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Lab study

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Identification of crystalline allantoin in the urine of African Cricetidae (Rodentia) and its role in their water economy

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Summary. All eleven cricetid species, examined in this investigation, produced an off-white crystalline precipitate in their urine when deprived of water, whereas not one murid examined did so. This crystalline compound was identified as allantoin, a common end product of purine catabolism. The quantity found in the solid precipitate alone accounted for 47% of the total nitrogen excreted and was approximately 14 times greater than the predicted quantity of allantoin from purine degradation. It appears that there is a shift in nitrogen excretion from urea to allantoin in the Cricetidae.

Water-deprived cricetids had higher urine osmolalities, urea concentrations and lower daily percentage body water turnovers than the murids. This can be explained by the substantial water savings associated with excreting solid allantoin. The discrepancy in the mode of nitrogen excretion between the two families inhabiting the Namib Desert can be attributed to their different evolutionary histories, the Cricetidae being pre-adapted for survival in deserts.

Introduction

Rodents are an important faunal element in arid environments. Much attention has been directed towards the physiological, morphological and behavioural attributes which favour their survival in these environments (Schmidt-Nielsen 1964; MacMillan et al. 1972; Borut and Shkolnik 1974; Ghobrial and Nour 1975; Mares et al. 1977; Christian 1979). Their physiological adaptations, however, might in part reflect their different evolutionary histories and their various taxonomic affinities.

During an investigation of the effect of water deprivation on arid-adapted rodents from the Namib desert, Buffenstein (1977) observed that when deprived of water, the cricetids *Gerbillurus paeba* and *Gerbillurus tytonis* produced large quantities of off-white needle-like crystals whilst, the murid *Rhabdomys pumilio* did not. Owing to the relative abundance of this substance in the urine of water-stressed *Gerbillurus* and the possibility that it might play an important role in its renal physiology, its investigation was undertaken. In addition, water economy and renal efficiency of several species of rodents from the arid regions of southern Africa were compared in an attempt to elucidate the physiological role of this substance.

Abbreviations: *WTR* water turnover rate

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Materials and methods

Animals. Rodents were live-trapped in Sherman traps baited with a mixture containing peanut butter, dried fruit and seed. The following murid and cricetid species, based on the classification of Missone (1974), were collected from the arid regions of the Cape Province (South Africa) and Namibia: (i) Muridae: *Acomys subspinosus, Aethomys chrysophilus, Aethomys namaquensis, Praomys natalensis, Rhabdomys pumilio* and *Thallomys paedulcus*; (ii) Cricetidae: *Desmodillus auricularis, Gerbillurus paeba, Gerbillurus setzeri, Gerbillurus tytonis, Saccostomys campestris* and *Tatera leucogaster*. In addition, we collected two arid-adapted cricetid species from north-eastern Kenya, *Gerbillus pusillus* and *Tatera robusta*, and four cricetid and four murid species from mesic areas in the Cape Province, namely *Gerbillurus paeba, Malocothrix typica, Steatomys krebsii* and *Tatera afra* and *Acomys subspinosus, Aethomys namaquensis, Mus musculus* and *Rhabdomys pumilio*.

The rodents were housed individually and supplied at lib. with golden millet and water in the form of green vegetables. They were left to acclimate to their new environment (12 L:12 D: 22–26 °C, 45–60% humidity) for two months prior to deprivation of water (hydropenia).

Hydropenia. The water supply was gradually decreased by reducing the daily ration of green vegetables to zero over eight days. Animals were weighed regularly on a Mettler balance to the nearest 0.01 g. In non arid-adapted species, if weight loss exceeded 30% of the initial body mass, water in the form of green vegetables was again supplied.

Urine. 24 h urine samples were collected under light liquid paraffin on the sixth day of hydropenia in non arid-adapted species and once the mass of all arid-adapted rodents had stabilised (six weeks after commencement of water deprivation). During the twenty-four hour periods of urine collection, millet seed was supplied *ad lib.* for two hours at the beginning of the scotophase. Urine was pipetted out and stored in plastic vials (Eppendorf 1.5 ml) and frozen (−15 °C) until analysis. As some contamination with faeces was inevitable, contamined urine was stored separately. Care was taken to collect all the urine and its crystalline component. Urine of all 22 species was examined for this crystalline component.

