Ammonotely in a passerine nectarivore: the influence of renal and post-renal modification on nitrogenous waste product excretion

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Summary

Most aquatic vertebrates are ammonotelic, whereas terrestrial vertebrates are typically uricotelic or ureotelic. However, the principal form of nitrogenous waste product in the urine of an animal may vary, depending on environmental conditions. Anna's hummingbird (*Calypte anna*) was found to switch from uricotely at high ambient temperature (T_a) to ammonotely at lower T_a, when energy demands and consequent nectar intake rates were high. In extension of this, we hypothesised that nectarivorous birds would switch from uricotely to ammonotely when water intake rates were high or when protein or salt intake rates were low. We examined the influence of water, electrolyte and protein intake and of T_a on the excretion of ammonia, urea and urate (uric acid and its salts) in nectarivorous Palestine sunbirds (*Nectarinia osea*). The proportion of ammonia in ureteral urine and excreted fluid was not influenced by total water or salt intake or by T_a. Protein intake did not influence nitrogenous waste product concentrations in ureteral urine. However, when protein intake was reduced, the proportion of ammonia in excreted fluid was higher because of the reduced urate concentration. This reduction in urate concentration leads to 'apparent' ammonotely. We suggest that ammonotely may not be a unique feature of nectarivorous birds. It could occur in any species in which breakdown of urate in the hindgut allows the uric acid-nitrogen concentration in the excreta to fall below that of the ammonia-nitrogen concentration.

Key words: nitrogen, excretion, ammonia, Palestine sunbird, *Necatarinea osea*, bird, ammonotely.

Introduction

Patterns of nitrogen excretion in animals broadly follow phylogenetic lines but are also linked to the environment (Campbell, 1991). Ammonotely (where ammonia accounts for more than 50 % of waste nitrogen in the urine) typically occurs in aquatic animals, while their terrestrial counterparts tend to be ureotelic (urea-N >50 % of total urine-N) or uricotelic (uric acid-N >50 % of total urine-N) (Wright, 1995). Birds were thought to be ubiquitously uricotelic. However, this view has recently been challenged by Preest and Beuchat (1997), who found that, under certain experimental conditions, Anna's hummingbirds (*Calypte anna*) were ammonotelic. Ammonotely occurred in half the experimental birds at low air temperature (10 °C), when energy demands were high and nectar and, thus, water intake rates increased.

Ammonia, urea and uric acid differ in toxicity, solubility and production costs. Ammonia, as the direct end-product of amino acid metabolism, has no additional production costs and is highly soluble, but also highly toxic, requiring 400 ml of water to dilute each gram to a non-toxic concentration (Wright, 1995). Terrestrial animals, being water-limited, usually detoxify ammonia and excrete it in the form of urea or a purine such as uric acid (e.g. Campbell, 1991). Urea and uric acid are less toxic than ammonia and require 10 and 50 times less water, respectively, for storage and excretion at non-toxic concentrations (Wright, 1995). Urea contains two nitrogen atoms per molecule and uric acid contains four, whereas ammonia contains just one. Thus, solutions of urea, ammonia or uric acid that contain the same amount of nitrogen differ in osmotic potential.

The ammonia-detoxifying mechanism in birds results in the production of uric acid (Campbell, 1994), but ammonia and urea themselves are also found in avian urine (Sturkie, 1986). The proportions of these three substances in the urine have been shown to vary according to the protein content of food and the amount of water available to birds (e.g. Ward et al., 1975; McNabb et al., 1980). Uric acid requires energy and amino acids for production. The major advantage of excreting uric acid and its salts (hereafter referred to as urates) rather than other nitrogenous waste products is in water conservation. Little water is required to expel them because they contain more nitrogen per molecule and have a lower toxicity and solubility. Some urate may thus precipitate out of the urine once it has left the kidneys (Sturkie, 1986), although urate is held in colloidal suspension in urine, in excess of its aqueous

solubility limits, by protein. Potential costs of uricotely include the energy cost of the waste product, the potential loss of additional proteins used to package the urates for transport through the renal tubules (Janes and Braun, 1997), and the possibility of excessive ion loss *via* cations that associate with urinary urates (McNabb et al., 1973; Laverty and Wideman, 1989; Dawson et al., 1991).

The presence of urea in avian urine is indicative of the catabolism of excess dietary amino acids, citrulline and arginine, and not of a functional urea cycle (Klasing, 1998). The urea cycle, which produces urea from ammonia, is incomplete in birds (Griminger and Scanes, 1986). This means that, if birds have flexibility in their pattern of nitrogenous waste excretion, it is likely to be in varying the proportions of urates and ammonia rather than in varying urea excretion.

Nitrogenous waste products of nectarivorous birds

A nectar diet is rich in water (60–90 %; e.g. Baker, 1975; Calder and Hiebert, 1983; Roxburgh, 2000), but poor in electrolytes (5–80 mmol l^{-1}; Roxburgh, 2000; Calder and Hiebert, 1983) and protein (<0.04 % dry mass; Martinez del Rio, 1994). Thus, nectarivorous birds typically have high water turnover rates (e.g. Williams, 1993; Powers and Conley, 1994; McWhorter and Martinez del Rio, 1999) and low protein requirements relative to allometric predictions (e.g. Brice and Grau, 1991; Roxburgh and Pinshow, 2000). For such birds, excreting their waste nitrogen as urates should not have the same advantages as it does for birds that are water-stressed. Nectarivores may often have little to gain from the low solubility and possible precipitation of urates, although at high temperature and/or high sugar concentration they may be water-limited (Calder, 1979). When the ratio of waste nitrogen to urine volume is low, the excretion of an increased proportion of ammonia is feasible, thereby potentially avoiding the costs (described above) of urate excretion.

We therefore predicted that three characteristics of nectarivore physiology may influence or alter the proportions or concentrations of nitrogenous waste produced in the kidney: (i) high water turnover rates, (ii) a need to conserve electrolytes and (iii) low protein intake rates.

