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Review

Do mammals, birds, reptiles and fish have similar nitrogen conserving systems?

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Abstract

Comparative physiological studies are a powerful tool for revealing common animal adaptations. Amino acid catabolism produces ammonia which is detoxified through the synthesis of urea (mammals, some fish), uric acid (birds), or urea and uric acid (reptiles). In mammalian herbivores and omnivores, urea nitrogen is salvaged by a series of steps involving urea transfer into the intestine, microbial mediated urea hydrolysis with synthesis of amino acids utilizing the liberated ammonia and transfer of the amino acids back to the host. A similar series of steps occur in omnivorous/granivorous and herbivorous birds, although in this case urine, containing uric acid, is refluxed directly into the intestine where microbes degrade the uric acid and utilize the liberated ammonia for amino acid synthesis. These amino acids are transferred back to the host. In reptiles and ureotelic fish not all of these steps have been experimentally confirmed. Reptiles like birds, reflux urine into the intestine where it is exposed to the microflora. However, the capacity of these microbes to breakdown the uric acid and urea and utilize ammonia for amino acid synthesis has not been documented. Ureotelic fish transfer urea into the intestine where urease (presumably of bacterial origin) hydrolyzes the urea. However, the amino acid synthesizing capacity of the intestinal microflora has not been studied. The series of steps, as outlined, would define the prevailing nitrogen conservation system for herbivores and omnivores at least. However, it would appear that some animals, in particular the fruit-eating bat and perhaps the fruit-eating bird, may have evolved alternative, as yet uncharacterized, adaptations to a very limited nitrogen intake.

Keywords: Mammals; Birds; Reptiles; Fish; Nitrogen conservation

1. Introduction

One of the advantages of a comparative approach to animal physiology is the perspective it affords in understanding general principles of organismal function. Animals take in nutrients and excrete waste products. Dietary amino acids in excess of the amounts needed for growth and maintenance of protein turnover are preferentially degraded over carbohydrates and lipids (Campbell, 1991) since animals cannot store these excess amino acids. Metabolism of carbohydrates and lipids forms essentially a single waste product, carbon dioxide, while amino acid degradation also releases nitrogen as ammonia, a relatively toxic molecule (Cooper and Plum, 1987). Whereas aquatic species can excrete ammonia directly, semiaquatic and terrestrial animals detoxify ammonia, at a considerable energetic cost, through the synthesis of urea (mammals) or uric acid (birds) or urea and uric acid (reptiles). In mammals, a fraction of the urea synthesized is cycled to the

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gastrointestinal tract, while the remainder is excreted in the urine. Within the intestinal lumen the urea is hydrolyzed by microbes, thus, generating ammonia as a potential nitrogen source. Can this sequence of urea transport into the gut, subsequent hydrolysis, with liberation of ammonia be considered a nitrogen conservation system? If so, is a similar nitrogen conservation system a design feature of other vertebrates? As the majority of data are available for mammals, I will review this group first.

2. Mammals

Hydrolysis of urea in the intestinal tract has been demonstrated in mammalian groups generally belonging to the omnivore and herbivore categories of dietary adaptation (Cocimano and Leng, 1967; Stevens and Hume, 1998). The magnitude of urea hydrolysis has been measured as either the difference between the rates of urea synthesis and urinary urea excretion (Walser and Bodenlos, 1959; Fuller and Reeds, 1998; Young et al., 2000) or as the percentage of injected ^{14}C-urea collected as $^{14}CO_2$ in expired air (Harlow, 1987; Harlow and Buskirk, 1991). Urea hydrolysis is catalyzed by the enzyme urease and urease activity has been detected in the gut mucosa of humans (Summerskill, 1966). In fact, an anatomic gradient was observed in that activity was much higher in the stomach than in the small intestine and higher in the small intestine than in the colon (Summerskill, 1966). However, the observations of Walser and Bodenlos (1959) in humans, Deguchi et al. (1978) in germ-free piglets, and Levenson et al. (1959) in the germ-free rat indicate that the importance of mucosal urease is probably trivial and that bacterial urease is responsible for almost all urea hydrolysis (Stevens and Hume, 1998).

Three categories of dietary adaptation have been described for mammals, based on the type of food they consume (Dehority, 1997). In turn, gastrointestinal tract structure appears to be directly related to each animal's dietary adaptation. The carnivores (or faunivores), animals consuming primarily animal matter, possess a simple unilocular stomach, a tortuous small intestine, short conical cecum, and simple, smooth-walled colon. Omnivores (or frugivores) consume fruits, flowers, seeds and tubers, and generally supplement their diets with varying amounts of animal matter. This group has no distinctive structural specialization unique to their intestinal tract. The last category, the herbivores (or folivores) are animals that consume leaves, grasses, twigs, barks and gums, all of which can only be digested by symbiotic microorganisms. These animals have chambers in their gastrointestinal tract where microbial fermentation can occur. Foregut fermentation occurs in a plurilocular stomach, which is divided into two, three or four chambers. Hindgut fermentation occurs in the cecum and/or colon.

