Nitrogenous Excretion in Chelonian Reptiles

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It seems to be well established, during the course of evolution, the migration of animals from aquatic to terrestrial environments has been at least partially dependent upon their ability to detoxicate the ammonia arising from the α-amino nitrogen of the food proteins (Baldwin, 1948). Among vertebrates, detoxication is usually accomplished by the formation of either urea or uric acid. Ureotelism occurs in the Amphibia and Mammalia, and uricotelism in the Sauropsida (snakes, lizards and birds).

The nitrogenous excretion of the Chelonian reptiles (tortoises and turtles) has not hitherto been systematically studied, and such data as are available are often contradictory. Magnus & Müller (1835) found evidence for the presence of urea in the urine of Testudo nigrta (nigra), while Schiff (1825) and Marchand (1845) similarly demonstrated the presence of urea in the urine of T. denticulata (tabulata). Both of these species are land dwellers. Several workers have studied T. graeca, another terrestrial form, Clementi (1929) stating that 90% of the excreted nitrogen is in the form of urea, an observation which is substantially supported by the results of Drilhon & Marcoux (1942). Münzel (1938), on the other hand, found that uric acid and urea were both produced in this species, the former predominating. He also analyzed the urine of the semi-aquatic species, Emys orbicularis (europaea) and found that the main excretory product was urea, although ammonia and uric acid were also present in considerable quantities. Both Lewis (1918) and Khalil (1947) have worked on the sea turtle, Chelonia mydas, but obtained contradictory results. Lewis (1918) found that this species is essentially ureotelic, whereas Khalil (1947) states that ammonia is the main excretory product. Needham (1931), basing his argument on the work of Clementi (1929), Lewis (1918), and Wiley & Lewis (1927), concluded that Chelonia are essentially ureotelic. He believes that, because they are laid in a damp environment, the eggs can absorb water from the surrounding mud or sand and are therefore presumably permeable to urea also. In many species, however, the eggs are laid in very dry surroundings where a permeable egg would hardly be able to conserve or acquire enough water for development.

It therefore seemed worth while to investigate the nitrogenous excretory products of Chelonia from a variety of habitats in an attempt to clarify this somewhat obscure position. Species from a variety of aquatic, amphibious and terrestrial habitats were therefore studied to discover whether there might be some correlation between nitrogen metabolism and environmental conditions.

EXPERIMENTAL

Material. Dr E. Hindle, F.R.S., was kind enough to put at our disposal the large collection of Chelonia belonging to the Zoological Society of London.

For the collection of urine the smaller specimens were kept and fed in rat metabolism cages of the usual type for 1 or 2 days. The wire floor separated the urine from the faeces, and fragments of food were removed by filtration through a loose plug of glass wool. The urine was collected in bottles containing a small amount of 0·2 N-H2SO4. The analysis
of urine collected in this way was usually begun within 24 hr. of excretion, but when longer waiting was necessary a few drops of toluene were also added, and the whole kept in the refrigerator.

Larger specimens were kept in their usual surroundings, as it was found that the excitement caused by being handled was usually sufficient to induce urination. Analysis of this urine was begun within a few hr. of its excretion and toluene added, if it had be be kept overnight.

The urine in all species was clear except in Testudo graeca which produced a cloudy urine, possibly because of the large amount of uric acid present.

Methods. Total N was estimated by the Kjeldahl method; digestion was continued for 16 hr. with the catalyst of Chibnall, Rees & Williams (1943), and the NH₃ formed determined by distillation in the apparatus of Markham (1942). Free NH₃ was also estimated by this method, the distillate in both cases being collected in a 2% (w/v) boric acid solution containing indicator as described by Sobel, Hirschman & Besman (1945). Urea was determined by incubation with urease (prepared from jack-bean meal by extraction with 0-3% (w/v) KH₂PO₄), followed by distillation of the NH₃ produced. Uric acid was estimated colorimetrically; 2 ml. each of the urea and cyanide solutions as used by Brown (1945) were added to the sample followed by 1 ml. of the arsenophosphotungstic reagent of Benedict & Franke (1922) and the whole diluted to 50 ml. Readings were made after 50 min.