Refluxing and post-renal modification of urine

In birds, unlike in mammals, the ureters open into the cloaca, which serves as a common receptacle for the urinary, digestive and reproductive systems. There, the urine may mix with the faeces and be actively refluxed by reverse peristalsis into the hindgut. This allows the urine contact with populations of micro-organisms that inhabit the hindgut and with the epithelial tissue of the colon, both of which can modify the composition of the urine (Braun, 1999). Refluxing allows for the uptake of electrolytes and water from urine in the hindgut (Goldstein and Braun, 1986). Protein that is present in ureteral urine may be broken down and reabsorbed, and recycling of the nitrogen in urates or urea may occur (Karasawa, 1999). Thus, the kidneys and hindgut work in concert to produce the final excreted fluid.

The implications of refluxing for nectar feeders in particular (although applicable to birds in general) are that, if it occurs, the protein present in the urine can be broken down and amino acids reabsorbed. Urates may also be broken down, releasing trapped electrolytes that are then available for reabsorption, together with the products of urate breakdown.

In the light of the above, we tested whether ammonotely would occur in a small nectarivorous passerine bird, the Palestine sunbird (*Nectarinia osea*). The specific questions we addressed in this study were: does ammonotely occur and, if so, under what conditions; i.e. what influences the proportions and concentrations of nitrogenous waste products in ureteral urine and excreted fluid? And, is there a difference between ureteral urine and excreted fluid; i.e. is there evidence for post-renal modification of urine in Palestine sunbirds? To answer these questions, we examined the influence of dietary water, electrolytes and protein on the excretion of the three major nitrogenous waste products, ammonia, urea and urate. We also compared ureteral urine with voided cloacal fluid to determine changes that occur by modification in the hindgut. Finally, we examined the effect of ambient temperature on the proportions of ammonia found in ureteral and excreted urine to repeat the experiment of Preest and Beuchat (1997) on Anna's hummingbirds.

Materials and methods
Experimental animals

Palestine sunbirds (*Nectarinia osea* Bonaparte 1856) (six males, three females, body mass 6.7±0.1 g; means ± S.D.) were captured on the Sede Boqer Campus at Midreshet Ben-Gurion and on the Tuviyahu Campus in Beer Sheva of Ben-Gurion University of the Negev in Israel (Israel Nature and National Parks Protection Authority permit 7686). The birds were housed in outdoor aviaries on the Sede Boqer Campus (30°51′N, 34°46′E) and maintained on a diet of two artificial nectar solutions, which they were offered *ad libitum*: a 20–25 % sucrose equivalent solution made up of sugar and honey, and a solution of sugar (15 % sucrose equivalent) and a soy protein infant formula (Isomil, Abbott Laboratories, Netherlands; diluted to approximately 2.5 g of protein per 100 g of sugar). Birds were also offered freshly killed fruit flies (*Drosophila* sp.) at least twice a week. Water was available *ad libitum*.

Experimental protocol

Three separate experiments were performed. In the first experiment, we tested the effect of total water, protein and salt intake on excretion of nitrogenous wastes. In the second, we tested the effect of the salt concentration of the diet on nitrogenous waste products, and in the third we tested the effect of ambient temperature (T_a) on excretion of nitrogenous wastes. All three experiments used the following basic protocol.

During experiments, birds were individually housed in small cages in a controlled-temperature room (25 °C; 13 h:11 h L:D, to mimic the natural light cycle at the time of the experiments)

and were offered artificial nectar containing sugar, protein and electrolytes, the exact concentrations of which depended on the treatment. The amount of nectar taken in by the birds was determined by weighing the feeders before and after feeding and correcting for evaporative losses by measuring the mass change of feeders that were not available for birds to feed on. Each treatment lasted for 3 days followed by a minimum 1-week recovery period. Birds were allowed to adjust to the cages and diet for 3 days, after which excreted fluid was collected in mineral oil for 2 h, between 07:00 and 09:00 h. Immediately thereafter, ureteral urine and blood samples were collected from each bird, and the birds were returned to the outdoor aviaries.

Protocols specific to each experiment

Experiment 1: the influence of total intake of water, protein and salt on nitrogenous waste products

Artificial nectars offered to sunbirds in each treatment had one of three levels each of water, protein and salt. The highest levels corresponded to the upper extreme of protein, salt and water intake rates that free-living birds would experience [based on measurements of the sugar and salt concentrations of nectar (e.g. Baker, 1975; Calder and Hiebert, 1983; Roxburgh, 2000) and of the protein requirements of Palestine sunbirds (Roxburgh and Pinshow, 2000)]. Medium levels were typical of foods encountered by free-living birds and, in the case of protein, met the birds' protein requirements. Low levels of protein were below the protein requirements of sunbirds, and low salt and water levels were below average values for floral nectars.

Sucrose concentrations were 0.29, 0.58 and 1.46 mol l^{-1} (10, 20 and 50 % sucrose by mass). Birds compensated for low sugar concentrations by drinking more artificial nectar. In this way, energy intake remained constant while intake of water varied across diets. Water intake rates averaged 2, 8 and 17 ml day^{-1}. Salt and protein concentrations were adjusted so that the intake of salt and protein would be similar for each dietary level. These nutrients were added to the diet to produce protein (isolated soy protein) intakes of 9, 26 and 61 mg day^{-1} and salt (NaCl and KCl) intakes of 3, 15 and 30 mg day^{-1} for low, medium and high treatments, respectively.

Experiment 2: the influence of salt concentration on nitrogenous waste products

Birds were offered a 0.58 mol l^{-1} sucrose solution containing a 2.62 g kg^{-1} solution of isolated soy protein with three different concentrations of salts, 0.8, 39.6 and 78.4 mmol l^{-1} each of NaCl and KCl. Five birds were used in this experiment. Each bird was tested on every diet, with at least 1 week between treatments.