Prior to the analysis, the urine was centrifuged for ten minutes and the liquid fraction decanted into Eppendorf vials of known mass. The total volume of urine excreted per day was determined by weighing the urine and a separate 10 µl aliquot, thus converting mass into volume. If the urine had a crystalline component, the latter was dried at 28 °C, weighed, filtered and thoroughly washed with ethanol and left to dry.

Blood collection. Before resupplying free water, blood samples were taken from the canthal sinus of the anterio-dorsal aspect of the orbit, according to Halpern and Pacaud (1951). After haematocrit readings were taken, plasma was separated from the packed cells and frozen in sealed capillary tubes until analysis.

Urine and plasma analysis. The plasma and urine of nine species were analysed in detail: Osmolality was determined using a vapour pressure osmometer (Wescor model 5100B). The spectrophotometric method of Chaney and Marbach (1962) was used for measuring urea concentrations. Uric acid was determined by the enzymatic colorimetric assay of Kageyama (1971). Allantoin dissolved in the urine was estimated according to Young and Conway (1942) and Vrbaski et al. (1978) in four species. Considerable difficulty was experienced when assaying the quantities of allantoin dissolved in the urine. The photometric assay of Vrbaski et al. was found to be very sensitive to the presence of urea as is indicated by the linear relationship between urea concentration (x, in $g \cdot l^{-1}$) and optical density (y):

$$y = 0.095 + 0.196\,x \qquad r = 0.99$$

Allantoin in solution was therefore estimated by subtracting the known concentrations of urea (Table 2) from the 'allantoin' concentrations determined photometrically (Table 4).

Chemical analysis of the crystalline precipitate. The crystalline precipitate from the urine of *D. auricularis, G. paeba* and *T. leucogaster* was examined by 1H-nuclear magnetic resonance (1H-nmr), infra red (IR) and mass spectrometry. The IR spectra were run as Nujol mulls. The 1H-nmr spectra were recorded in hexadeuterodimethyl sulfoxide at 100 Mhz, using tetramethylsilane as an internal standard. Labelled water, D_2O, was used in determining whether the protons were exchangeable. Mass spectra were measured at 70 eV. In addition, uncorrected melting points were determined.

Melting points were determined on the crystalline compounds from the urine of all the other rodent species to ascertain whether the same compound was present.

Water turnover. Daily water turnover rates were determined in a separate group of animals collected in the same areas. These animals were left to acclimate to laboratory conditions for one month prior to the commencement of hydropenia. Percentage water turnover rates (WTRs) were measured by the isotopic dilution technique (Richmond et al. 1962; Holleman and Dietrich 1973).

Each animal was injected intraperitoneally with 10 µCi tritiated water (TOH) whilst still being maintained on an ad lib. water supply, just prior to the commencement of water stress. Water turnover rates were calculated from changes in the TOH concentration in consecutive blood samples taken at four day intervals, commencing after ten days without free water.

Blood samples collected from the orbit were centrifuged for 10 min and then the heparinised capillary tube was broken to provide a clear plasma sample. The volume of this sample was calculated from the tube length occupied by plasma and the inside diameter of the capillary tube. These samples were washed in 100 µl of 10% trichloroacetic acid in order to precipitate the plasma proteins. They were then stored in Eppendorf vials for subsequent analysis. Distilled water and stock TOH samples were treated in a similar manner.

The Eppendorf vials were centrifuged to obtain a protein free supernatant and a 50 µl sample of clear supernatant was thoroughly mixed with 10 ml Packard Instagel scintillation cocktail. Radioactivity was measured in a Packard model 3385 liquid scintillation counter. A Wang 700 bench top computer programmed to correct for quenching was used to convert cpm to dpm. The WTR was calculated using the equations of Yousef et al. (1974).

