NOTE / NOTE

New observations on urine contents in water-deprived Negev Desert rodents

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Abstract: In past studies, several rodent species of the murid subfamilies Gerbillinae and Cricetomyinae from the Namib Desert, when deprived of water, excreted allantoin precipitate in their urine. Shifting nitrogen excretion from urea to allantoin allows them to save much water. This phenomenon has not been reported in other rodents, and whether it is a trait that is common among desert rodents, but undocumented, or is unique to these Namib Desert species, is not known. We found no allantoin precipitate in the urine of any of five additional species of water-deprived murid rodents of two subfamilies from the Negev Desert. There was no discernible precipitate in the urine of *Gerbillus andersoni allenbyi* (Gerbillinae), *Gerbillus pyramidum* (Gerbillinae), or *Acomys cahirinus* (Murinae). Sodium oxalate was found in both the precipitate and the liquid urine of *Psammomys obesus* (Gerbillinae), and the as yet unidentified precipitate in the urine of *Gerbillus dasyurus* (Gerbillinae) was not allantoin. This preliminary study suggests that not all gerbilline rodents have the capacity to switch from urea to allantoin excretion. The Namib Desert gerbilline and cricetomyine rodents may be examples of closely related mammalian taxa that have evolved a new metabolic pathway to produce a nitrogenous product that results in sizable water savings, i.e., by switching from urea to allantoin.

Résumé : Des études antérieures ont démontré qu'en l'absence d'eau, plusieurs espèces de rongeurs Muridae des sous-familles Gerbillinae et Cricetomyinae du désert de Namibie excrètent des précipités d'allantoïne dans leur urine. Le passage de l'excrétion de l'azote sous forme d'urée à l'excrétion sous forme d'allantoïne leur permet d'épargner beaucoup d'eau. Le phénomène n'a pas été signalé chez d'autres rongeurs et on ne sait pas si c'est un caractère commun, mais pas encore signalé, aux rongeurs des déserts ou alors une caractéristique particulière des espèces du désert de Namibie. Nous n'avons pas retracé la présence de précipités d'allantoïne dans l'urine de cinq espèces additionnelles de rongeurs muridés des deux sous-familles provenant du désert du Néguev et privées d'eau. Il n'y avait pas de précipité décelable dans l'urine de *Gerbillus andersoni allenbyi* (Gerbillinae), ni de *Gerbillus pyramidum* (Gerbillinae), ni d'*Acomys cahirinus* (Murinae). Nous avons trouvé de l'oxalate de sodium dans le précipité ainsi que dans l'urine liquide de *Psammomys obesus* (Gerbillinae), de même qu'un précipité encore non identifié, mais qui n'est pas de l'allantoïne, dans l'urine de *Gerbillus dasyurus* (Gerbillinae). Notre étude préliminaire indique que ce ne sont pas tous les rongeurs gerbillinés qui sont capables d'excréter de l'allantoïne au lieu de l'urée. Les rongeurs gerbillinés et cricétomyinés du désert de Namibie sont peut-être des exemples de taxons de mammifères très apparentés qui ont développé de nouvelles voies métaboliques pour élaborer un produit azoté qui entraîne une conservation substantielle d'eau, i.e. en remplaçant l'urée par l'allantoïne.

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Introduction

Nucleic acids are made of two nitrogen-containing nucleic bases, pyrimidines and purines. Pyrimidine metabolism usually produces ammonia, while purines are metabolized to a variety of nitrogenous compounds, including urea, uric acid, and allantoin (Voet et al. 1999).

The pathways of purine nucleotide catabolism in non-human animals have not been as carefully studied as the metabolism of proteins (Schmidt-Nielsen 1997). These pathways differ

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among animals, but all produce uric acid. Uric acid can be further degraded to urea and glyoxylic acid via a metabolic pathway that produces allantoin and allantoic acid as intermediates. The enzymes necessary for these conversions, allantoicase, allantoinase, and urate oxidase, were lost during evolution in different vertebrate taxa (Voet et al. 1999).