Experiment 3: the influence of T$_a$ *on nitrogenous waste products*

Birds were placed in a controlled-temperature room at 10 °C for a 3-day experimental period, followed by a 1-week recovery period in the outdoor aviary, after which they were returned to the controlled temperature room at 30 °C for a further 3 days. During the experimental period, birds were offered artificial nectar containing 0.58 mol l^{-1} sucrose and 11 mmol l^{-1} each of sodium chloride and potassium chloride. The nectar offered to the birds at 10 °C contained 0.86 g soy protein kg^{-1}, whereas the diet at 30 °C contained 1.46 g kg^{-1}. To keep the protein intake of the birds as constant as possible, we increased the protein content of the 30 °C diet, because birds ate less at 30 °C than at 10 °C.

Fluid sampling and analyses

Blood, ureteral urine and excreted fluid samples were collected during experiments. Urate, urea and ammonia concentrations of all samples were determined using Sigma diagnostic kits (no. 685 for uric acid and no. 640 for urea and ammonia). Sodium and potassium concentrations were measured using a flame photometer (Corning 435) and chloride concentration with a chloride titrator (Corning 925). Osmotic potential was measured with a vapour pressure osmometer (Wescor 5100C). Ureteral urine samples were collected from birds by briefly inserting a closed-end cannula, made from polyethylene tubing, into the bird's cloaca. The closed end prevented contamination of ureteral urine by intestinal fluids. Urine drained into the cannula *via* a window positioned under the ureteral papillae (Thomas et al., 1984). Blood samples were collected in heparinised capillary tubes from a brachial vein punctured with a 27-gauge needle.

In addition to analysing whole excreta samples, we also diluted excreta samples 1:1 in a 0.5 mol l^{-1} LiOH solution to dissolve all urate precipitates and solubilise any trapped ions (Laverty and Wideman, 1989). We reanalysed these samples for potassium and sodium, and compared them with samples in which urates had not been dissolved to determine the proportion of salts bound to uric acid.

Experimental design and statistical analyses

All values are expressed as mean ± standard deviation (S.D.).

Experiment 1: the influence of total intake on nitrogenous waste products

The first experiment had a fractional factorial design. This design uses only a fraction of all possible dietary treatment combinations; it was used because, with three dietary factors, there are too many combinations of water, protein and salt intake to be tested on every bird (Table 1). Thus, every possible dietary combination was tested, but not every bird was tested on every diet. The data were analysed using a four-way fractional factorial analysis of variance (ANOVA) (Mead, 1988; SYSTAT ver. 7.0, SPSS Inc.). Repeated measurements of birds were taken into account by using bird as a factor in the ANOVA together with water, protein and salt intake.

Nine birds were used, with six treatments on each bird. These numbers were chosen to keep the experimental design balanced, i.e. all dietary factors were tested equally. This design allowed us to test second- and third-order interactions between the three dietary factors (Mead, 1988), but did not

Table 1. *The four-way fractional factorial experimental design used in the experiment to test the effect of protein, water and salt intake on nitrogenous waste product excretion in Palestine sunbirds*

Factors	Number
Bird	9
Protein	3 (high, medium, low)
Water	3 (high, medium, low)
Salt	3 (high, medium, low)
Potential number of treatments on each bird	27 (3×3×3)
Actual number of treatments on each bird	6
Total number of measurements	54 (6×9)

allow testing of any interactions that included the identity of the birds. Proportional data were arcsine-square-root-transformed before analysis (Sokal and Rohlf, 1981).

Experiments 2 and 3: the influence of salt concentration and T_a on nitrogenous waste products

Data from these experiments were analysed using a non-parametric, bootstrap randomisation technique (Manly, 1997; Resampling Stats ver. 5.0.2, Resampling Stats. Inc.). This technique was chosen because sample sizes were small and birds were used repeatedly in different treatments. Probability values were calculated by comparing the observed sum of squared mean differences between treatment groups with a randomly generated sum of squared mean differences. This was repeated 1000 times, the final probability value being the proportion of randomly generated sums of squared mean differences that equalled or exceeded the observed sum of squared mean differences.

Results

Experiment 1: the influence of total intake of water, salt and protein on nitrogenous waste products

Water and energy intake on different diets

The amount of water taken in by the sunbirds was significantly different for each dietary sugar concentration, but was not affected by salt or protein intake (analysis of covariance, ANCOVA, using body mass as a covariate; $F_{2,24}=578.1$, $P<0.001$ for sugar concentration; $F_{2,24}=1.89$, $P=0.17$ for protein concentration; $F_{2,24}=0.25$, $P=0.78$ for salt concentration). Birds consumed 17 ± 2.0 ml ($N=18$) of water per day on the low-sugar diet and 7.9 ± 0.8 ($N=18$) and 2.1 ± 0.4 ml ($N=16$) per day on the medium- and high-sugar diets, respectively.

The energy gained on different diets did not differ significantly (ANCOVA using body mass as a covariate; $F_{2,24}=0.80$, $P=0.46$ for sugar concentration, $F_{2,24}=1.31$, $P=0.29$ for protein concentration; $F_{2,24}=0.36$, $P=0.70$ for salt concentration). Thus, sunbirds compensated fully for changes in the sugar concentration of their diet so that mean daily energy intake was 32.5 ± 4.8 kJ (assuming 15.41 kJ g^{-1} for sucrose), irrespective of artificial nectar intake rate.