The sites of urea hydrolysis are the forestomach (e.g. foregut-fermenting herbivores) or the cecum and/or colon (hindgut-fermenting herbivores, omnivores, Stevens and Hume, 1998). In the case of carnivores, less information is available, but urea hydrolysis has been demonstrated in the American marten (*Martes americana*) (Harlow and Buskirk, 1991) and presumably occurs in the cecum and/or colon.

The inter-relationship between urea production, hydrolysis, and excretion has been studied in mammals generally belonging to the dietary categories omnivore and herbivore. Jackson (1998) and Child et al. (1997) reported that in humans, urea production remains relatively constant over a wide range of nitrogen intakes. Over this same dietary range, there was a close inverse relationship between nitrogen intake and rate of urea hydrolysis. Jackson (1998) has proposed that a decrease in dietary protein is matched by an increase in rate of urea hydrolysis such that the sum of intake and hydrolysis is much less variable than intake itself. In this model, control of urea hydrolysis accounts for a relatively constant rate of urea production independent of nitrogen intake over a wide range. Young et al. (2000), also working with humans have published quite different results. They reported that urea production was linearly correlated with nitrogen intake and that leucine oxidation was highly correlated with the rate of urea production. Urea hydrolysis also increased linearly with nitrogen intake, but there was considerable variation in the rate among individuals. According to these authors, control of body nitrogen balance is via regulation of urea production, not hydrolysis. The reasons for the discrepancy between these two sets of results are unclear and according to Young et al. (2000) do not appear to be due to differences in experimental protocol. Urea kinetic parameters (production, hydrolysis and excretion) as a function of nitrogen intake have been measured in other mammalian species. In some of these studies

sufficient data points are available to do linear regression analysis. The results of these studies and also those of Jackson (1998) and Young et al. (2000) are summarized in Table 1. In the study by Cocimano and Leng (1967) performed in sheep (*Merino ewes*), a ruminant, all three parameters are linearly correlated with nitrogen intake. However, hydrolysis has the least slope, and the weakest correlation coefficient. For the greater bilby (*Macrotis lagotis*), (Gibson and Hume, 2002) a small omnivorous marsupial (and foregut fermenter) urea production and excretion increase linearly with nitrogen intake, whereas urea hydrolysis appears to be relatively independent of nitrogen intake. In several other studies, not sufficient data points are available for linear regression, but trends are discernible. In the tammar wallaby (*Macropus eugenii*), a herbivore (Chilcott et al., 1985) urea kinetic measurements were made with respect to two different nitrogen intakes, 0.54 and 0.18 g/kg/d. Urea production (mmol/kg/d) increased from 5.14 to 13.8 and urea excretion (mmol/kg/d) increased from 1.24 to 9.1 when the animals were changed from the low to high nitrogen intake. Urea hydrolysis (mmol/kg/d) increased very little (3.90–4.65) with this same dietary change. The rock hyrax (*Procavia habessinica*) (Hume et al., 1980) is a small herbivore and also a hindgut fermenter. When nitrogen intake was reduced from 358 to 186 mg/kg/d, urea production fell from 260 to 175 mg N/kg/d and urea hydrolysis from 158 to 101.5 mg N/kg/d. Finally, Barboza et al. (1997) measured urea kinetic parameters in bears (*Ursus americanus* and *Ursus arctos*), an omnivore, during autumn hyperphagia and winter dormancy. Urea production decreased from 9.75 to 1.74 mmol urea N/kg/d comparing autumn and winter periods. Urea hydrolysis changed very little between these same two periods, increasing modestly from 1.06 to 1.67 mmol urea nitrogen/kg/d. In all these studies, the subjects appeared to be in a steady state with respect to a given nitrogen intake. Except for the bear during winter dormancy, water was available ad lib and there appeared to be no obvious caloric dietary restrictions.

In summary, except for the observations of Jackson (1998), the results of the other studies are consistent with the following model. A decrease in nitrogen intake is associated with a fall in the rates of amino acid oxidation, urea synthesis and urea excretion. The rate of urea hydrolysis also falls with a reduction in nitrogen intake, although the slope is much less than that for the rates of urea synthesis and excretion. In the case of the greater bilby or the bear, urea hydrolysis appears to be independent of nitrogen intake. Hence, when the rate of urea hydrolysis is expressed as a proportion of the rate of urea synthesis, this proportion increases as nitrogen intake falls. For example, in the tammar wallaby, urea hydrolysis was 34% of production at the high nitrogen intake, but rose to 76% at the low nitrogen intake. In the greater bilby, urea hydrolysis increased from 49 to 69% of production between the highest and lowest nitrogen intakes. If we compare sheep (Table 1) consuming a nitrogen intake of 1.03 vs. 0.11 g/kg/d, (the range of intakes studied) the rate of urea hydrolysis was 33% of the rate of synthesis at the high nitrogen intake, but 92% at the low intake. When viewed in this manner, urea hydrolysis appears to be a nitrogen salvage mechanism. The factors or mechanisms 'controlling' the rate of hydrolysis are not known, but candidate processes will be considered later.