Buffenstein et al. (1985) found that several species of rodents from the Namib Desert produced large amounts of a crystalline precipitate in their urine after they were water-deprived for 4 weeks. This precipitate consisted of pure allantoin and accounted for 29% of the mass of the urine and 30% of the total nitrogen excreted per day. Such a large quantity of allantoin could not be accounted for by purine degradation alone. The production of allantoin instead of urea in urine could result in significant saving of water. In the same region, Downs and Perrin (1991) also observed allantoin production in water-deprived murid rodents (*Gerbillurus tytonis*, *Gerbillurus paeba paeba*, *Gerbillurus vanillus vanillus*, and *Gerbillurus setzeri*, and all of the subfamily Gerbillinae) from the Namib Desert; species additional to those reported by Buffenstein et al. (1985). Buffenstein et al. (1985) and Downs and Perrin (1991) left unanswered the question of whether water-stress-induced allantoin production is unique to a few Namib Desert rodent species.

To investigate this question we conducted a study of murid rodents living in the Negev Desert in southern Israel to test whether they also produce excess allantoin when subjected to water deprivation. We hypothesized that if switching to allantoin production under water stress emerged early in the evolution of gerbilline rodents, we would observe this response in the Negev Desert Gerbillinae as well. The absence of allantoin production in Negev Desert gerbilline rodents would lead us to predict that the rodents from the Namib Desert, possibly the oldest extant desert in the world (Ward and Corbett 1990; Van der Wateren and Dunai 2001), have evolved unique metabolic pathways that serve to save water.

Material and methods

We obtained rodents that were caught in the wild, but were being maintained in captivity, from other departments of the Jacob Blaustein Institute for Desert Research, Ben Gurion University of the Negev. They included four species of the subfamily Gerbillinae: the fat sand rat (*Psammomys obesus*, mean body mass 225 g, $n = 3$); Anderson's gerbil (*Gerbillus andersoni allenbyi*, mean body mass 30 g, $n = 3$); Wagner's gerbil (*Gerbillus dasyurus*, mean body mass 30 g, $n = 3$); and the greater Egyptian gerbil (*Gerbillus pyramidum*, mean body mass 41 g, $n = 2$), and one species from the subfamily Murinae: the Cairo spiny mouse (*Acomys cahirinus*, mean body mass 30 g, $n = 3$). We housed the animals individually in cages held in a temperature-controlled room (ambient temperature 33.0 ± 1°C and light cycle 12 h light : 12 h dark). For 10 days prior to beginning experiments, we fed all species except *P. obesus* dry millet seeds and fresh alfalfa as a water source ad libitum. Most rodents that inhabit arid and semi-arid areas do not drink free water, but rely on preformed water in their food or on metabolic water production (Degen 1997). We fed the *P. obesus* 150 g of freshly harvested saltbush (*Atriplex halimus* L.) leaves per day, this being their natural diet in Israel (Frenkel and Kraicer 1972). The experimental period included 1 week of normal hydration (in which we fed the diets described above) and 4 weeks of water deprivation. All species except *P. obesus* were deprived of water by removing the alfalfa from their diets. *Psammomys obesus* were fed oven-dried (50°C) saltbush leaves, which contained about 20% less water than fresh leaves. During the 4 weeks of water deprivation we weighed the animals every third day. We decided a priori that we would consider animals dehydrated when they had lost 4–5% of their initial body mass, and if an animal had lost 15–20% of its body mass, we returned it to the initial, water-containing (alfalfa or fresh saltbush) food regimen. *Psammomys obesus* did not lose significant body mass until the second week of water deprivation, therefore we did not collect urine samples from them during the first week. Conversely, all *A. cahirinus* lost over 15% of their initial body mass by the second week of water deprivation and we discontinued their use in experiments at that point. All animals were cared for according to the guidelines of the Canadian Council on Animal Care.