Statistical analyses included Student's *t*-test and analysis of variance according to Zar (1974).

Results

Species producing a crystalline precipitate

All members of the Cricetidae examined produced a substantial crystalline deposit in their urine when deprived of water. None of the Muridae examined excreted a crystalline waste (Fig. 1).

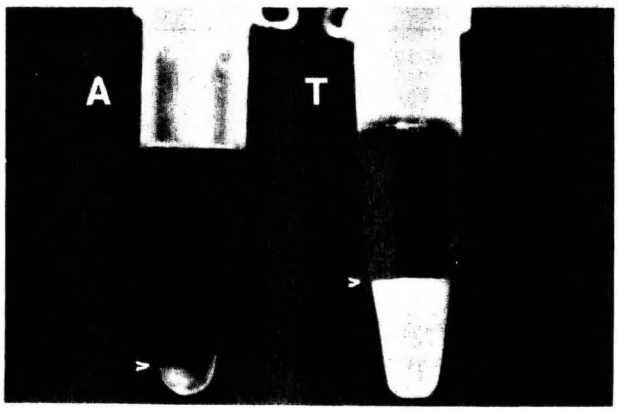

Fig. 1. Twenty-four hour urine samples from (*A*) *Aethomys namaquensis* and (*T*) *Tatera leucogaster* deprived of water for six weeks

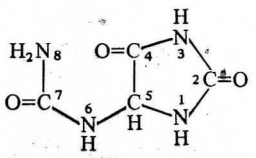

Fig. 2. Structure of allantoin

Chemical analysis of the crystalline compound

The crystals of the precipitate in the urine of *D. auricularis, G. paeba* and *T. leucogaster* were insoluble in most of the common organic solvents with the exception of warm dimethylsulfoxide and dimethylformamide and were only very slightly soluble in water. The spectra obtained from the precipitate of the three species were identical and showed no trace of impurity.

The IR spectrum showed absorption at 3,470 to 3,370 cm^{-1} and 1,785 to 1,710 cm^{-1} indicating an NH group and a CO group, respectively.

The 100 Mhz 1H-nmr spectrum clearly defined six protons. The one proton singlets at *d* 10.34 and 8.05 were assigned to H-3 and H-1 respectively. Both were D_2O exchangeable. The one proton doublet at *d* 6.90 ($J_{H,H}=9$ Hz) was attributed to H-6. The two proton doublet at *d* 5.78 was due to the NH_2 group and the one proton doublet at *d* 5.28 ($J_{H,H}=9$ Hz) was assigned to H-5. The high resolution mass spectrum gave a molecular ion at m/e 158.04458. $C_4H_6N_4O_3$ requires m/e 158.04399. Major fragment ions were recorded at m/e 141 (M-17), 130 (M-CO), 115 (M-CONH), 87, 60, 55, 44. The crystals had a melting point of 232–233 °C. Crystals from all the species that produced an off-white precipitate in their urine had similar melting points.

From the melting point and the spectral data, the crystalline compound was identified as allantoin, data agreeing closely with published values. The structure of this compound is given in Fig. 2.

Urine concentrating capacity; allantoin excretion

All arid species were quite capable of existing for fairly long periods (six weeks) without free water. *Desmodillus auricularis* did not lose weight when deprived of water, whereas *R. pumilio* lost the most (25%) before mass stabilised. All other arid-adapted species showed intermediate responses (Buffenstein 1984, 1985).

Urine osmolality increased progressively over the period of water restriction, reaching a maximum after one month. *Desmodillus auricularis* produced the most concentrated urine (5,500 mOsm · kg^{-1}) when deprived of water and also showed the highest urine/plasma (14:1) ratio (Table 1). The cricetids as a whole showed higher ($P \leqq 0.01$) urine concentrations when deprived of water than the murids. Urea concentrations (Table 2) rose concomitantly with increased osmolality. Urea contributed most of the osmolality of the urine in all species. Consequently the cricetids investigated had higher maximum concentrations of urea than those of the murids. Both the murids and the cricetids showed similar maximum concentrations of uric acid when hydropenic.

