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Renal physiology of two southern African *Mastomys* species (Rodentia: Muridae): a salt-loading experiment to assess concentrating ability

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Abstract

Aspects of renal physiology were examined to test the hypothesis that two cryptic species of the genus *Mastomys* (*Mastomys natalensis* and *Mastomys coucha*) are geographically separated by differences in aridity tolerance. Laboratory-bred females of each species were subjected to different levels of salinity in their water source (distilled water, 0.9% NaCl, and 1.5% NaCl; 10 conspecifics in each group) from weaning until sexual maturity. Individuals of the two species exhibited similar rates of water consumption and urine production. The salinity treatments caused sodium diuresis in both species, evident in increased urine volume, decreased osmolality and increased osmotic output. Urine concentration, kidney mass and kidney relative medullary area (RMA) did not differ between species. The results of our study do not support the hypothesis that differences in osmoregulatory ability separate these two cryptic species. Nor do they support the use of salt loading to elicit maximum urine concentrations in mammals.

Keywords: *Mastomys coucha*; *Mastomys natalensis*; Osmoregulation; Kidneys; Urine concentration

1. Introduction

In South Africa, the widely distributed rodent genus *Mastomys* has been traditionally considered to comprise a single species, *Mastomys natalensis*, but subsequent studies have revealed the existence of two cryptic species, *M. natalensis* (A. Smith, 1834) and *Mastomys coucha* (A. Smith, 1836) (Gordon, 1984). These species are two of the most common and widely distributed southern African rodents (Green et al., 1980; Gordon, 1984; Smit et al., 2001). As one of the first species to recolonise disturbed habitats, *M. natalensis* can undergo rapid population increases, making it a major agricultural pest (Leirs et al., 1996; Stenseth et al., 2001; Jackson and van Aarde, 2004). Because of this and both species' medical importance as vectors of bubonic plague (Grats, 1997), they have been the focus of considerable research.

Information available on their distribution suggests that within South Africa *M. natalensis* is confined to high rainfall areas (greater than 600 mm annual precipitation) along the eastern coastal region, whereas *M. coucha* predominantly occurs at higher altitudes in central regions with lower and more variable rainfall (Skinner and Smithers, 1990; Venturi et al., 2004). Potential factors influencing these distributions have not been examined. Water is a limiting factor for many terrestrial rodent populations (e.g., Reaka and Armitage, 1976; Moro and Bradshaw, 1999a) and physiological adaptation of small rodents to arid conditions is achieved mainly through concentrating ability of their kidneys (Beuchat, 1996). The present paper tests the hypothesis that observed differences in the distribution of *M. natalensis* and *M. coucha* can be

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attributed, at least in part, to differences in kidney function, stemming from the observation that there is a strong correlation between the pattern of distribution of the two species and variability in precipitation.

The urine concentrating ability of mammalian kidneys is dependent on renal medullary size, a consequence of nephron morphology. Animals from more arid habitats generally have better urine concentrating ability than those from more mesic regions (Beuchat, 1993, 1996). We predicted that *M. coucha*, occurring in areas of lower and more variable rainfall (Venturi et al., 2004), would exhibit a superior ability to concentrate urine, facilitated by a more pronounced medulla, than the more mesically distributed *M. natalensis*. This hypothesis was tested in a series of experiments designed to examine renal function and response to an osmoregulatory challenge. Renal function was quantified by examining differences between laboratory-reared females of the two species in water consumption, urine volume and urine osmolality in response to mild salt loading. In addition, kidney mass and morphology were compared under the same conditions. Salt loading is one of the treatments commonly used to elicit maximum osmolality in studies of urinary concentrating ability in mammals (Beuchat, 1996). Our choice of a salt-loading experiment was based on the knowledge that *Mastomys* are omnivorous (Field, 1975, De Graaf, 1981) and cannot withstand water deprivation, an alternative way of testing concentrating ability, for more than 2–3 days (T.P. Jackson, personal observation). The use of post-F1 offspring reared under identical conditions allowed us to focus on genetic rather than phenotypic differences between the two species (Tracy and Walsberg, 2001a).