Urine collection

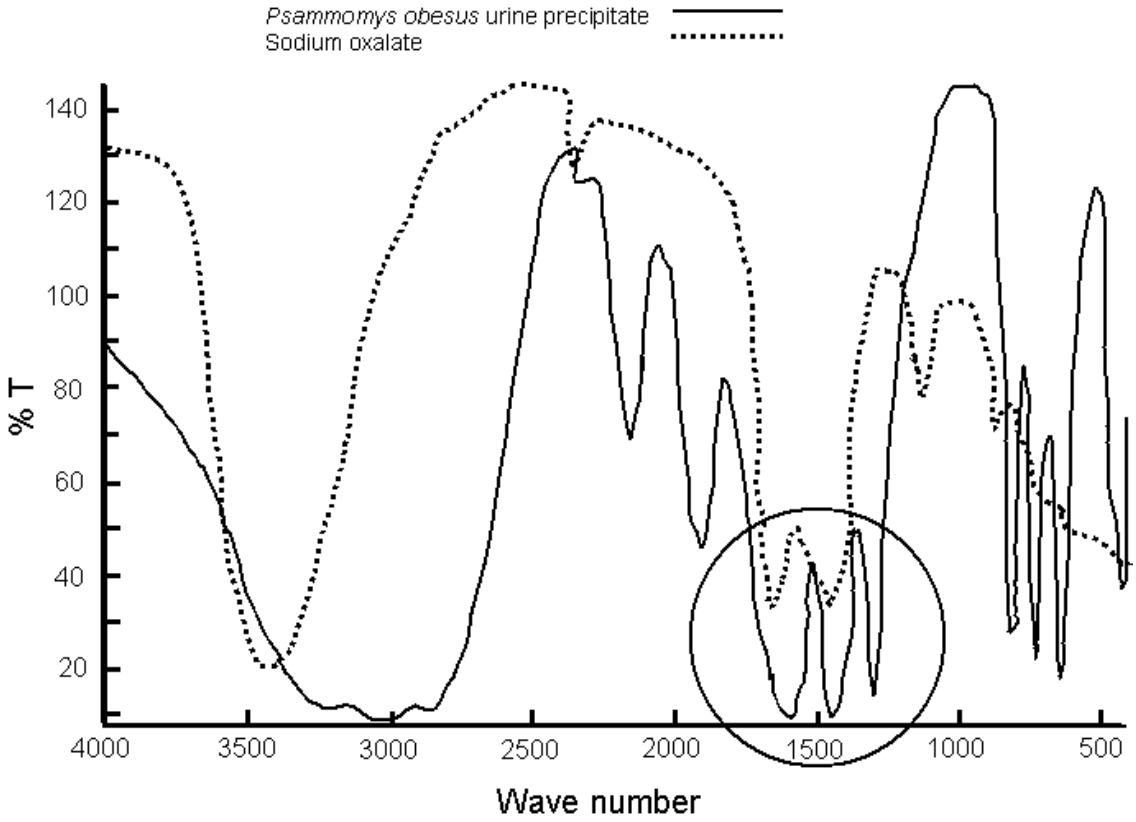
We collected urine once during the pre-experimental period of normal hydration and once weekly for 4 weeks during the water-deprivation period. Early in the morning (at the end of their scotophase), we placed the animals in metabolic cages that had stainless-steel wire-mesh floors; no food was available. Under the mesh we placed aluminum trays containing paraffin oil to prevent evaporation from the small urine droplets, and the urine was aspirated from these trays every 30 min. The animals remained in the metabolic cages until we had collected a urine sample large enough to analyze (>60 μL). We collected urine only if it was uncontaminated with feces.

We transferred urine to preweighed Eppendorf tubes containing 75 μL of phosphate buffer at pH 5.5. This acidified the samples, preventing loss of ammonia. During collection we kept the samples in a refrigerator below 10°C and stored them at 6°C until analysis the following day.