When deprived of water for two weeks, daily water turnover rates of the cricetids were approximately 77% of those of the murids (Table 3).

Table 1. Maximum urine and plasma concentrations in arid-adapted rodents deprived of water for six weeks

Species	*n*	Concentration mOsm l^{-1}		Urine/Plasma ratio
		Urine $\bar{x}\pm$SE	Plasma $\bar{x}\pm$SE	
Cricetidae				
Desmodillus auricularis	15	5,507±339	396±16	14
Gerbillurus paeba	32	4,837±178	413±12	12
Gerbillurus setzeri	14	5,368±148	480±22	11
Gerbillurus tytonis	16	5,404±222	456±18	12
Gerbillus pusillus	12	4,084±210	505±21	9
Tatera leucogaster	30	5,000±172	404±10	12
Muridae				
Acomys subspinosus	4	3,784±186	586±18	7
Aethomys crysophilus	2	3,468±372	424±22	8
Aethomys namaquensis	28	3,725±136	419±14	9
Praomys natalensis	4	3,740±540	400±28	9
Rhabdomys pumilio	6	3,805±230	444±21	9

Table 2. Maximum concentrations of urea and uric acid in the urine of some cricetid and murid rodents from the Namib desert

Species	Urea concentration (mM)		Uric acid concentration (mM)	
	$\bar{x}\pm$SE	*n*	$\bar{x}\pm$SE	*n*
Cricetidae				
Desmodillus auricularis	3,929±149	10	0.58±0.60	7
Gerbillurus paeba	3,900±491	8	0.23±0.08	5
Gerbillurus setzeri	3,353±203	9	0.39±0.07	7
Gerbillurus tytonis	3,169±284	8	0.21±0.09	8
Gerbillus pusillus	2,351±215	15	–	–
Steatomys krebsii	2,513±195	6	–	–
Tatera leucogaster	3,592±911	12	0.45±0.05	13
Muridae				
Aethomys namaquensis	2,449±199	10	0.36±0.06	10
Rhabdomys pumilio	2,077±208	13	0.20±0.03	9

Table 3. Daily water turnover rates in arid-adapted rodents

Species	WTR (%) $\bar{x} \pm SE$	n
Cricetidae		
Desmodillus auricularis	4.30±0.67	7
Gerbillurus paeba	4.01±0.74	6
Gerbillurus tytonis	3.91±0.98	6
Tatera leucogaster	3.26±0.46	11
Muridae		
Aethomys namaquensis	4.82±0.41	21
Rhabdomys pumilio	5.18±0.43	6

The concentration of allantoin dissolved in the urine of *A. namaquensis* and *D. auricularis* fell within the solubility range of allantoin whereas the urine of *G. paeba* and *T. leucogaster* was slightly supersaturated. Allantoin in the crystalline precipitate was found to be pure and contributed substantially to both urine mass (Table 4) and to the amount of nitrogen excreted (Table 5).

Discussion

Allantoin excretion

Most mammals, with the exception of the Dalmation dog and some primates, including man, excrete their purine catabolites in the form of allantoin. This is produced predominantly in the liver by uricase (Byers et al. 1947). *Rattus* sp. on a normal diet excrete approximately 1.5 mmol·kg^{-1} body mass·day^{-1} (Greger et al. 1976). This quantity falls well within the water solubility range (6.3 mmol·l^{-1}). Using tracer techniques Greger et al. (1975) showed that the renal handling of allantoin in *Rattus* sp. consists of free filtration with neither reabsorption nor secretion occurring along the nephron.