Proportions of ammonia in ureteral urine and excreted fluid

The proportion of ammonia in ureteral urine was not significantly different in any of the treatment groups (three-factor fractional ANOVA; $F_{2,14}=0.69$, $P=0.52$ for water; $F_{2,14}=1.06$, $P=0.37$ for protein; $F_{2,14}=0.33$, $P=0.73$ for salt; Table 2). The proportion of ammonia in excreted fluid was significantly influenced by the amount of protein in the diets (three-factor fractional ANOVA; $F_{2,17}=5.51$, $P=0.01$; Table 3). Birds eating low and medium levels of protein had significantly higher proportions of ammonia in their excreted

Table 2. *Concentrations of nitrogenous waste products and percentage ammonia in ureteral urine of Palestine sunbirds in treatment groups differing in water, protein and salt intake rates*

Treatment factors	Concentrations of nitrogenous waste products (mmol N l^{-1})			% Ammonia
	Urate	Urea	Ammonia	
Water				
High	24.0±32.7 (18)	2.0±1.2 (17)	2.9±1.8 (17)	18.9±13.6 (17)
Medium	24.2±20.5 (14)	2.0±1.4 (9)	4.6±2.7 (11)	23.3±22.0 (9)
Low	42.0±21.3 (12)	4.5±1.9 (5)*	10.9±5.2 (7)*	22.3±12.1 (4)
Protein				
Low	19.0±22.3 (14)	1.8±1.5 (11)	5.8±5.8 (14)	28.3±21.8 (11)
Medium	22.4±21.2 (17)	2.7±1.9 (11)	4.5±3.2 (13)	19.8±11.3 (11)
High	40.8±32.5 (13)	2.9±1.3 (8)	4.4±2.4 (8)	11.6±3.8 (8)
Salt				
Low	30.4±42.6 (11)	2.1±2.1 (9)	4.7±6.2 (9)	26.2±25.5 (8)
Medium	25.9±18.7 (17)	2.6±1.5 (12)	5.2±4.1 (13)	18.9±10 (12)
High	25.1±20.2 (16)	2.5±1.5 (10)	5.0±2.9 (13)	18.5±12.6 (10)

Values are means ± S.D. (*N*).

* indicates significant differences between levels within each treatment factor, within each type of nitrogenous waste product, i.e. differences within each cell.

Table 3. *Concentrations of nitrogenous waste products and percentage ammonia in excreted fluid of Palestine sunbirds in treatment groups differing in water, protein and salt intake rates*

| Treatment factors | Concentrations of nitrogenous waste products (mmol N l^{-1}) | | | % Ammonia |
	Urate	Urea	Ammonia	
Water				
High	4.6±3.8	1.3±0.8	1.6±0.8	25.7±12.3
Medium	12.8±9.8	2.8±2.5	4.3±1.7	25.3±10.6
Low	26.5±12.8 (16)*	10.3±4.4 (16)*	12.9±5.2 (16)*	26.7±8.8 (16)
Protein				
Low	10.6±11.1 (17)	3.7±4.1 (17)	4.9±4.5 (17)	30.3±12.8 (17)
Medium	10.9±10.2	5.0±5.5	6.9±7.6	28.3±8.5
High	21.3±14.8 (17)*	5.1±4.5 (17)	6.2±4.5 (17)	18.9±6.2 (17)*
Salt				
Low	12.5±12.0 (16)	3.6±4.4 (16)	6.4±6.8 (16)	28.4±11.2 (16)
Medium	17.7±14.8	4.8±4.6	6.4±5.9	23.7±9.3
High	12.2±11.0	5.3±5.5	5.2±4.2	25.8±11.5

Values are means ± S.D. N=18 unless indicated otherwise (in parentheses).

* indicates significant differences between levels within each treatment factor, within each type of nitrogenous waste product, i.e. differences within each cell.

fluid than did birds on the high-protein diets (*post-hoc* pairwise comparison, P=0.02 and P=0.04, respectively). Water and salt intake rates had no influence on the proportion of ammonia in excreted fluid (ANOVA; $F_{2,17}$=0.29, P=0.75 for water; $F_{2,17}$=0.48, P=0.63 for salt).

Concentrations of nitrogenous waste products in ureteral urine and excreted fluid

In ureteral urine, the concentrations of ammonia and urea were significantly different at different water intake rates (ANOVA; $F_{2,14}$=10.81, P<0.01 for ammonia; $F_{2,14}$=4.62, P=0.03 for urea), but urate concentration did not differ significantly (ANOVA; $F_{2,14}$=1.71, P=0.22; Table 2). The excreted fluid concentrations of all three nitrogenous waste products were significantly different at different water intake rates (ANOVA; $F_{2,17}$=19.07, P<0.01 for urate; $F_{2,17}$=19.66, P<0.001 for urea; $F_{2,17}$=31.19, P<0.001 for ammonia; Table 3). In addition, the urate concentration of the excreted fluid was significantly higher on the high-protein diet than on the other two diets (*post-hoc* pairwise comparison, P=0.001 in both cases). Therefore, the change in the proportion of ammonia in the excreted fluid with increasing protein intake rate is apparently a result only of the change in the quantity of urate being excreted.

Incidence of ammonotely

Seven out of 52 excreted fluid samples were indicative of ammonotely (i.e. the amount of nitrogen excreted as ammonia exceeded the amount excreted as urate) (Fig. 1). Ammonotely was significantly more likely to occur in low-protein treatments than in medium- or high-protein treatments (*G*-test; Sokal and Rohlf, 1981; *G*=7.07, P<0.05). In all except eight excreted fluid samples, ammonia concentration exceeded that of urate. However, as uric acid contains four nitrogen molecules, the amount of nitrogen excreted in the form of urate usually exceeded that excreted as ammonia. Two out of 29 ureteral urine samples contained more ammonia-nitrogen than urate-nitrogen, and in both cases the corresponding excreted fluid sample was also ammonotelic. The remaining five ammonotelic excreted fluid samples had corresponding ureteral urine samples that were uricotelic.