Amino-acids were collectively determined by a slight modification of the method of Sobel et al. (1945); the NH₃ formed was distilled by Markham's (1942) method instead of being removed by aeration. Creatinine was estimated by the Folin picrate method, using the sodium picrate solution recommended by Borsook (1935) and hippuric acid by the colorimetric method of Denigès (1939). Before estimating either allantoin or guanine + xanthine it is necessary to uric remove acid, which gives a colour in both the colorimetric methods used. This was achieved by heating in a boiling water bath for 10 min. with 0-2% nitric acid as recommended by Hitchings (1941). Allantoin was then estimated by the colorimetric method of Young & Conway (1942), while guanine + xanthine were precipitated as silver salts by the method of Gulland, Jordan & Threlfall (1947), and estimated colorimetrically with the phenol reagent of Folin (1927) as used by Hitchings (1941).

RESULTS

Eight species of Chelonia from a variety of habitats were examined. The averages of the results for each species are tabulated in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of determinations</th>
<th>Total N (mg./sample)</th>
<th>Ammonia</th>
<th>Urea</th>
<th>Uric acid</th>
<th>Aminos- acids</th>
<th>Allantoin</th>
<th>Guanine + xanthine</th>
<th>Creatinine</th>
<th>Nitrogen as percentage of total nitrogen</th>
<th>Percentage total N accounted for</th>
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<tr>
<td>Kinosternon subrubrum</td>
<td>1</td>
<td>7-0</td>
<td>24-0</td>
<td>22-9</td>
<td>0-7</td>
<td>10-0</td>
<td>1-1</td>
<td>1-0</td>
<td>Trace</td>
<td>59-7</td>
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<tr>
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<td>18-5</td>
<td>24-4</td>
<td>4-5</td>
<td>20-6</td>
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<td>14-4</td>
<td>47-1</td>
<td>2-5</td>
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<td>Trace</td>
<td>88-0</td>
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</table>

Table 1. Nitrogen partition in excreta of Chelonian reptiles

(Where more than one determination was carried out the non-bracketed figures are mean values, whilst the figures in brackets show extreme values obtained.)
under much drier conditions. The nitrogenous excretion of the two species is, nevertheless, very similar. Both excrete 40–60 % of the total nitrogen as urea; ammonia is responsible for only 6 % of the excreted nitrogen and the uric acid level remains at 5 %.

Three species of the wholly terrestrial family Testudinidae were studied and here an interesting division was discovered. *Testudo denticulata*, which inhabits damp swampy ground in Brazil, excretes some 30 % of its total nitrogen as urea and only 7 % as uric acid, but *T. graeca* and *T. elegans*, both of which live under almost desert conditions, excrete uric acid as their main nitrogenous end product. Although these species excrete 50–60 % of their total nitrogen as uric acid, they still produce considerable quantities of urea (10–20 %) in contrast to other uricotelic forms where urea synthesis is completely suppressed.

Apart from ammonia, urea and uric acid, amino-acids accounted for 10–20 % of the total nitrogen in all species. Allantoin forms 1–2 % of the total (except in *T. denticulata* where it accounts for 8 %); guanine + xanthine was consistently low at less than 4 % (except in *T. graeca* where it accounted for 9 % of the total); and creatinine was found in traces only. Hippuric acid, which is 10–25 % in Khalili's (1947) analysis of the urine of *Chelonia mydas*, was not detectable in any of the species studied.

In all, 60–95 % of the total nitrogen excreted has been accounted for in each species.