We analyzed dissolved allantoin using a modification of Young and Conway's (1942) method described by DelGiudice et al. (2000). Samples of the urine of *P. obesus* and *G. dasyurus* were centrifuged at 14 000 rpm in a microcentrifuge for 10 min. The supernatant was discarded and the precipitate freeze-dried. These samples were dissolved in hexadeutorodimethyl sulfoxide using tetramethylsilane as an internal standard and were analyzed by 1H-nuclear magnetic resonance (NMR) at 100 MHz. The NMR spectra were compared with the spectrum of pure allantoin (Sigma No. A7878).

Only the urine of *P. obesus* contained sufficient precipitate for further chemical analysis. A pellet of the precipitate was analyzed with a Nicolet Avatar 320 Fourier-transformed infrared spectrophotometer, and the infrared spectrum obtained was compared with the infrared spectra of pure ammonium oxalate (Sigma No. A8545), calcium oxalate (Sigma–Aldrich No. 45,599-7), and sodium oxalate (Sigma No. S9265). Both urine-precipitate and liquid-urine samples were analyzed by ion chromatography using a Dionex™ model 100 ion

Fig. 1. Fourier-transformed infrared spectra of urine precipitate from *Psammomys obesus* and a sodium oxalate standard. The matching peaks in the circled area indicate that the urine precipitate is most likely sodium oxalate. %T, percent transmittance.



chromatograph. Cations (Li^+, Na^+, NH_4^+, K^+, Ca^{2+} and Mg^{2+}) were analyzed using a CS12 column with a CSRS-1 suppressor with 20 mM MSA as a mobile phase with a flow rate of 1.5 mL/min. Organic anions were analyzed using an AS12A column and a ASRS-1 suppressor with 2.7 mM sodium carbonate buffer as the mobile phase with a flow rate of 1.0 mL/min.

We used urine osmotic potential as a relative measure of hydration state and measured it in duplicates with a freezing point depression osmometer (Precision Systems, Osmette II).

Results

As expected, urine osmotic potential increased steadily throughout the experiment and was in the range 4000–5000 mosmol·(kg H_2O)$^{-1}$ during normal hydration and increased to the range 6000–7000 mosmol·(kg H_2O)$^{-1}$ during water deprivation.

The urine of all the rodents tested in this study contained some dissolved allantoin, but neither the urine of *G. a. allenbyi* nor that of *A. cahirinus* and *G. pyramidum* contained any precipitate at any level of hydration. In *G. dasyurus*, the concentration of dissolved allantoin in urine increased from 22.5 to 202 mg/dL after 2 weeks of water deprivation, and this increase coincided with the first appearance of a precipitate in their urine. The urine of *P. obesus* contained large amounts of precipitate (about 20% of total urine mass) at all levels of hydration. The urine of *G. dasyurus* contained smaller quantities of precipitate (reaching about 11% of urine mass), which began to appear in the urine only after 2 weeks of water deprivation. The precipitate in the urine of both species was not allantoin as determined by NMR and ^{13}C analysis. Unfortunately, the small amount of urine collected from *G. dasyurus* did not allow further analysis of this precipitate.

The Fourier-transformed infrared spectrum of the *P. obesus* urine precipitate was compared with the spectra of three oxalate salts: ammonium, calcium, and sodium (Fig. 1). The close fit in the diagnostic region (circled area in Fig. 1) of these spectra indicates that the precipitate in the urine of *P. obesus* is an oxalate salt, more specifically sodium oxalate. The main cations in the sample were sodium, potassium, and magnesium and the main anions were chloride and sulfate. Oxalate was found in both the liquid and the precipitate portions of the urine.