To date no mention has been made in the literature of the crystalline precipitate of allantoin found in the urine of hydropenic cricetid rodents. In fact little quantitative work has been done on the excretion of allantoin. This appears to be primarily due

Table 4. Allantoin excretion in Namib desert rodents deprived of water for four weeks

Species	Allantoin[a]		Crystalline allantoin			
	n	mM	n	mmol day^{-1}	mg day^{-1}	% Urine mass
Cricetidae						
Desmodillus auricularis	8	612±126	9	0.35±0.03	56.0±5.2	29.63±2.8
Gerbillurus paeba	8	788±164	13	0.29±0.002	45.3±3.1	28.49±2.1
Tatera leucogaster	11	870±194	16	1±0.43	175.0±7.0	28.94±9.3
Muridae						
Aethomys namaquensis	9	615±202	28	0.00±0.00	0.0±0.0	0.00±0.0

[a] Corrected values, urea deducted, cf. Materials and methods

Table 5. Daily nitrogen excretion in urea and allantoin and estimated water savings by excreting crystalline allantoin instead of urea

	Aethomys namaquensis		*Desmodillus auricularis*		*Gerbillurus paeba*		*Tatera leucogaster*	
	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
Mass (g)	47.9	–	59.9	–	29.9	–	53.7	–
Urine vol. (10^{-1} ml day^{-1})	4.0	–	1.5	–	1.4	–	5.0	–
Total urea (10^{-3} mmol day^{-1})	979.6	79.97	550.1	55.45	443.6	52.50	1,796.0	53.74
Total uric acid (10^{-4} mmol day^{-1})	1.3	0.01	0.8	0.01	0.3	0.04	2.3	0.01
Total allantoin in solution (10^{-3} mmol day^{-1})	245.2	20.02	92.0	9.27	111.3	13.17	435.5	13.03
Total allantoin solids (10^{-3} mmol day^{-1})	0.0	0.0	350.0	35.28	290.0	34.32	1,110.0	33.22
Total nitrogenous wastes measured (10^{-3} mmol day^{-1})	1,226.1	–	992.9	–	845.2	–	3,343.8	–
Nitrogen in urea (10^{-3} mg day^{-1})	27.4	66.67	15.4	28.07	12.4	35.81	50.2	36.76
Nitrogen in dissolved allantoin (mg day^{-1})	13.7	33.33	19.6	35.74	6.2	17.90	24.4	17.86
Nitrogen in crystalline allantoin (mg day^{-1})	0.0	0.0	19.8	36.18	16.0	46.29	61.95	45.36
Measured urinary nitrogen (mg day^{-1})	41.1	–	54.8	–	34.6	–	136.55	–
Water saving from crystalline fraction (10^{-3} ml)	0.0	0.0	38.0	25.33	31.0	21.95	119.0	23.8

to difficulties encountered in the use of quantitative assays for allantoin. Only non-specific photometric assays are available (Larson 1931; Young and Conway 1942). These techniques are cumbersome and in the present study were found to result in considerable error. The main drawback of these techniques and also of more recent assays is interference from other urinary wastes, especially urea (present study, Abraham et al. 1976; Borchers 1977). When using the method of Vrbaski et al. (1978) we found that the estimated amounts of allantoin were most unrealistic (0.5–1.1 kg allantoin·l^{-1}). The cricetids appeared to excrete 1.5 times as much 'allantoin' as the murids. This ratio was similar to that of the urea concentrations and can therefore be attributed to urea rather than allantoin levels. From this it can be assumed that levels of dissolved allantoin in the urine are similar in both rodent families (in normal, not water-deprived animals) and this would be the result of normal purine catabolism.

By contrast, the quantity of crystalline allantoin excreted by the cricetids when deprived of water (Fig. 3), accounted for 29% of the daily mass of urine excreted and approximately 30% of the total nitrogen excreted per day (Tables 4 and 5). Allantoin excretion determined from the precipitate alone was substantially higher than that estimated by Pak et al. (1973) from body weight for rats. *Tatera leucogaster* produced 24 times the amount predicted for a rat of the same body weight in the solid fraction of allantoin alone. *Gerbillurus paeba* and *D. auricularis* excreted 939% and 753%, respectively, of that predicted by mass using the allometric equation of Pak et al. (1973).