2. Materials and methods

2.1. Animals and their maintenance

The original stock of *M. natalensis* was from Richards Bay (28°43′ S, 32°12′ E) while *M. coucha* were trapped at the University of Pretoria's Experimental Farm (25°45′ S, 28°14′ E). Species status of individuals was confirmed by starch gel protein electrophoresis (G. Campbell, personal communication). Animals were bred (about eight generations for *M. natalensis* and three generations for *M. coucha*) at 25 °C on a 14L/10D cycle. Humidity in the holding facility, monitored using HOBO relative humidity sensors (Onset Computer, Bourne, MA), was found to fluctuate around 36±8.5% RH. The same conditions were maintained throughout the experimental period. Experimental animals were housed individually in standard rodent cages (41×24.5×12 cm) fitted with wire mesh tops, and placed randomly (with regard to treatment) in holding racks. Bedding, in the form of sawdust, was changed on a weekly basis. We provided mouse pellets containing 12% water and 18% protein (Epol Animal Feed Manufacturers, Pretoria West, South Africa) and drinking water ad libitum.

2.2. Saline treatments

The experimental protocol comprised two saline treatments and a control (water), together with mouse pellets. We used 30 females of each species in our study, with 10 conspecifics randomly allocated to each treatment. We administered water and two saline treatments (0.9% and 1.5% NaCl) by means of agar blocks (20 g of dry agar gel per litre distilled water) to mice between 21 and 70 days of age (weaning age and sexual maturity respectively; Meester, 1960). A pilot study showed that concentrations of 2% NaCl and higher deterred animals from drinking, so that some died from dehydration (S. Ntiyantiya, unpublished data). We therefore opted to use lower concentrations that would still challenge these particular species osmotically. Agar blocks were replaced daily and we measured agar consumption during the seventh week of treatment by recording the change in mass after 24 h, with corrections for evaporative water loss from control blocks of the relevant salinity. These values were converted to daily water consumption (g water/day) by multiplying by the proportion of water in the agar blocks. All animals were weighed at weekly intervals throughout the experimental period.

2.3. Urine collection

At 75 days of age, individuals were transferred to metabolic cages (Labotec Suppliers, Halfway-House, South Africa). We collected urine for three consecutive nights under liquid paraffin in plastic containers placed under these cages. During the day, we transferred animals back to their rodent cages, and provided individuals with mouse pellets and agar blocks of different salinities as before. We could not provide either food or water overnight, as experience showed that animals crumbled the food pellets and agar blocks, contaminating the collection medium. Thus, eating and drinking were limited to daytime and water consumption was not measured during the three days of urine collection. Urine samples were pipetted into 1.5-mL microcentrifuge tubes and frozen until analysis. For the 30 females of each species on the three treatments, urine was analysed separately for the three days of the collection period.

2.4. Analysis of urine osmolality

Osmolality of urine samples was analysed in duplicate and in random order using a Vapro Vapour Pressure Osmometer (Wescor, UT). Whenever possible, osmolality was measured using undiluted urine samples. However, those samples that exceeded the maximum limit of the

Table 1
Statistical analyses comparing *M. coucha* and *M. natalensis* maintained on different salinity treatments

Variable	Species		Treatment		Covariate (body mass)	
	F	*P*	*F*	*P*	*F*	*P*
Animal mass (g)	11.95	0.001	6.14	0.004	N/A	N/A
Kidney mass (g)	0.60	NS	0.73	NS	24.45	0.000
Relative medullary area	1.32	NS	3.80	0.032	0.001	NS
Water consumption (g/day)	0.18	NS	0.28	NS	0.069	NS
Urine volume (mL/h)	0.30	NS	2.43	NS	0.089	NS
Urine concentration (mmol/kg water)	0.0038	NS	4.91	0.012	0.43	NS
Total osmotic output (mmol/h)	1.06	NS	0.98	NS	0.095	NS

Body mass was analysed using two-way ANOVA, and the other variables were analysed using ANCOVA, with body mass as a covariate. No interaction effects were apparent in any of the analyses.

extended range thermocouple head (3600 mmol/kg) were diluted by a factor of 2:1 (sample: H_2O) or 1:1 for the most concentrated samples. Osmolality of a solution is not necessarily a linear function of concentration unless the solution is very dilute (see McFarland and Wimsatt, 1969; Sweeney and Beuchat, 1993), though Tracy and Walsberg (2001b) found that dilution of synthetic urine of kangaroo rats introduced negligible errors in osmolality measurements.