A comparison of the nitrogenous waste products of excreted fluid and ureteral urine

The ammonia, urate and urea concentrations of excreted fluid samples were compared with those of ureteral urine samples. A complicating factor when comparing the concentrations of nitrogenous waste products is that the
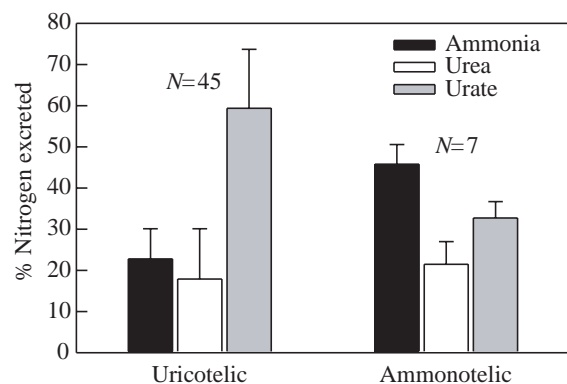

Fig. 1. The proportion of total nitrogen voided as urate, urea and ammonia in excreted fluid samples from Palestine sunbirds. Birds were either uricotelic (when urate was the main waste product) or ammonotelic (when ammonia was the main waste product). In seven of 52 cases, birds were ammonotelic; in the remaining 45 cases, birds were uricotelic. Values are means + S.D.

volume of ureteral urine and excreted fluid may not be the same. T. J. McWhorter (unpublished data) found that in Palestine sunbirds not all water in the diet was taken up across the gut wall and processed by the kidneys and, thus, that the greater the water intake rate, the lower the volume of ureteral urine would be relative to the volume of excreted fluid. We therefore assumed that, at low water intake rates (i.e. a diet with a high sugar concentration), ureteral urine and excreted fluid volume would be almost identical, or even that water is reabsorbed from the hindgut, making the volume of excreted fluid less than that of ureteral urine. At medium and high water intake rates, excreted fluid volume would be increasingly greater than ureteral urine volume. We analysed the data and interpreted our results in the light of these assumptions.

We compared ureteral urine and excreted fluid using repeated-measures ANOVA, with water intake rate (low, medium or high) as an additional factor. The urate concentration of excreted fluid was significantly lower than that of ureteral urine ($F_{1,27}=10.58$, $P<0.01$), regardless of water intake rate (interaction $F_{2,27}=0.94$, $P=0.40$) (Fig. 2A). There were significant differences in the urea concentration of ureteral urine and excreted fluid ($F_{1,27}=11.41$, $P=0.01$), and these differences were dependent on water intake rate (interaction $F_{2,27}=14.91$, $P<0.01$). In general, the ammonia concentration did not differ between ureteral urine and excreted fluid ($F_{1,27}=1.16$, $P=0.29$). However, there were significant differences between diets with different water intake rates (interaction $F_{2,27}=5.22$, $P=0.01$).

Both urea and ammonia concentrations were higher in excreted fluid than in ureteral urine at low water intake rates (Fig. 2B,C). At medium water intake rates, urea and ammonia concentrations were similar in ureteral urine and excreted fluid, while at high water intake rates, urea and ammonia concentrations were lower in excreted fluid than in ureteral urine.

The proportions of ammonia were significantly higher in excreted fluid than in ureteral urine (repeated-measured ANOVA; $F_{1,27}=4.31$, $P=0.05$). The percentage of ammonia in ureteral urine was $20.7\pm16.0\%$ ($N=30$), whereas it was $27.4\pm12.1\%$ in matching excreted fluid samples.

Osmotic potential and cation concentrations of excreted fluid and ureteral urine

The changes in osmotic potential between ureteral urine and excreted fluid samples followed a similar pattern to that found for concentrations of ammonia and urea. That is, whereas there were no overall significant differences between ureteral urine and excreted fluid (repeated-measures ANOVA; $F_{1,22}=2.83$, $P=0.11$), there were significant differences between diets with different water intake rates (interaction $F_{2,22}=20.85$, $P<0.001$; Table 4). When water intake rate was low, excreted fluid osmotic potential was higher than that of ureteral urine, while at high water intake rates, excreted fluid osmotic potential was less than that of ureteral urine.

The osmotic potential of both ureteral urine and excreted fluid was strongly correlated with salt and water intake rates,
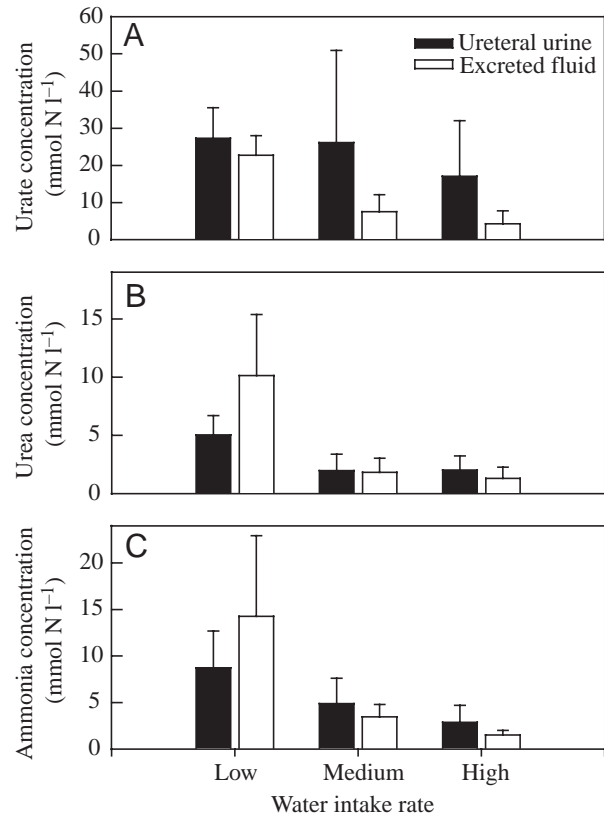

Fig. 2. The changes in concentration of nitrogenous waste products (in mmol nitrogen l^{-1}) in the ureteral urine and excreted fluid of Palestine sunbirds with changes in the water intake rate. Values are means + s.D. See Tables 2 and 3 for N values. (A) Urate concentration; (B) urea concentration; (C) ammonia concentration.

with the lowest osmolalities occurring on the low-salt high-water diets (47 ± 20 mosmol kg^{-1}; $N=6$; for excreted fluid) and highest on the high-salt low-water diets (754 ± 233 mosmol kg^{-1}; $N=5$; for excreted fluid). Neither the sodium nor the potassium concentration of ureteral urine was significantly different from that of excreted fluid (repeated-measures ANOVA, $F_{1,15}=0.03$, $P=0.87$ for sodium; $F_{1,15}=0.99$, $P=0.34$ for potassium; Table 4).

