text{uric acid.} \]

Snail species excreting all three of these purines must have guanase and xanthine oxidase with fairly low activities. Xanthine oxidase of Eulalandina and Mesomphix, however, must be very active, so that, on formation, xanthine is immediately converted into uric acid and does not appear in the kidney.

Ammonia and urea are present in all material examined, but do not represent appreciable excretory nitrogen. Ammonia found in the kidney may be merely blood ammonia, since the amount found in Eulalandina excreta is significantly lower (p < 0.005, t test) and could be due to bacterial action on the excreta.

Spaag and Campbell (1968) have reported the elimination of appreciable quantities of ammonia nitrogen from C. lactea and Helix aspersa in the form of ammonia gas. The amounts from activating snails compare favorably with amounts excreted by non-terrestrial molluscs (Potts, 1967). Ammonia gas from active snails is 50% less than that from satiating snails. Active individuals of the two species from the present work were analysed in a fashion similar
to the method of Speeg and Campbell (1963) as were several active \textit{H. superba}. No ammonia gas was recovered, while 100% recovery of known ammonia were obtained. It is possible that these active snails produced amounts too low to be detected, or the ammonia gas is not produced continually, none being produced at the time of analysis.

\textit{Euglandina} excretory amino acid nitrogen is very low when compared with literature values, such as the 5.2% TNPN in \textit{H. pomatia} found by Delsucay (1931). However, Jezewski, et al. (1963a) found no amino acids in either kidney or excreta of \textit{H. pomatia}.

It is evident that amino acids are not an important constituent of \textit{Euglandina} excreta. They are a very important fraction of kidney nitrogen, however, in both \textit{Euglandina} and \textit{Mesomphix}. There are few data available on amino acid content of kidneys or other land snail organs. Florkin (1966) found 212.2 mg free amino acids/100 g of \textit{H. pomatia} hepatopancreas. Kerkut and Gottrell (1962) reported 1 M amino acids/ml serum and 18.2 M/g wet weight of brain of \textit{H. pomatia}. Russell (1965) found 4.9 - 9.6 mg non-protein nitrogen/100 ml serum in various species of \textit{Helicittus}. These data are difficult to compare with those of the present study, but reported values seem to be lower than those found here.

The pattern of amino acids present in \textit{Euglandina} kidney and excreta, and that of \textit{Mesomphix} is very similar.
Awapara (1962) reports *Englandina exigua* and its herbivorous prey *Bulinus* also show relatively little difference in their amino acid patterns. One significant difference was noted between *Englandina* and *Mesomphix* in the present work. Histidine was present in *Englandina* kidney and excreta in large amounts, as evidenced in intensity of staining on the chromatogram. *Mesomphix* kidney contained large amounts of phenylalanine, with much less histidine. No reason for this difference is immediately evident. The function of the high amino acid levels found in snails, as well as in other invertebrates, is unknown except in those where it is correlated with osmotic regulation.

The pattern of nitrogen excretory products of the species studied here is similar in many respects to previously studied snails. Purines make up the bulk of the nitrogen. Uric acid is preponderant, but guanine is present in significant amounts. Ammonia, urea and amino acids are found in low quantities in *Englandina* excreta. Kidneys of both species contain little ammonia and urea, but large amounts of amino acids. Both species rely heavily on the storage capabilities of their kidneys, *Mesomphix* doing so to the exclusion of excretion. The pattern of nitrogen compounds is remarkably similar in the two snails, one a carnivore, the other a scavenger and prey of the former. Differences are attributable to longevity and
possibly to protein in the diet of the respective species.

It has been increasingly evident that land snails are "purinotelic" and not just uricotelic. The analysis and interpretation of snail nitrogen excretion should be attempted only with the realization that several different purines may be involved.
SUMMARY

1. The kidneys and excreta of two pulmonate land snail species, Mesomphix vulgatus and Englandina rosea, were analyzed for non-protein nitrogen compounds.

2. Mesomphix was found to void no excretory material.

3. The purines, uric acid and guanine, represent 64.9% of the total non-protein nitrogen (TNPN) of Mesomphix kidney TNPN, 63.9% of Englandina kidney TNPN, and 83.8% of Englandina excreta TNPN.

4. Mesomphix kidney contained 42.9% uric acid and 22.0% guanine. Englandina kidney was 55.9% uric acid and 8.0% guanine, while the excreta was 52.9% uric acid and 6.9% guanine.

5. Amino acids represented 24.7% of the TNPN of Mesomphix kidney and 19.3% of Englandina kidney. Englandina excreta contained only 0.6% of the TNPN as amino acids.

6. Urea and ammonia were present in the kidneys and excreta, but both materials together represented less than 4% of the TNPN. No gaseous ammonia was released by either species.

7. Almost all of the TNPN of the kidneys and excreta was accounted for: 93.3% in Mesomphix kidney, 86.6% in Englandina kidney, and 92.0% in Englandina excreta.
8. It was considered that *Mesomphix vulgatus* is a short-lived species and an individual may excrete very little if any nitrogen during its lifetime.

9. The kidney of *Mesomphix* contains a significantly higher percent TKN as guanine than does *Euglandina* kidney. This allows more nitrogen to be stored with an expenditure of less energy per molecule of purine, enabling *Mesomphix* to rely on storage of nitrogen rather than excretion.

10. *Euglandina*, while able to store large amounts of nitrogen in the kidney, mainly as uric acid, voids waste nitrogen excretory products within 12 hours of feeding.

11. The term "purinotelic," rather than uricotelic, seems a better one to apply to land snail excretion.
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BIIOGRAPHICAL SKETCH

David George Badman was born November 30, 1941, at Chippewa Falls, Wisconsin; he was graduated from Chippewa Falls Senior High School. He attended the University of Wisconsin, and in June, 1963, he received the degree of Bachelor of Science in Zoology. In September, 1963, he enrolled in the Graduate School of the University of Florida. He worked as a graduate assistant in the Department of Zoology until August, 1969. He received the degree of Master of Science with major in Zoology in August, 1966. From September, 1966, until the present time he has pursued his work toward the degree of Doctor of Philosophy. He spent the spring and summer of 1965 at Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington, supported by a National Science Foundation Fellowship.

David George Badman is married to the former W. Sue Griffith. He is a member of the Phi Sigma Society, The Society of the Sigma Xi, the American Association for the Advancement of Science, and the American Society of Zoologists.
This dissertation was written under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1969

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Dean, College of Arts and Sciences

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