2.5. Kidney morphology

At the end of the trial, animals were euthanised using fluothane. Kidneys were dissected out, weighed and fixed in Bouin's solution for 24 h before being transferred to 70% ethanol for later processing. The left kidney from each animal was embedded in paraffin wax, sectioned sagittally at 7–10 μm and stained using Masson's trichrome staining technique (Humason, 1979). Sections were placed in a photographic enlarger and the image projected onto paper, so that the outlines of the kidney and the cortico-medullary junction could be traced. The two regions (cortex and medulla) were cut out and their respective areas were measured to the nearest 0.01 cm^2 using a leaf area meter. Relative medullary area (RMA) was calculated as the ratio of the medullary area to the cortical area: Of several renal indices used to estimate mammalian urine concentrating ability (see Beuchat, 1993), RMA can be considered the most precise (correlation coefficient=0.93, Brownfield and Wunder, 1976).

2.6. Statistical analysis

We analyzed the data for animal mass using two-way ANOVA, with species and treatment as independent variables. Data for kidney mass, RMA, water consumption, urine volume, urine osmolality and total osmotic output were analyzed with ANCOVA (Zar, 1996), again with species and treatment as independent variables and body mass as a covariate. We carried out post hoc analyses (Tukey HSD test for unequal samples) where significant differences ($P<0.05$) were recorded. All data are given as mean±S.E. As some animals lost weight during the 3 days of urine collection, and this change in body mass may have differed among groups, we present results only for the first day of urine collection.

3. Results

For both species, body mass after 7 weeks of treatment showed a reduction with increased salinity (Table 1; Fig. 1a and b). The only statistically significant differences, however, were between *M. coucha* females on 1.5% NaCl that

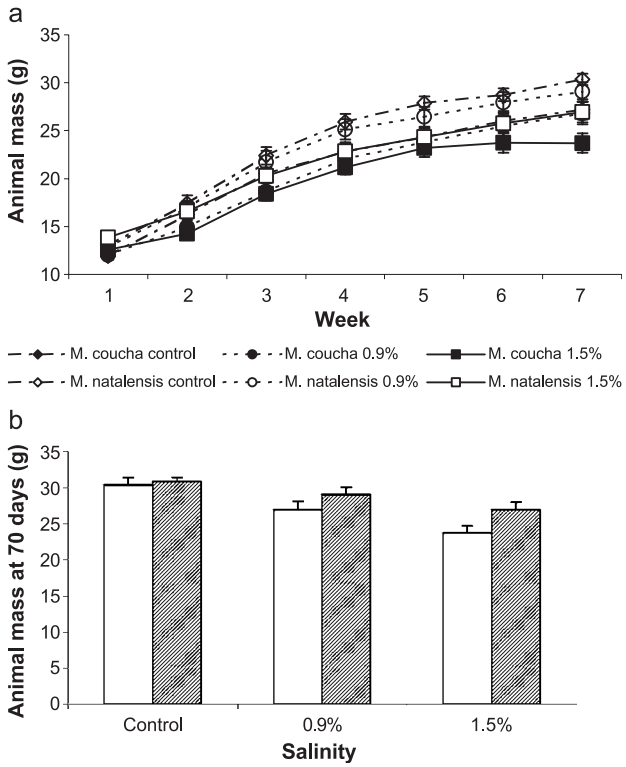

Fig. 1. (a) Body mass (g) of *M. coucha* and *M. natalensis* recorded over 7 weeks of treatment and (b) body mass (g) of *M. coucha* (clear bars) and *M. natalensis* (hatched bars) at the start of urine collection after seven weeks on treatments of differing salinity. All values are means±S.E. (N=9–10).