Discussion

The dissolved allantoin in the urine of the rodents examined was most probably the by-product of purine catabolism. In contrast to the other species tested, the concentration of dissolved allantoin in the urine of *G. dasyurus* increased almost 10-fold after 2 weeks of water deprivation. However, this increase did not result from an increase in the absolute amount of allantoin, but from a decrease in urine volume. Interestingly, the concentration of dissolved allantoin in the urine of *G. dasyurus* dropped sharply in the fourth week of water deprivation. We were unable to ascertain whether the initial increase in dissolved allantoin was related to water

deprivation per se or to the formation of a precipitate, because the small amount of precipitate formed was not sufficient for analysis.

Buffenstein et al. (1985) proposed that Namib Desert rodents switch from urea production to allantoin production as a water-saving mechanism during water stress. This suggestion implies that these rodents possess biochemical pathways that are new to science, since there are no known metabolic pathways for the biosynthesis of allantoin from urea. The switch from urea production to allantoin production results in significant water savings, up to 20% of daily water excretion, but has a high energetic cost (Randall et al. 1997). Therefore, it may only be feasible when water savings must occur and energy is available.

There are a few examples of animals that excrete uncharacteristic (for their taxa) nitrogenous products in their urine in order to realize water savings. The tortoise *Testudo mauritanica* shifts between urea and uric acid according to temperature and hydration state (Drilhon and Marcoux 1942). Most frogs are ammonotelic during the aquatic tadpole stage and switch to urea production after metamorphosis (Schmidt-Nielsen 1997). The African tree frog *Chiromantis xerampelina* excretes mainly uric acid (Loveridge 1970) and the urine of the waterproof frog (*Phyllomedusa sauvagii*) contains large amounts of semisolid urates. In these frogs, the ability to excrete uric acid instead of urea reduces the daily requirement for urine formation from 60 to 3.8 mL water per kilogram body mass (Shoemaker et al. 1972).

A precipitate was present in the urine of *P. obesus* at all levels of hydration and therefore it was unlikely that its presence was related to water conservation. This precipitate was determined to be sodium oxalate, which should not be surprising, since saltbush in the Negev Desert contains large amounts of oxalate as well as NaCl (Ellern et al. 1973). Shirley and Schmidt-Nielsen (1967) found that *P. obesus* fed ^{14}C calcium oxalate did not increase their urinary Ca^{2+} excretion and expired about 25% of the ^{14}C that they were fed in the form of $^{14}CO_2$. Furthermore, when they were fed sodium ^{14}C oxalate they metabolized almost 100% of the oxalate. However, when injected with ^{14}C oxalic acid some animals excreted most of it in their urine, while others oxidized most of it. Shirley and Schmidt-Nielsen (1967) suggested that the ability to metabolize oxalate is important for *P. obesus*. Our results are consistent with this suggestion; it may be that *P. obesus*, like pack rats (*Neotoma albigula*), have the ability to use the calcium in calcium oxalate (Shirley and Schmidt-Nielsen 1967) and then excrete the oxalate in its soluble form (sodium oxalate) in their urine. The results from ion-exchange chromatography indicated that the precipitate contained almost no Ca^{2+}, but large amounts of Na^{+}, and that oxalate was found both in the precipitate and the liquid portions of the urine. In view of this, as well as the Fourier-Transformed infrared analysis of the urine precipitate, we concluded that the precipitate in the urine of *P. obesus* was sodium oxalate. By excreting sodium oxalate in their urine, *P. obesus* are able to tolerate a high dietary intake of sodium and oxalate and therefore consume saltbush leaves almost exclusively, in spite of their high salt and oxalate content. The question of whether *P. obesus* can absorb calcium from calcium oxalate requires further investigation.

In summary, the Namib Desert gerbilline and cricetomyine rodents may be examples of closely related mammalian taxa that have evolved a new metabolic pathway to produce a nitrogenous product, i.e., allantoin from urea, resulting in sizable water savings. The present study suggests that not all gerbilline rodents have this capacity, and we hypothesize that while evolving in the most ancient extant desert in the world, Namib Desert rodents have evolved unique mechanisms for minimizing urinary water loss.

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