What is the fate of ammonia released by intestinal hydrolysis of urea? If all of the ammonia nitrogen is returned to the liver and resynthesized into urea, then the process of urea hydrolysis with liberation of ammonia is not a nitrogen conservation system. Unfortunately, there are only limited data available with respect to this question. Jackson et al. (1984) estimated that in humans on a normal diet (1.25 g protein/kg/d), approximately 42% of hydrolyzed urea was resynthesized back to urea. On the other hand, studies on humans by Young and El-Khoury (1994) and El-Khoury et al. (1996) have led these authors to conclude that almost all ammonia released via urea hydrolysis is returned to the urea cycle. These experiments were performed in subjects ingesting a 1 g/kg/d protein diet. Patterson et al. (1995) observed that in (fasting) humans less than one-half (46%) of labelled nitrogen appeared in urea after oral administration of $^{15}NH_4C1$. The remainder (54%) was incorporated into nonessential amino acids. In sheep on a low nitrogen intake (0.11 g nitrogen/kg/d) approximately 8% of urea nitrogen was recycled back into urea (Cocimano and Leng, 1967). In the rock hyrax (Hume et al., 1980), approximately 41% of urea nitrogen in animals on the high nitrogen diet (358 mg/kg/d) and approximately 47% of urea nitrogen in animals taking the lower nitrogen intake (186 mg/kg/d) was reutilized for urea synthesis. For the tammar wallaby

Table 1
Linear regression relationships between urea production, excretion, and hydrolysis and dietary nitrogen intake

Species	Reference	Urea production	Urea hydrolysis	Urea excretion
Human $n=12$	Jackson, 1998	Not given	Hydrolysis (mgN/kg/d) $= -0.4 \times$NI (mg/kg/d) $+ 138$ ($R = -0.74$)	Not given
Human $n=34$	Young et al., 2000	Urea production (mgN/kg/d) $= 0.89 \times$NI (mgN/kg/d) + intercept (not given) ($R=0.98$)	Not given	Not given
Sheep $n=6$	Cocimano and Leng, 1967 (from table 2)	Urea production (g urea/kg/d) $= 1.49 \times$NI (g/kg/d) $+ 0.06$ ($R=0.95$)	Urea hydrolysis (g urea/kg/d) $= 0.35 \times$NI (g/kg/d) $+ 0.16$ ($R=0.79$)	Urea excretion (g urea/kg/d) $= 1.15 \times$NI (g/kg/d) $- 0.12$ ($R=0.97$)
Greater bilby (*Macrotis lagotis*) $n=7$	Gibson and Hume, 2002 (from table 4)	Urea production (mg N/ kg/d) $= 0.31 \times$NI (mg/kg/d) $+ 130.6$ ($R=0.92$)	Urea hydrolysis (mgN/kg/d) $= 0.029$ NI (mg/kg/d) $+ 127$ ($R=0.12$)	Urea excretion (mgN/kg/d) $= 0.28$ NI (mg/kg/d) $+ 1.63$ ($R=0.78$)

n refers to the number of separate data points; NI refers to nitrogen intake.

(Chilcott et al., 1985), a high nitrogen intake (0.54 g/kg/d) was associated with 85% of urea nitrogen being re-utilized for urea synthesis; whereas the figure was 59% when a lower nitrogen intake (0.18 g/kg/d) was used. It is difficult to generalize from this limited data set, especially given the conflicting results of Jackson et al. (1984) and El-Khoury et al. (1996), but possibly 40% or more of cycled urea nitrogen is available for purposes other than resynthesis of urea, particularly in animals on a low nitrogen intake.

What happens to the ammonia that is not used to resynthesize urea? In ruminants, the microflora of the forestomach is capable of synthesizing amino acids and protein from simple sources of nitrogen such as ammonia. (Virtanen, 1966; Smith, 1989; Cotta and Russell, 1997). Urea is recycled into the rumen by way of salivary secretions, and by transfer across the rumen epithelium (Cotta and Russell, 1997). There, microbial urease hydrolyzes urea to ammonia and carbon dioxide. The ammonia is incorporated into microbial protein, which can be digested and the constituent amino acids absorbed through the small intestine of the host (Stevens and Hume, 1998). As reviewed by Orskov (1982) microbial protein supplies a large fraction of the amino acids required by the host ruminant. In fact, as shown by Virtanen (1966) rumen bacteria are capable of synthesizing all the amino acids necessary for protein synthesis, and cows fed a diet of purified carbohydrates with urea and ammonium salts as the sole sources of nitrogen are well maintained and capable of relatively high milk production. In addition, anaerobic fermentation of carbohydrate is the most important source of energy for microbial synthesis of protein. In non-ruminants, the data are not as complete. Colonic bacteria, like ruminal microbes, can synthesize amino acids and protein using ammonia as a nitrogen source (Morrison and Mackie, 1997). However, since the small bowel is thought to be the major site of amino acid absorption (Fuller and Reeds, 1998), amino acids derived from microbial protein synthesized in the cecum/colon may not be readily available to the non-ruminant host.