**DISCUSSION**

The Chelonian reptiles seem to be biochemically separable into three groups. The first group contains the aquatic and semi-aquatic species, and these excrete both ammonia and urea in approximately equal quantity. In the second group, consisting of those terrestrial species which are restricted to damp, marshy ground, there is little excretion of ammonia but some 50–60 % of the total nitrogen is excreted as urea. The third group includes the terrestrial species which live in very dry habitats and which excrete some 60 % of their waste nitrogen in the form of uric acid. Although this group is essentially uricotelic, the ureotelic habit of Chelonia in general has not been completely suppressed, for about 10–20 % of the total nitrogen is still excreted as urea. The family Testudinidae seems to be the most interesting from this point of view, some of its species being typically ureotelic while others excrete urea and uric acid together, the latter predominating.

According to Needham (1931), the change from ureotelism to uricotelism is determined by the conditions under which embryonic development takes place, uricotelism being associated with development within a 'cleidoic' egg. The eggs of the ureotelic species *Thalassochelys corticata* (Karashima, 1929), *Emys orbicularis* (*europaea*; see Hochstetter, 1906), and *Caretta* (Hildebrand & Hatzel 1927), all require a damp environment for successful development. Karashima's (1929) data show that the eggs of *Thalassochelys corticata* absorb water to the extent of 42 % of their original water content from the environment during development; and Nakamura (1929), also working on this species, showed that the total nitrogen of the egg decreased by 17 % before hatching. These eggs are therefore permeable to water and probably to urea also, so that the urea formed by the embryo can diffuse out into the environment. In addition, certain other uroeletic species such as *Chrysemys picta picta* (Cunningham, 1923), *C. picta bellii* (Stromsen, 1923), *Emys orbicularis* (Rollinat, 1932), and several others moisten the ground with fluid from the bladder or some closely associated gland before laying their eggs. The reason for this may be to produce an artificially damp environment for the eggs, but it has been suggested (Stromsen, 1923), that it may be only a method of softening the ground to facilitate the digging of the nest.

Young's (unpublished) work on the egg of *Testudo graeca*, however, shows conclusively that in this species the shell is even more impermeable than that of the domestic fowl. The eggs, which develop under very dry conditions, are presumably supplied with sufficient water to last through their embryonic life. Being impermeable to water, they are almost certainly impermeable to urea also. The urea produced by a urotelic embryo would therefore accumulate within the egg, and perhaps might reach a toxic level. It is in those species like *T. graeca*, which live and lay their eggs in almost desert habitats, that uricotelism has largely replaced ureotelism. It seems probable, therefore, that this is an adaptation to embryonic life within a 'cleidoic' egg, but further data on the permeability and conditions of hatching of the eggs of other species are required to confirm this view. If, as appears probable, it is found that the development of uricotelism takes place side by side with the development of a 'cleidoic' egg within this group, this would be further evidence in support of Needham's (1931) belief that the two factors are interdependent.

This biochemical evidence supports the usual theory of Chelonian evolution based on morphological and palaeontological grounds. The tortoises and turtles are believed to have evolved from an early amphibious stock which must presumably have been ureotelic. Some of these early forms returned to an aquatic environment where they again began to excrete some ammonia, while retaining partially their ureotelic nature, thus giving evidence of their amphibious ancestry. Others remained in damp terrestrial habitats and are still typically ureotelic.
A third group migrated to drier terrestrial habitats, and here, apparently because of the conditions of embryonic development, they became largely uricotelic. In the Sauropsida, which are all uricotelic, very little urea indeed is produced, and this is not of synthetic origin, being produced from exogenous urea found in the food and from the amidine group of arginine (Clementi, 1932). The fact that these Chelonian species, while being mainly uricotelic, still excrete some 10–20% of their total nitrogen as urea, suggests that the stable uricotelic state found in the Sauropsida has not yet been achieved. It may be that their biochemical evolution towards uricotelism is still in progress.

**SUMMARY**

1. The nitrogenous excretion of eight species of Chelonian reptiles was studied.
2. The analyses showed that urea accounted for 10–20%, or more, of the total nitrogen excreted by all the species examined.

**REFERENCES**


Marchand, R. F. (1845). *J. prakt. Chem.* 34, 244.