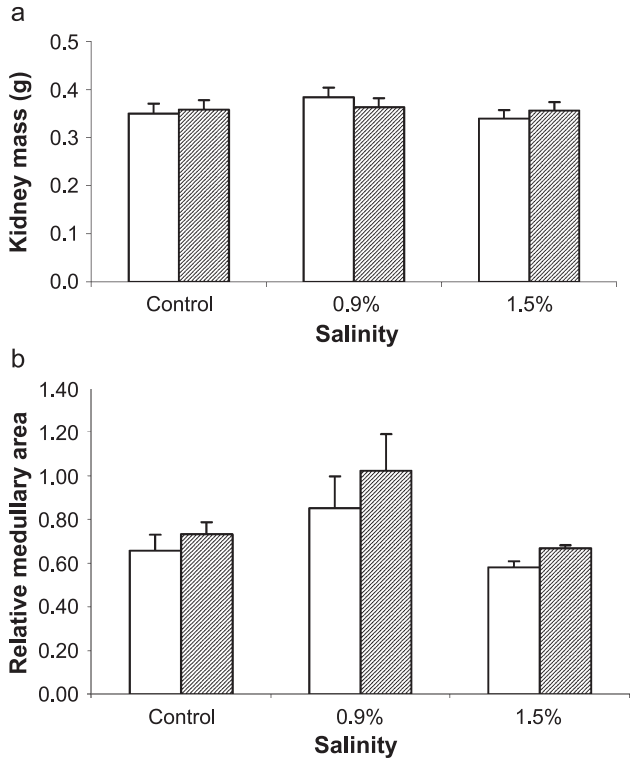

Fig. 2. Kidney variables (a) mass of both kidneys (g) and (b) relative medullary area (RMA) of *M. coucha* (clear bars) and *M. natalensis* (hatched bars) maintained on different salinity treatments. Values are means±S.E. (*N*=9–10 for kidney mass and 4–7 for RMA).

were lighter than *M. natalensis* females on control and 0.9% NaCl treatments. Within treatments, species' body mass did not differ significantly.

Under our experimental conditions, the mass of both kidneys averaged 0.34–0.38 g and did not differ significantly between species or treatments (Table 1; Fig. 2a). Body mass did, however, significantly effect kidney mass, though this effect did not differ between species or treatment. No inter-specific differences were recorded for RMA, though a significant treatment effect was recorded. Animals on 1.5% NaCl had significantly lower RMA values than the animals on 0.9% NaCl, but neither group differed significantly from the control animals, which were intermediate in terms of RMA (Table 1; Fig. 2b).

Water consumption rate was not affected by either species or treatment during the seventh week of our experiment (Table 1; Fig. 3). However, for the period of urine collection at the start of the eighth week, urine production apparently increased with NaCl concentration, though this increase was not statistically significant (Fig. 4a). Urine concentration significantly affected by treatment, with the control females producing more concentrated urine than females on 1.5% NaCl (Table 1; Fig. 4b). Mean values for urine osmolality of control animals on the water treatment were 3896±349 mmol/kg for *M. coucha* and 3666±436 mmol/kg for *M. natalensis*. Total osmotic output

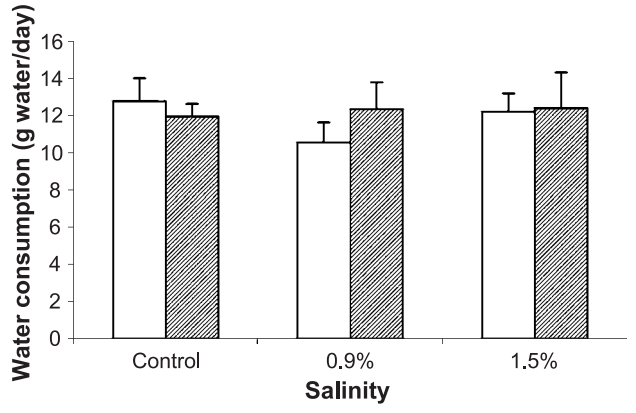

Fig. 3. Water consumption rate (g/day) of *M. coucha* (clear bars) and *M. natalensis* (hatched bars) during the seventh week on treatments of differing salinity. Values are means±S.E. (*N*=9–10).