Allantoin production in rats has been found to be influenced by several parameters: Morgan and Hanson (1964) found that larger quantities were excreted when there was an increase in protein synthesis through growth or lactation. Kiriyama and Ashida (1964) suggested that allantoin production is dependent on the age of the animal, dietary protein, body size and the rate of growth, whereas Pak et al. (1973) obtained contradictory results. These authors concluded that the amount of allantoin excreted in the urine was independent of the age of the animal and of the diet consumed, but was directly related to (a) the weight and the rate of gain in weight and (b) body nitrogen content and the rate of gain of nitrogen content. Allantoin, although present in relatively high concentrations in the seeds and germinating sprouts of several plant families (Tracey 1955; Barash 1972), was not present in millet seeds in any detectable quantity in this investigation. In the light of these findings, it is most interesting to observe that no precipitate of allantoin occurs in the Muridae, whilst the Cricetidae maintained on an identical diet and under the same experimental conditions, produce allantoin in quantities greater than the solubility range (Fig. 1). These large quantities cannot be merely a result of purine degradation, as the rodents used in this study were all mature, non-lactating animals which were able to survive indefinitely on a diet of air-dried seed alone. All measurements reported here were taken after weight had stabilised. Slight changes in weight occurred in both families and cannot therefore account for the differences found.

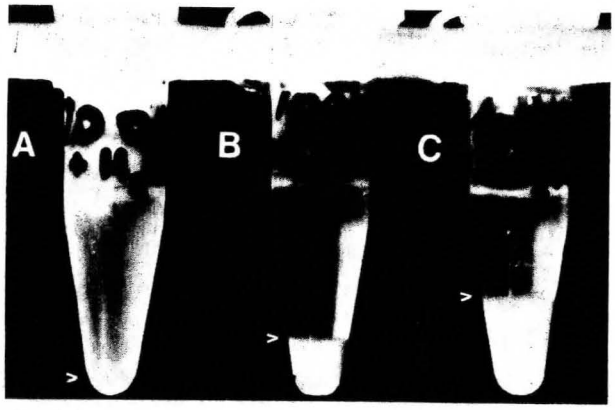

Fig. 3. Urinary crystalline deposits from an individual *Tatera leucogaster* when (*A*) provided with ad lib. water, (*B*) water stressed, (*C*) water deprived for ten days. Note the approximately three-fold increase in the amount of crystalline precipitate

Significance of allantoin excretion

Both Cricetidae and Muridae are primarily ureotelic. Urea accounted for 67% of the measured nitrogen excretion in hydropenic *A. namaquensis*, whereas it constituted only 28% of the measured nitrogen in *D. auricularis* deprived of water (Table 5). By not converting allantoin to urea and glyoxylic acid, or conversely by converting urea to allantoin, three moles of water are saved for every one mole of urea (cf. Lehninger 1970) in addition to the reduced solvent requirements. From this one can deduce that approximately 20% of the water which would have been excreted in the urine is saved. This saving in water is reflected in the significantly different WTRs ($P \leqq 0.01$) between the murids and the cricetids of the Namib. The WTR saving is similar in magnitude (Table 5) to the savings calculated from the solid moiety. Cricetid WTRs were even slightly lower than the lowest WTRs recorded by Yousef et al. (1974) for heteromyid rodents. These low WTRs were accom-

panied by concomitantly high maximum osmolalities and urea concentrations.

Shifts from ammoniotelism to ureotelism are a common phenomenon in the lower vertebrates. Shifts from ureotelism to uricotelism when temperatures are elevated and water is limiting, have also been observed in some frogs (Loveridge 1970) and in tortoises (Drilhon and Marcoux 1942). The cricetids, however, appear to be unique amongst the mammals in showing a shift from urea to allantoin.

The ability to produce a concentrated urine has been viewed as an indicator of efficiency of water conservation (Schmidt-Nielsen 1979). Our results suggest that the African cricedits are better adapted for desert niches than the murids. The murids are relatively recent invaders of the harsh Namib desert (Meester 1965; Missone 1969) and their distribution is restricted to the more mesic regions on the eastern side of the Namib, rocky outcrops and densely vegetated areas (pers. obs.; Coetzee 1969; Christian 1979; Withers 1979). It is possible that markedly increased rates of allantoin excretion confer an adaptive advantage to the long-term residents (Cricetidae) of the Namib.