Plasma urate concentrations

Plasma urate concentrations did not vary with salt or water intake rates, but were significantly higher on the high-protein diet (ANOVA; $F_{2,17}=20.2$, $P<0.01$). Plasma urate concentrations were 1.1 ± 0.6 mmol N l^{-1} ($N=17$) on the high-protein diet and 0.4 ± 0.2 mmol N l^{-1} ($N=17$) and 0.5 ± 0.3 mmol N l^{-1} ($N=18$) on the low- and medium-protein diets, respectively. Urea and ammonia concentrations were too low and the sample volumes were too small to allow reliable measurement using the Sigma diagnostic kits.

Experiment 2: the influence of dietary salt concentration on nitrogenous waste products

The electrolyte concentration of the diet did not affect the proportion of ammonia in excreted fluid ($P=0.27$), although the

Table 4. *Osmotic potential and sodium and potassium concentrations of ureteral urine and excreted fluid of Palestine sunbirds in treatment groups differing in water intake rates*

	Water intake rate	Ureteral urine	Excreted fluid
Osmotic potential (mosm kg^{-1})	Low	517±202 (6)	739±106 (6)
	Medium	187±116 (10)	117±70 (10)
	High	110±55 (9)	54±19 (9)
Sodium concentration (mmol l^{-1})	Low	99±65 (5)	100±51 (5)
	Medium	22±13 (6)	27±19 (6)
	High	26±12 (7)	16±7 (7)
Potassium concentration (mmol l^{-1})	Low	124±155 (5)	83±53 (5)
	Medium	36±30 (6)	22±14 (6)
	High	27±17 (7)	13±6 (7)

Values are means ± S.D. (*N*).

proportion of ammonia in low-salt treatments was less than in the medium- or high-salt treatments (Table 5). However, the proportion of ammonia in ureteral urine was significantly lower in the low-salt treatment than in the other two treatments (*P*=0.008).

The ammonia and urea concentrations in ureteral urine did not differ among treatments (*P*=0.49 and *P*=0.63, respectively). However, the urate concentration of ureteral urine was significantly higher in the low-salt treatment than in the medium- and high-salt treatments (*P*=0.05). Plasma urate concentrations were also significantly different among treatments (*P*=0.03), with birds on the high-salt diet having lower plasma urate concentrations than birds on the low-salt diet. Feeding rates of sunbirds were not significantly different among the three treatment groups (ANOVA; $F_{2,11}$=2.9, *P*=0.1).

Table 5. *Urate, urea and ammonia concentrations and percentage ammonia of ureteral urine, excreted fluid and plasma of Palestine sunbirds on diets differing in salt concentration only*

Salt	Urate	Urea	Ammonia	% Ammonia
Low				
UU	34.6±16.4[a]	2.2±1.6	3.7±3.3	8.0±3.9[a]
EF	12.4±8.7	1.5±0.8	3.0±1.2	19.8±7.4
Plasma	0.8±0.5[c]			
Medium				
UU	11.1±5.7[b]	1.7±1.6	2.8±1.9	16.9±5.1[b]
EF	5.7±4.1	1.5±1.1	2.4±0.6	28.4±11.5
Plasma	0.5±0.3[c,d]			
High				
UU	12.7±14.3[b]	1.4±0.8	4.6±1.9	26.9±12.1[b]
EF	5.3±2.2	1.4±0.4	2.5±0.6	27.9±8.6
Plasma	0.1±0.1[d]			

Values are means ± S.D. (*N*=5).

EF, excreted fluid; UU, ureteral urine.

Letters indicate significant differences within columns, between treatment levels.

Experiment 3: the influence of T_a *on nitrogenous waste products*

Sunbirds drank significantly more artificial nectar at 10 than at 30°C (*P*<0.001): 11.5±0.8 ml day^{-1} compared with 8.24±1.1 ml day^{-1}, respectively. Temperature had no significant influence on the proportion of ammonia in excreted fluid (*P*=0.19) or ureteral urine (*P*=0.68) (Fig. 3A). While the average concentration of all three nitrogenous waste products was greater at 30 than at 10°C, only the ammonia concentration of excreted fluid was significantly greater (*P*<0.001) (Fig. 3B).

Sodium and potassium trapping with urates

Neither the sodium nor the potassium concentrations of excreted fluid and ureteral urine were different when samples were solubilised with LiOH compared with when they were not, suggesting that insignificant amounts of these ions were trapped with urate (paired *t*-test for excreted fluid, t_8=2.1, *P*=0.07 for sodium; t_8=1.7, *P*=0.13 for potassium; for ureteral urine, t_5=1.9, *P*=0.13 for sodium; t_5=0.6, *P*=0.7 for potassium).

Samples collected from wild sunbirds

Eight free-ranging sunbirds were caught with mist-nets or drop nets on the Sede Boqer campus in August and September, and blood and ureteral urine samples were collected from these birds. Birds were released immediately thereafter. It was possible to collect ureteral urine samples from four of these birds. The mean proportion of ammonia in ureteral urine was 8.7±5.3 % (*N*=4) and mean ammonia, urate and urea concentrations were 4.7, 48.4 and 2.2 mmol N l^{-1} respectively. Plasma urate concentration was 1.6±0.5 mmol N l^{-1} (*N*=8).