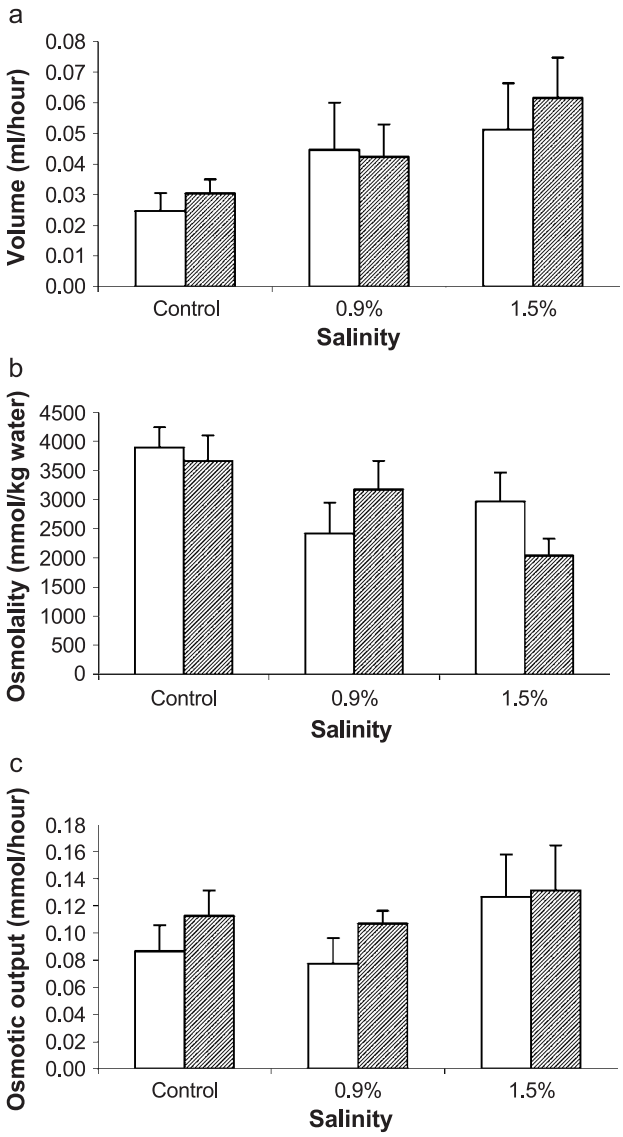

Fig. 4. (a) Production (mL/h) and (b) osmolality (mmol/kg water) of urine collected from mice after 7 weeks of salt loading. Total osmotic output (mmol/h) is shown in (c). Clear bars=*M. coucha*, hatched bars=*M. natalensis*. Values are means±S.E. (*N*=9–10).

was calculated from the data for urine volume and osmolality (Fig. 4c) and, although osmotic output increased with salinity, there were no significant differences between species or among treatments (Table 1).

4. Discussion

Traits measured on individual animals from different geographic areas may vary because of acclimation, developmental plasticity or genetic differences (Tracy and Walsberg, 2001a). In this study of physiological variation between two species of *Mastomys*, we excluded acclimation effects by using laboratory-reared animals. Although there were clear developmental effects of saline treatment on renal physiology, there were no genetic differences apparent between the two species (with the single exception of body mass compared across treatments).

Induced osmotic stress also resulted in reduced body mass in two species of *Acomys* (*Acomys russatus*, Ron and Haim, 2001; *Acomys cahirinus*, Scantlebury et al., 2002) but had no effect on body mass in house mice (*Mus domesticus*) and short-tailed mice (*Leggadina lakedownensis*) (Moro and Bradshaw, 1999b). In the present study, the decrease in body mass with increasing salinity was more pronounced for *M. coucha*, the arid-occurring species, than for *M. natalensis*, but the trend was not significant within species. That is, conspecifics on different treatments did not differ significantly in body mass. The reduction in body mass associated with increasing salinity may be a result of dehydration caused by salt-induced diuresis. A previous study of these rodents has clearly demonstrated that *M. natalensis* is larger than *M. coucha*, this difference being apparent from weaning (Jackson and van Aarde, 2003).