The generalisation that the murids are less well adapted for desert survival in the arid zones of Africa cannot be applied universally as the Australian murids hold the record for the most concentrated urine (MacMillen and Lee 1969); MacMillen et al. 1972; Hewitt et al. 1981). Missone (1969) whilst examining the evolutionary history of the murids suggested that Africa and Indo-Australia have been separated from each other sufficiently long to allow an almost complete generic separation. This might account for the difference in renal performance between the African and Australian murids.

Excretion of allantoin whilst saving considerable amounts of water, is not totally advantageous, as the synthesis of allantoin results in the removal of twice as much carbon (1C/1N as opposed to 1C/2N in urea). Carbon loss is energetically linked and excretion of allantoin is therefore more costly than urea. Nevertheless, the excretion of large quantities of allantoin can play an important role in enabling the cricetids to thrive in areas inaccessible to the murids. Even mesic cricetids have retained the ability to excrete allantoin and this might therefore be used as a taxonomic tool in assigning doubtful groups of rodents to either of these families.

These findings pose several interesting questions which remain unanswered. Firstly, what shifts the biochemical pathway from urea to allantoin? Secondly, are the crystals of allantoin formed intrarenally and if so, what processes are employed to enable their passage through the urinary system? Most importantly, why, with the abundance of information on renal function of arid dwelling rodents, has this phenomenon not been previously recorded? Does this suggest that this means of water conservation is unique to the African cricetids and if so, why?

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References

Abraham J, Simeone FA, Hopkins FA (1976) A sensitive assay for allantoin. Anal Biochem 70:377–380

Barash J (1972) Accumulation of urea and allantoin during purine utilization by germinating spores of *Geothrichum candidum*. J Gen Microbiol 72:539–542

Borchers R (1977) Allantoin determination. Anal Biochem 79:612–613

Borut A, Shkolnik A (1974) Physiological adaptation to the desert environment. In: Robertshaw D (ed) Environmental physiology. International Review of Science, Physiology, Ser. 7. Butterworths, London MTP, pp 185–229

Buffenstein R (1977) Adaptive renal efficiency in three species of rodent. Unpublished honours project, University of Cape Town

Buffenstein R (1984) Energy and water balance during torpor and hydropenia in the pigmy gerbil, *Gerbillus pusillus*. J Comp Physiol B 154:535–544

Buffenstein R (1985) The effect of a high fibre diet on energy and water balance in two Namib desert rodents. J Comp Physiol B 155:211–218

Byers SO, Friedman M, Garfield MM (1947) The blood uric acid and allantoin of the rat after nephrectomy and hepatectomy. Am J Physiol 15:677–681

Chaney AL, Marbach EP (1962) Modified reagents for determination of urea and ammonia. Clin Chem 8:131–135

Christian DP (1979) Physiological correlates of demographic patterns in three sympatric Namib desert rodents. Physiol Zool 52:329–339

Coetzee CG (1969) The distribution of mammals in the Namib desert and adjoining inland escarpment. Sci Papers Namib Desert Res Station 40:23–36

Drilhon A, Marcoux F (1942) Etude biochimique du sang et de l'urine d'un chélonien: *Testudo mauritanica*. Bull Soc Chim Biol Paris 24:103–107

Ghobrial LI, Nour TA (1975) The physiological adaptations of desert rodents. In: Prakash I, Ghosh PK (eds) Rodents in desert environments. Junk, The Hague, Monographiae Biol 28:413–414

Greger R, Lang F, Deetjen P (1975) Handling of allantoin by the rat kidney. Clearance and micropuncture data. Pflügers Arch 357:201–207

Greger R, Lang F, Deetjen P (1976) Renal excretion of purine metabolites, urate and allantoin, by the mammalian kidney. Int Rev Physiol Kidney Urinary Tract Physiol 11:257–281