The bear, for example, does not eat, drink, urinate, or defecate during winter dormancy (Lundberg et al., 1976). During this period lean body mass is conserved (Lundberg et al., 1976), amino acid oxidation occurs with production of urea, yet plasma urea levels fall (Barboza et al., 1997). Dormant bears re-utilize almost 100% of their urea nitrogen (Barboza et al., 1997) and the rate of protein synthesis is greater in the dormant compared to the active state (Lundberg et al., 1976; Barboza et al., 1997). According to Nelson (1989), released urea nitrogen is recycled through nonessential and essential amino acids, body protein, and back into urea. No data are presented as to whether the nonessential and essential amino acids are microbial in origin. If these amino acids are derived from microbial protein, then what is the energy source for microbial protein synthesis, since the bear has no intake while dormant?

Varcoe et al. (1975) measured the utilization of urea nitrogen for albumin synthesis in normal males and females as well as patients with chronic renal failure. In four normals ingesting a 70 g protein/d diet mean rates of urea synthesis and hydrolysis were 0.24 and 0.031 mmol/h/kg. In two individuals receiving a 30 g protein/d intake, mean rates were synthesis 0.16 (mmol/h/kg) and hydrolysis 0.045 (mmol/h/kg). However, despite the higher rate of hydrolysis associated with the lower protein intake only 0.13% of the total nitrogen required for the measured rate of albumin synthesis was provided by urea nitrogen in both sets of individuals. The experiments of Varcoe et al. (1975) do not indicate the pathway by which urea nitrogen is incorporated into albumin. Deguchi et al. (1978) detected ^{15}N (from orally administered ^{15}N-urea) in the tissue proteins (TCA precipitates) of the liver, kidney, heart, muscles, and colon mucosa in specific pathogen-free piglets, but not germ-free piglets. All animals received a diet of between 20 and 26 g protein/d. This study confirmed the role of bacterial urease in the process of urea hydrolysis and that urea nitrogen could be incorporated into tissue proteins, although the magnitude of this incorporation is unclear. Patterson et al. (1995) administered $^{15}NH_4CI$ orally to (fasting) humans. The oral route was used to evaluate the metabolic fate of ammonia that would arise from microbial mediated urea hydrolysis. They found that 54% of the labelled nitrogen appeared in plasma amino acids. The highest enrichment was achieved for those amino acids for which ammonia is a direct biosynthetic precursor, arginine, glutamate and glutamine. No ^{15}N was detected in essential amino acids which implies that the liver was the site of amino acid synthesis. However, microbial amino acid synthesis cannot be excluded. Metges et al. (1999a)

measured the incorporation of urea nitrogen into microbial proteins and plasma-free amino acids in humans. Subjects were given a diet of 160 mg nitrogen/kg/d and either $^{15}NH_4CI$ or [$^{15}N_2$] urea. For our purposes, we will consider only the results with [$^{15}N_2$] urea. Following oral administration of labelled urea, ^{15}N enrichment was detected in dispensable and indispensable free amino acids. The lower ratio of plasma to microbial ^{15}N enrichment found for threonine and histidine, compared with all of the other amino acids, was interpreted by the investigators to mean that these labelled amino acids were derived exclusively from microbial synthesis within the intestine. For the other labelled amino acids, microbial synthesis was not clearly demonstrated. Comparative experiments in which labelled NH_4Cl was administered orally to germ-free and conventional rats (Torrallardona et al., 1996), confirmed that de novo lysine synthesis was entirely of microbial origin. Labelled lysine was detected in liver and plasma proteins as well as the carcass of conventional but not germ-free rats. The investigators estimated that absorbed microbial lysine accounted for two-thirds of lysine maintenance requirements. In a similar study in humans, Metges et al. (1999b) detected ^{15}N enriched plasma lysine and ^{15}N enriched microbial protein lysine after oral administration of [$^{15}N_2$] urea. Subjects were given a diet of 160 mg nitrogen/kg/d. Given the observations of Torrallardona et al. (1996), this study confirms that urea nitrogen can be incorporated into microbial synthesized lysine and this amino acid can be absorbed by the host. It is clear then, that in non-ruminants, intestinal microbes (possibly in the small intestine or cecum/colon) can utilize urea nitrogen for protein synthesis and that amino acids derived from microbial protein can be transferred to the host. The magnitude of this transfer as discussed by Metges et al. (1999b) is difficult to estimate. Jackson (1998) has also demonstrated that colonic bacteria can synthesize lysine using urea nitrogen and that this amino acid can be transferred to the host. Although the small intestine is the major site of amino acid absorption (Fuller and Reeds, 1998), there is evidence that amino acids can be absorbed across the cecum/colon (Fuller and Reeds, 1998; Metges et al., 1999a). Jackson (1998) has suggested that a peptide transporter rather than an amino acid transporter might be the mechanism for transfer of amino acids from colonic lumen to host.