Discussion
Nitrogenous waste products in sunbird excreta

Of the excreted fluid samples, 13 % were indicative of ammonotely (Fig. 1), and ammonotely occurred significantly more frequently in birds that ate smaller daily quantities of protein. In general, higher proportions of ammonia in excreted
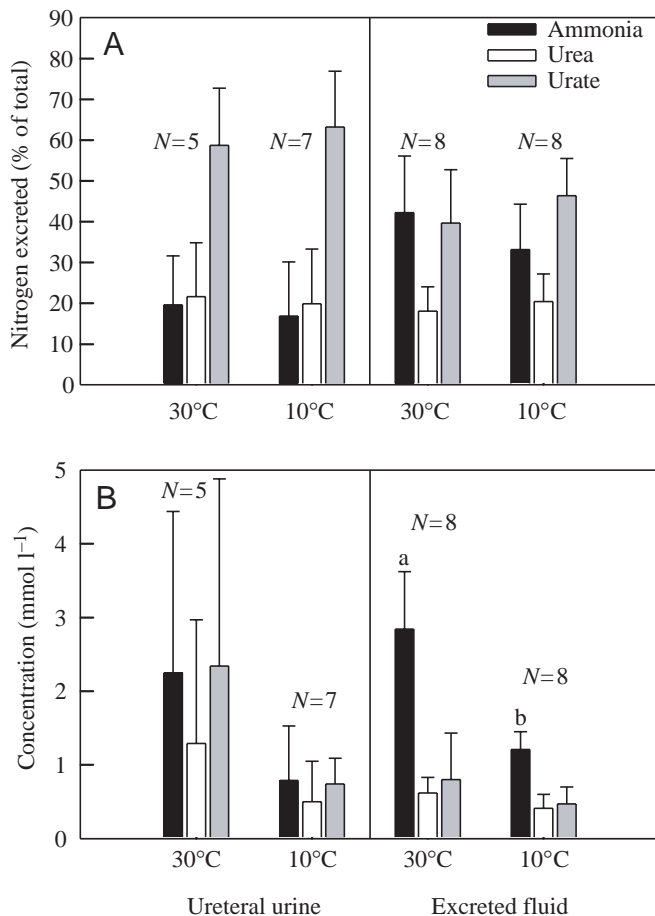
Fig. 3. The proportion (A) and concentration (in mmol l^{-1}) (B) of nitrogen excreted as ammonia, urea and urate in ureteral urine and excreted fluid of Palestine sunbirds at 30 and 10 °C. Values are means + S.D. Only ammonia concentrations of excreted fluid were significantly different at the two temperatures as indicated by different letters above columns.

fluid occurred on lower-protein diets. However, these higher proportions of ammonia resulted from a decline in urate concentration rather than from increased ammonia concentrations because neither ammonia nor urea concentration was affected by protein intake. Unexpectedly, no changes occurred in the composition of the ureteral urine in response to protein intake. In addition, no changes in the composition of ureteral urine or excreted fluid occurred in response to changes in salt or water intake rates apart from a decline in the concentrations of nitrogenous waste products as water intake increased.

Table 6 summarises data on the concentrations and proportions of urate, urea and ammonia in the ureteral urine of five bird species. Palestine sunbirds have concentrations of nitrogenous waste products that are considerably lower (ranging from only 0.5 to 15 %) than those for the other species. However, the proportions of nitrogenous waste products in ureteral urine are comparable with those of the other species.

Post-renal modification of ureteral urine

A comparison of excreted fluid and ureteral urine in Palestine sunbirds shows that post-renal modification of urine occurred in the hindgut. Excreted fluid contained on average 64 % of the urate present in ureteral urine. In comparison, in Gambel's quail (*Callipepla gambelii*), the voided excreta contained 68 % of the urate present in the ureteral urine (Anderson and Braun, 1985). As mentioned above, excreted fluid volume was probably greater than ureteral urine volume. It could be argued that the decline in urate concentration of excreted fluid relative to ureteral urine is simply an effect of dilution by addition of liquid excreta from the gut. However, the difference in the dilution patterns of urate *versus* urea and ammonia suggests that urate breakdown occurred.

On a high-sugar/low-water diet, urate concentrations were lower in excreted fluid than in ureteral urine, whereas urea and ammonia concentrations were greater (Fig. 2). On this diet, Palestine sunbirds probably absorbed almost all their water from the food in the gut (T. J. McWhorter, unpublished data). Volumes of ureteral urine and excreted fluid were thus unlikely to differ significantly unless reabsorption of water from ureteral urine had occurred in the hindgut. Reabsorption of water from ureteral urine has been demonstrated in, for example, house sparrows *Passer domesticus* (Goldstein and Braun, 1986) and Gambel's quail (Anderson and Braun, 1985). Whether water was reabsorbed or not, our suggestion that urate was broken down is supported. If water had been reabsorbed in the hindgut, one would expect that ammonia, urate and urea concentrations would all increase. But this was not the case. Urate concentration dropped by an average of 9 %, while ammonia and urea concentrations increased by 104 and 97 %, respectively.

On the low-sugar/high-water diet, we expected the volume of ureteral urine to be significantly less than that of excreted fluid because not all water in the food is taken up across the gut wall (T. J. McWhorter, unpublished data). Thus, dilution of all three nitrogenous waste products should have occurred (Fig. 2). However, urate concentration fell to a much greater extent than ammonia or urea concentration (an average decrease of 62 % *versus* 34 % and 25 % for ammonia and urea, respectively), again providing evidence indicating the breakdown of urate.

The breakdown and recycling of urate is known to occur in the caeca of chickens, turkeys and other galliforms (Clench, 1999). The caeca contain large microbial populations that are capable of breaking down urates (Laverty and Skadhauge, 1999). The products of this breakdown (ammonia) are either incorporated into microbial protein or may be absorbed by the caecal epithelium. Mortensen and Tindall (1981) have shown that caecal ammonia can be used in the enzymatic synthesis of glutamic acid and that this amino acid can then be absorbed.

However, many (if not most) bird species, such as the Palestine sunbird, do not possess caeca or have vestigial caeca. How do these birds recycle the nitrogen in urates? It is possible that uricolytic microbial populations occur in the colon of these birds and that urate is broken down and recycled in the same

Table 6. *Proportions and concentrations of ammonia, urea and urate in the ureteral urine of several bird species*

Species	Ammonia		Urea		Urate	
	(%)	(mg N ml^{-1})	(%)	(mg N ml^{-1})	(%)	(mg N ml^{-1})
Palestine sunbird (*Nectarinia osea*) (this study)	11–28	0.04–0.12	8–13	0.03–0.07	61–80	0.24–0.38
Yellow-vented bulbul (*Pycnonotus xanthopygos*) (Roxburgh, 2000)	8.4	0.12±0.02	10.3	0.14±0.02	81.3	1.12±0.29
Rooster (*Gallus domesticus*) (McNabb and McNabb, 1975)	11–22	1.2–1.3	2–12	0.09–1	68–77	3–10.5
Turkey vulture (*Cathartes aura*) (McNabb et al., 1980)	9–16	2–6	4–8	1–3	77–87	10–53
Emu (*Dromaius novaehollandiae*) (Dawson et al., 1991)	1–15		3–14		53–85	

Ranges indicated minimum to maximum treatment means.
Values for bulbul are means ± S.D. (*N*=13).

way as in birds with caeca. C. A. Beuchat (personal communication) found uricolytic bacteria in the hindgut of hummingbirds, and such bacteria are probably responsible for urate breakdown in sunbirds as well. The mechanisms of nitrogen recycling, particularly in birds that do not possess caeca, are unknown.