Halpern BH, Pacaud A (1951) Technique de prélèvement d'echantillons de sang chez les petits animaux de laboratoire

par fonction du plexus ophthalmique. CR Séanc Soc Biol 145:1465–1466

Hewitt S, Wheldrake JF, Baudinette RV (1981) Water balance and renal function in the Australian desert rodent *Notomys alexis*: the effect of diet on water turnover rate, glomerular filtration rate, renal plasma flow and renal blood flow. Comp Biochem Physiol 68A:405–440

Holleman DF, Dietrich RA (1973) Body water content and turnover in several species of rodents as calculated by the tritiated water method. J Mammal 54:456–465

Kageyama N (1971) A direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. Clin Chim Acta 31:421–426

Kiriyama S, Ashida K (1964) Effect of the quality of dietary protein on nitrogen compounds in the urine of rats. J Nutr 82:127–134

Larson HW (1931) A colorimetric method for the determination of allantoin. J Biol Chem 94:727–738

Lehninger A (1970) Biochemistry. Worth Publishers, New York

Loveridge JP (1970) Observations on nitrogenous excretion and water relations of *Chiromantis xerampelina* (Amphibia, Anura). Arnoldia 5:1–6

MacMillen RE, Baudinette RV, Lee AK (1972) Water economy and energy metabolism of the sandy inland mouse, *Leggadina hermansburgensis*. J Mammal 53:529–539

MacMillen RE, Lee AK (1969) Water metabolism of Australian hopping mice. Comp Biochem Physiol 28:493–514

Mares MA, Blair WF, Enders FA, Greegor D, Hulse AC, Hunt JH, Otte D, Sage RD, Tomoff CS (1977) The strategies and community patterns of desert animals. In: Orians GH, Solbrig OT (eds) Convergent evolution in warm deserts. Dowden Hutchinson and Ross, Cambridge Massachusetts, pp 107–163

Meester J (1965) The origins of the southern African mammal fauna. Zool Afr 1:87–93

Missone X (1969) African and Indo-Australian Muridae. Evolutionary trends. Tervuren, Belgique, Ann, Musée Royal Afrique centrale, Sér Sci Zool 172:1–219

Missone X (1974) Rodentia, Part 6. In: Meester J, Setzer HW (eds) The mammals of Africa: an identification manual. Smithsonian University Press, Washington, pp 1–39

Morgan EH, Hanson A (1964) Serum and urine allantoin in pregnancy and lactation in the rat. Acta Physiol Scand 60:164–169

Pak N, Donoso G, Tagle MA (1973) Allantoin excretion in the rat. Br J Nutr 30:107–112

Richmond CR, Langham WH, Trujillo TT (1962) Comparative metabolism of tritiated water by mammals. J Cell Comp Physiol 59:45–53

Schmidt-Nielsen K (1964) Desert animals: physiological problems of heat and water. Clarendon Press, Oxford

Schmidt-Nielsen K (1979) Animal physiology: adaptation and environment, 2nd edn. Cambridge University Press, Cambridge

Tracey MV (1955) Urea and ureids. In: Paech K, Tracey MV (eds) Modern methods of plant analysis, vol 4. Springer, Berlin Göttingen Heidelberg, pp 119–141

Vrbaski MM, Grujic'-Injac B, Gajic' D (1978) A new method for allantoin determination and its application in allantoin determination in *Agrostemma githago* L seeds. Anal Biochem 91:304–308

Withers PC (1979) Ecology of a small mammal community on a rocky outcrop in the Namib desert. Madoqua 2:229–246

Yousef MK, Johnson HD, Bradley WG, Seif SM (1974) Tritiated water turnover rate in rodents: desert and mountain. Physiol Zool 47:153–162

Young EG, Conway CF (1942) On the estimation of allantoin by the Rimini-Schryver reaction. J Biol Chem 142:839–852

Zar JH (1974) Biostatistical analysis. Prentice Hall, New Jersey