How might the rate of hydrolysis be regulated? Specific urea transporters have been detected in the human colon and sheep rumen (Ritzhaupt et al., 1998) as well as the rabbit colon (You et al., 1993). Perhaps a low protein diet up-regulates these transporters, as has been described for the urea transporters present in the mammalian kidney collecting duct (Sands, 1999). Clearly, more information is necessary concerning how the function of these transporters is controlled. Another potential locus of regulation is the intestinal microflora itself. The biosynthetic activity of ruminal and colonic bacteria is sensitive to the ambient ammonia concentration (Morrison and Mackie, 1997). Current understanding of the control of biosynthetic pathways in ruminal and colonic bacteria is still largely descriptive (Morrison and Mackie, 1997). There is no information available as to how the transfer of urea into the intestine and the biosynthetic activity of gut microbes is co-ordinated.

In summary, the mammalian groups studied that have essentially been herbivores and omnivores, respond to a decreased nitrogen intake by reducing the rates of urea synthesis and urinary urea excretion. The rate of intestinal urea hydrolysis also falls, but to a lesser extent than that of production. Hence, in the case of a low nitrogen intake a greater percentage of synthesized urea undergoes hydrolysis than that occurring at a high nitrogen intake. The transfer of urea into the rumen or colon appears to be via specific transporters although it is not known how the function of these transporters is regulated. Ammonia released by bacterial urease can either be returned to the liver and resynthesized into urea or incorporated into amino acids by ruminal/colonic bacteria. In ruminants, microbial proteins synthesized in the forestomach serve as an important source of amino acids for the host animal. For non-ruminants the evidence is less clear. Amino acids, such as lysine, threonine, and histidine can be synthesized by the colonic gastrointestinal microflora utilizing urea nitrogen and transferred to the host. Presumably the same scheme holds for other amino acids. How urea transport into the intestine and microbial biosynthetic activity are co-ordinated is not clear. Although many details are unknown, the sequence of transfer of urea into the intestine, hydrolysis of urea by the microflora with liberation of ammonia, microbial synthesis of amino acids/protein using the ammonia as a nitrogen source and transfer of

amino acids back to the host is clearly a nitrogen conservation system. If we consider this sequence as analagous to a metabolic pathway, then metabolic control analysis (Fell and Thomas, 1995) would suggest that physiological control of this sequence would involve regulation at multiple sites. However, the quantitative importance of this nitrogen conservation system in the non-ruminant (except perhaps for the bear) is still controversial.

Although intestinal urea hydrolysis with recycling of urea nitrogen constitutes the strategy by which herbivores and omnivores deal with a reduced nitrogen intake, some mammals appear to have evolved alternative adaptations to situations of limited nitrogen availability. For example, fruit-eating bats have a diet rich in carbohydrates but very poor in protein (Korine et al., 1996). The gastrointestinal tract of these mammals is relatively simple. There is no cecum or appendix and macroscopically, there is no obvious delimitation between small and large intestine (Klite, 1965; Tedman and Hall, 1985; Makanya et al., 1997). In essence, the intestinal tract contains no obvious fermentation chambers. Intestinal transit time is very rapid at between 12 and 34 min (Klite, 1965; Tedman and Hall, 1985) and intestinal bacterial counts are approximately 5–6 orders of magnitude lower than that of laboratory white mice (Klite, 1965). The lack of a cecum or distinct large intestine, the rapid transit time and the low intestinal bacterial counts are conditions which make the likelihood of quantitatively significant microbial mediated urea hydrolysis low. However, the actual magnitude of urea hydrolysis in fruit-eating bats has never been directly measured. How then, do these bats maintain nitrogen balance? According to Delorme and Thomas (1996, 1999), fruit bats appear to be extremely efficient in limiting nitrogen losses in the feces and it is this low metabolic fecal nitrogen loss that is the principal adaptation by which fruit bats survive on a low nitrogen diet. In addition, a rapid intestinal transit time allows the bat to quickly process large quantities of food even though the short digesta retention time probably compromises extraction efficiency.