Osmotic potential and post-renal modification of ureteral urine

The osmotic potential of the excreted fluid of Palestine sunbirds in the lowest salt and sugar treatments were 47±20 mosmol kg^{-1}. This matches the lowest values found in free-living hummingbirds (Calder and Hiebert, 1983) and in freshwater fish (e.g. Talbot et al., 1992) and amphibians (e.g. Shpun et al., 1992). In the highest sugar and salt treatments, excreted fluid osmotic potential rose to 754±233 mosmol kg^{-1}, which is approximately double the plasma osmotic potential. The osmotic potential of ureteral urine ranged from a mean value of 61 mosmol kg^{-1} to a mean value of 585 mosmol kg^{-1}. The ability to produce urine that is twice as concentrated as plasma is within the range typically found for birds (e.g. Skadhauge, 1974).

In Palestine sunbirds, we found no significant trapping of electrolytes in urates. In contrast, in Gambel's quail, 16 % of the sodium and 36 % of the potassium found in ureteral urine were trapped in urates (Anderson and Braun, 1985), while in domestic hens (*Gallus domesticus*) 9 % of sodium and 23 % of potassium were trapped (Long and Skadhauge, 1983). In comparison with other birds, electrolyte concentrations in nectarivore urine are typically very low (e.g. Calder and Hiebert, 1983; Beuchat et al., 1990), as are concentrations of urate (e.g. Table 6; Preest and Beuchat, 1997). Some cations

must be bound to uric acid because uric acid has a pK$_1$ of 5.4 (Goldstein and Skadhauge, 2000) and thus, under most physiological conditions, must occur as a monobasic urate salt. However, the concentration of cations bound to uric acid is unlikely to be more than a few mmol l^{-1} because of the low concentrations of uric acid/urate (3.5±3.2 mmol l^{-1} in excreted fluid). We rarely observed precipitated urate in sunbird excreta, and thus co-precipitation is also unlikely to be important in these birds.

Although we predicted that a reduction in the salt concentration of the diet would lead to an increase in the proportion of ammonia excreted, this was not the case. As little or no precipitation of urates occurred and thus no trapping of electrolytes, our original prediction that a decline in urate concentration or conversely an increase in ammonia concentration would occur was not valid.

However, we did find that, when the salt concentration of the diet was low, the proportion of ammonia in the ureteral urine was significantly lower than in birds offered diets with higher salt concentrations (Table 5). Urate concentration in ureteral urine increased significantly at low salt concentrations. The plasma urate concentration of Palestine sunbirds was higher on the low-salt diet and decreased as salt intake increased; it was unusually low in birds on the high-salt diet. The reason for this fall in plasma urate concentration is not clear.

Comparison with ammonotely in hummingbirds

In contrast to the results of the study on hummingbirds by Preest and Beuchat (1997), we found that T_a had no effect on the proportions of ammonia found in either excreted fluid or ureteral urine. Preest and Beuchat (1997) found that 50 % of

hummingbirds tested at 10 °C were ammonotelic, while the birds were all uricotelic at 20 and 40 °C. The experimental protocols of Preest and Beuchat's study were different from ours. Anna's hummingbirds were offered a 0.7 mol l⁻¹ sucrose solution, which contained neither protein nor electrolytes. Birds were not acclimated to the experimental T_a (other than 20 °C) for more than a few hours (M. R. Preest, personal communication). In our study, birds were offered a 0.58 mol l⁻¹ sucrose solution containing some salts and protein and were acclimated to the experimental T_a for 3 days. In addition, hummingbirds appear to absorb essentially all water from their food and process it in the kidney (McWhorter and Martinez del Rio, 1999), whereas there is evidence that Palestine sunbirds modulate water uptake from their food (T. J. McWhorter, C. Martinez del Rio and B. Pinshow, unpublished data). Thus, water loads processed by the kidney may be much larger at lower T_a for hummingbirds than for sunbirds, especially considering the hummingbirds' lack of acclimation to the experimental T_a. This may account for differences in ammonia and urate excretion.

Other studies report that birds excreted large quantities of ammonia. Moss and Parkinson (1972) found that red grouse *Lagopus lagopus scoticus* eating heather excreted most of their digested nitrogen as ammonia and ornithurates, while Moss and Parkinson (1975) found that rock ptarmigan *Lagopus mutus* eating berries excreted approximately half their metabolised nitrogen as urates and half as ammonia. They did not collect ureteral urine from these birds, but is it likely that these birds harbour large populations of uricolytic micro-organisms in the caeca and that urate is extensively decomposed in the hindgut (Mortensen and Tindall, 1981).

Concluding remarks

The proportion of ammonia in the ureteral urine of Palestine sunbirds was not influenced by total water, salt or protein intake. In excreted fluid, however, the proportion of ammonia increased significantly with decreasing protein intake. This change was not due to a change in ammonia concentration but rather to a change in urate concentration. Urate appeared to be broken down in the hindgut, leading to a fall in urate concentration in excreted fluid. The reduction in the urate concentration of excreted fluid led to 'apparent' ammonotely. We suggest that ammonotely may not be a unique feature of nectarivores. It could occur in any species in which the breakdown of urate in the hindgut allows the uric acid nitrogen concentrations in the excreta to fall below that of ammonia nitrogen concentrations.

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