Unexpectedly, we found that the urine excreted by the control mice had the lowest volume and the highest concentration among the three treatments. The osmotic concentration of urine is, in general, inversely related to urine flow rate (McFarland and Wimsatt, 1969). In order to maintain homeostasis, mammals may reduce the load of excess solutes and nitrogenous wastes by flushing them out in urine. This was observed in the experimental mice: Both species of *Mastomys* responded to salt loading by sodium diuresis, with increased urine volume and total solute output. We did not measure sodium concentrations in the urine, but it is possible that these were less than the 150 and 260 mM Na^+ concentrations in the two saline treatments. A possible explanation for the discrepancy between the similar water consumption rates across treatments (Fig. 3) and differences in urine output (Fig. 4a) may lie in varying rates of food consumption. Considering that the moisture content of the food source was not controlled, differences in food consumption could mask differences in water consumption. If indeed the control animals' water intake from all sources did not differ from that of the groups on saline treatments, then it becomes difficult to account for the low urine volume of the control animals. Evaporative water loss is assumed to have been minimal in animals maintained at 25°C, but the control animals could have eliminated additional water in faeces. Plasma volume may also have varied between treatments. Degen (1997) discusses changes in body fluid compartments following dehydration in small mammals.

Our salinity treatments of 0.9% and 1.5% NaCl (0.15 and 0.26 M, respectively) were mild in comparison to those used in some other studies of osmoregulatory physiology of arid-occurring rodents. Water and salt balances of house mice (*Mus domesticus*) and short-tailed mice (*L. lakedownensis*) were investigated under both field and laboratory conditions by Moro and Bradshaw (1999a,b). Both species were capable of drinking saline solutions up to 1 M NaCl: Intake increased on 0.25 M NaCl compared to water, then decreased as salinity was increased further. Animals compensated for the increased salt intake by excreting more urine (whereas water restriction led to an immediate reduction of urine volumes). However, in contrast to the present study, urine osmolality also increased with salinity. Ron and Haim (2001) showed that acclimation of golden spiny mice (*Aco. russatus*) to a high salt concentration (7%) caused a dramatic decrease in urine volume, accompanied by a significant increase in urine osmolality, compared to mice on 1% NaCl. Wild-captured common spiny mice, *Aco. cahirinus*, also responded to increased dietary salinity (up to 3.5% NaCl) by increasing urine concentration, but a different response to salt loading occurred in their offspring: when offered agar containing increasing salt levels up to 2.5% NaCl, the offspring produced larger volumes of more dilute urine (Shanas et al., 2003). The offspring may have responded by consuming larger volumes of water, but drinking rate (agar consumption) was not measured. In a subterranean rodent with poor kidney concentrating ability, the naked mole-rat *Heterocephalus glaber*, mild salt loading similarly caused sodium diuresis (Urison and Buffenstein, 1994).

Concentrating ability of the mammalian kidney is usually investigated by exposing animals to dehydration, salt or protein loading, or vasopressin injection. Beuchat (1993) has stressed that these are not equivalent osmoregulatory challenges, and gives maximum urine osmolality for six mammal species subjected to both water deprivation and salt loading: In each case, water deprivation elicited the higher value for urine osmolality. The results of the present study emphasise that values for urine osmolality obtained after salt loading must be treated with caution. The mean urine osmolality of 3896 and 3666 mmol/kg, measured under control conditions for *M. coucha* and *M. natalensis*, respectively, may be considerably lower than the maximum values of which these species are capable.

In view of the diuresis induced by salt loading, it is perhaps not surprising that there was no effect on renal development. Dehydration, on the other hand, is more likely to lead to medullary hypertrophy. The values for RMA of *M. coucha* (0.62–0.84) were lower than those of *M. natalensis* and do not reflect arid adaptation, as they fall well below those of some desert rodents of Argentina (range from 0.75 to 1.73) as listed in Diaz and Ojeda (1999). Juveniles of Namib desert rodents (*Aethomys namaquensis* and *Tatera leucogaster*) maintained without an exogenous water supply expressed renal performance (as indicated by maximum urine concentration and relative medullary area) superior to that of siblings reared with a ready supply of water. RMA of *Aet. namaquensis* increased from 0.99 to 1.34 (Buffenstein and Jarvis, 1985), demonstrating developmental plasticity in the kidney function of these desert-adapted rodents.

Our data suggest that differences in geographical distribution of *M. natalensis* and *M. coucha* in South Africa cannot be attributed to differences in osmoregulatory capacity. Instead, other aspects of their physiology (e.g., dietary requirements and metabolism) or ecological phenomena (e.g., competitive exclusion) may be more important in maintaining the observed distributional differences. Microhabitat preference may be an important behavioural adaptation allowing *M. coucha* to subsist in the relatively drier areas, together with selection of food of high water content, such as succulent vegetation or invertebrates.

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