3. Birds

In birds, ammonia formed during amino acid catabolism is converted to uric acid for excretion (Campbell, 1991). Although the liver is the primary site for uric acid synthesis (Campbell, 1991) Chin and Quebbemann (1978) have demonstrated that in chickens a fraction of the uric acid excreted in the urine is synthesized in the kidney.

To the best of my knowledge, there are no studies in which uric acid production has been directly measured as a function of nitrogen intake. However, several studies have measured uric acid excretion in birds ingesting different nitrogen (protein) diets. Tasaki and Okumura (1964) measured nitrogen excretion in white leghorn cockerels (*Gallus domesticus*), an omnivore/granivore, ingesting differing protein intakes. Birds were adapted to the different diets for approximately 8 days. Urine was collected separately from feces. With increasing protein intake (casein content varying from 0 to 12%), total urinary nitrogen excretion rate and urinary uric acid excretion rate increased by 80 and 142%, respectively. The urinary excretion rate of urea and creatinine were unaffected by changes in protein intake. In the study by Chin and Quebbemann (1978), non-fasted chickens (*G. domesticus*) (diet containing 16% protein) had a urate excretion rate of 616 μg/kg/min of which 106 μg/kg/min (17%) was synthesized in the kidney. After an 18-h fast, chickens excreted 752 μg/kg/min urate of which 58 μg/kg/min (7.7%) was renal synthesized. In these experiments urine was collected directly from the kidney. Goldstein et al. (2001) studied the renal response of the house sparrow (*Passer domesticus*), an omnivore, to two dietary protein intakes. Birds were adapted for 4 weeks to either an 8 or 30% casein diet, which were isocaloric. Uric acid concentrations were measured in the plasma and in ureteral samples of urine. Plasma uric acid concentration was higher in the birds ingesting the higher protein diet (1.09 vs. 0.39 mmol/l) and uric acid excretion was greater in the birds taking the 30% casein diet (6.17 vs. 0.64 mmol/d). van Tets et al. (2001) measured nitrogen excretion in the yellow-vented bulbul (*Pycnonotus xanthopygos*) (a frugivorous passerine) at two different nitrogen intakes (high; 7.23 g/l soy protein and low 1.03 g/l soy protein). Birds were adapted for a minimum of 4 days. Decreased nitrogen intake was followed by increased food intake and a corresponding decrease in the (ureteral) urine concentration of total nitrogen and uric acid. Since urine flow rates were not given, actual uric acid excretion rates cannot be

calculated. Can we use uric acid excretion as an indirect measure of production rate? Unfortunately, only the study by Goldstein et al. (2001) of the three studies has sufficient data to allow us to calculate the renal clearance of uric acid. In the house sparrow, uric acid excretion rate increased by 9.6 times (0.64–6.17 mm/d) between low and high nitrogen intakes while renal uric acid clearance increased by only 3.6-fold (1.6–5.7 l/d). Hence, most of the increased uric acid excretion is the result of a change in production rather than simply a change in renal uric acid handling. It would seem reasonable, then, to conclude from the results of these three studies that a decrease in nitrogen (protein) intake is associated with a decrease in uric acid production analagous to the relationship between nitrogen intake and urea production observed in mammals. Is there a transfer of uric acid into the intestinal tract analagous to the transfer of urea observed in mammals? Most birds have a relatively short midgut and a hindgut that consists of a short straight colon, and often paired ceca (Stevens and Hume, 1998). The urine of birds which contains uric acid passes from the ureters via two ureteral openings into the urodeum of the cloaca (Goldstein and Skadhauge, 2000). In a number of avian species, such as domestic fowl (*G. domesticus*), rock ptarmigan (*Lagopus mutus*), domestic turkey (*Meleagrus gallopavo*), domestic goose (*Anser anser*), roadrunner (*Geococcyx californianus*), emu (*Dromaius novaehollandiae*), all of which are herbivores and granivores retrograde urine transport toward the ceca has been demonstrated (Bjornhag, 1989). Colonic motility patterns have been studied in turkeys (*M. gallopavo*) and eight other wild species which included gulls (*Larus delawarensis* and *Larus atricilla*), a night-heron (*Nycticorax nycticorax*), mallard (*Anas platyrhynchos*), hawk (*Buteo jamaicensis*), and owl (*Strix varia*) (Duke, 1989). These birds include carnivores, omnivores, and a herbivore. The retrograde peristalsis appears to originate in the coprodeum and be propagated orad. These antiperistalitic waves move only the more fluid portions of the colonic contents and Duke (1989) has concluded that urine is moved along the mucosa surface by antiperistalsis and colonic ingesta and large particulate matter are moved aborad through the center of the lumen by different large colonic contractions. As we shall discuss shortly, the hindgut contains microbes capable of degrading uric acid. Bjornhag (1989) has observed in hens that the percentage of produced urine transported retrogradely into the ceca is influenced by the nitrogen content of the diet. Hens were given isocaloric diets with high (19.8% crude protein), medium (15% crude protein) or low (10.6% crude protein) content of nitrogen. The average percentage of produced urine transported into the ceca was 6.1, 12.1 and 22.4, respectively, for hens ingesting a high, medium, or low protein intake. The hindgut (coprodeum, colon, ceca) of birds is capable of active sodium reabsorption and solute-linked water absorption (Thomas, 1982; Laverty and Skadhauge, 1999) and hence, can modify the composition and volume of refluxed urine. In birds, the hindgut is an important organ for both osmoregulation and as we will discuss nitrogen conservation (Laverty and Skadhauge, 1999; Braun 1999). In mammals, the kidney is the sole organ for regulating the composition of the extracellular fluid. However, in mammals, osmoregulation and nitrogen conservation do intersect at the level of the renal tubule. Urea transporters in the collecting duct facilitate urea reabsorption (Sands, 1999) which is important both for nitrogen conservation (Jackson, 1998) and for maintaining the urea medullary concentration gradient and, hence, the integrity of the counter current system. These urea transporters can be up-regulated by a low protein diet (Sands, 1999). The involvement of the avian hindgut, and the mammalian collecting duct urea transporters in both osmoregulation and nitrogen conservation are examples of the multifunctional characteristic (and, hence, economy) of body systems.

Microbes are found throughout the avian gastrointestinal tract (Vispo and Karasov, 1997) but in general as residence time of ingesta is usually short in the small intestine this segment of the gut is unsuitable for colonization by microbes (Vispo and Karasov, 1997). The large intestine (colon) and ceca are the most important sites of microbial activity and of the two, the cecum has been most extensively studied. Cecal bacteria capable of degrading uric acid have been demonstrated in many species of herbivores and granivorous birds such as the chicken (*G. domesticus*), turkey (*M. gallopavo*), duck (*A. platyrhynchos*), desert quail (*Callipepla gambelii*), and willow ptarmigan (*Lagopus lagopus lagopus*) (Mortensen and Tindall, 1981a; Braun and Campbell, 1989; Karasawa, 1989a; Mead, 1989). End products of microbial

uric acid degradation include short chain fatty acids, ammonia, and carbon dioxide (Karasawa, 1989a; Mead, 1989). Ceca in turkeys, chickens and other galliforms have active motility that stirs and periodically evacuates the luminal contents (Clench, 1999). Propagation velocity of cecal contractions is affected by the presence or absence of food in the lumen (Clench, 1999). In addition, the reverse peristaltic activity originating in the cloaca is modulated by the composition of urine in the cloaca (Braun, 1999). Antiperistalsis is reduced in the presence of urine with a significantly higher osmotic potential than that of plasma.

In summary, birds excrete urine containing uric acid into a cloaca and then move that urine retrogradely up the colon and into ceca in those species which possess this structure (Clench, 1999). The uric acid is degraded by colonic and cecal bacteria releasing ammonia, carbon dioxide and short chain fatty acids. Colonic antiperistalic activity is modulated by the nitrogen content of the diet. Hence, the movement of urine containing uric acid into the colon and ceca is a regulated process. The actual regulating/co-ordinating mechanisms are unknown. Cecal motility is another potential regulatory site. Analagous to mammals, the transfer of the nitrogenous end product (uric acid) to the gut microbial population appears to be a controlled process somehow linked to nitrogen intake.

What role does the recycling of urinary nitrogen into the gut play with respect to nitrogen balance in birds? There is only limited published data with respect to this question. Mortensen and Tindall (1981b) showed that cecal microbes in the willow ptarmigan were able to synthesize glutamic acid from alpha-ketoglutarate using ammonia nitrogen from the microbial decomposition of uric acid. However, these investigators were not able to demonstrate absorption of amino acids from the ceca. Karasawa and Maeda (1995) used urea as a 'non-specific' nitrogen source and introduced a [^{15}N] urea solution into a chicken cecal pouch. Little urea is synthesized by chickens due to the absence of the mitochondrial system for citrulline synthesis (Vorhaben and Campbell, 1972). Chicken gut microbes have urease activity (Karasawa 1989a). The experimental results indicated that the introduced urea was degraded to ammonia and that a fraction of the ammonia was used in the synthesis of amino acids and protein. The labelled amino acids and protein were also detected in cecal venous blood. Karasawa (1989b) and Karasawa and Maeda (1994) investigated the role of the ceca in chickens ingesting a low protein diet. Colostomization had no effect on nitrogen balance and nitrogen utilization in chickens fed a protein diet of 560 mg N/kg/d. However, colostomization significantly reduced nitrogen balance and nitrogen utilization in birds ingesting a diet containing 280 mg N/kg/d supplemented by oral urea (280 mg urea N/kg/d). Karasawa (1999) has interpreted these results as follows. Dietary urea is absorbed in the upper intestine and excreted in the urine. The urinary urea is moved retrogradely from the cloaca into the colon and ceca. In the ceca the urea is hydrolyzed and the ammonia utilized by the microflora for the synthesis of amino acids and protein. The amino acids and protein can be reabsorbed across the cecal wall. In colostomized chickens, dietary urea is excreted in the urine and lost since the colostomization prevents retrograde flow of urine from cloaca into the colon and ceca. Chickens ingesting a low protein diet and a urea supplement are able to maintain nitrogen balance through the mechanism outlined above. Colostomization prevented chickens on an identical low protein diet from using the dietary urea supplement to maintain nitrogen balance.

The role of the avian cecum in nitrogen recycling is further strengthened by the experiments of Obst and Diamond (1989). Cecal amino acid transport was observed in five bird species; domestic chicken (*G. domesticus*, an omnivore/granivore), sage grouse (*Centrocercus urophasianus*, a herbivore), Canada goose (*Branta canadensis*, a herbivore), red-necked phalarope (*Phalaropus lobatus*, a carnivore), and feral rock dove (*Columba livia,* an omnivore/granivore). These observations support the notion that nitrogen can be recycled by incorporation of excretory nitrogen into microbial synthesized amino acids which can then be reabsorbed across the cecal wall. Obst and Diamond (1989) argued that Mortensen and Tindall's (1981b) failure to detect cecal amino acid transport was due to technical reasons.

In summary, the data available for birds belonging to the omnivore/granivore and herbivore dietary categories are consistent with the presence of a nitrogen conserving mechanism having the same general features as that of mammals. However, it is quite plausible that not all birds conform to this model for nitrogen conservation. Fruit-eating birds, (like fruit-eating bats) subsist on a diet low in

protein (Martinez Del Rio, 1994). These birds have a rapid intestinal transit time, shorter than that of chickens, herbivorous or granivorous wild birds or hummingbirds (Karasov and Levey, 1990). The rapid passage of digesta through the intestine would allow fruit-eating birds to process a larger quantity of food. However, there does not appear to be any consensus as to how the fruit-eating bird has adapted to a limited nitrogen intake (Karasov and Levey, 1990; Martinez Del Rio, 1994).

4. Reptiles

The nitrogenous end products excreted by reptiles are more diverse than that of mammals or birds. Tortoises, for example, excrete urea and uric acid and both ureotelic and uricotelic (synthetic) systems have been demonstrated in their liver mitochondria (Campbell et al. 1987). Lizards and snakes excrete chiefly uric acid while in alligators and crocodiles the products are ammonia and uric acid. There are no published studies measuring uric acid and/or urea production as a function of nitrogen intake. There is also very limited data on the influence of nitrogen intake on nitrogen excretion. Hill and Dawbin (1969) measured nitrogen excretory products in *Sphenodon punctatus* ingesting differing protein intakes. This species excretes nitrogen chiefly in the form of urea and uric acid with lesser amounts of ammonia. The animals were distributed into three groups maintained on a high protein (heart muscle), intermediate (snails) or low protein (mealworms) diet. The exact number of animals used and the length of time they were adapted to the different diets are not explicitly stated. Total nitrogen excretion did not appear to vary with protein intake, but there was a change in the distribution of end products. With decreasing protein intake, there was a significant decrease in urea excretion, a smaller fall in ammonia excretion but an increase in the amount of uric acid excreted. The reduction in urea excretion would be consistent with a nitrogen conservation mechanism. The reason for the rise in uric acid excretion is unclear. One possible explanation is that the animals ingesting a low protein diet were also water depleted. Since uric acid can be excreted as a semisolid paste by this reptile, the shift to uric acid excretion could be a response to limited water availability.

Coulson and Hernandez (1959) examined the influence of diet on nitrogen excretion in alligators. The principal nitrogenous end products in this reptile are ammonia (as NH_4HCO_3) and uric acid. Alligators fed casein, gelatin or rabbit meat were compared to a fasted group. The length of time for adaptation is not given, but both groups were well hydrated. As given in figure 2 of this reference, fed animals excreted approximately 7.0 mM N/kg/d while the value for fasted animals was approximately 2.0 mM nitrogen/kg/d. There was also a change in the partition of nitrogenous end products. In the fed state ammonia (as NH_4HCO_3) and uric acid were excreted in about equal amounts. In the fasted state, 75% of the excreted nitrogen was in the form of ammonia. Hence, alligators conserve nitrogen when fasted by reducing total nitrogen excretion and by excreting more nitrogen as ammonia than uric acid.

In the reptile, as in the bird, urine is voided into the urodeum of the cloaca (Withers, 1992). Beyenbach (personal communication) using either ^{14}C-polyethylene glycol or ^{14}C-inulin as a marker of glomerular filtrate was able to measure radioactivity in the lumen of all intestinal sections (including the proximal small intestine) of active and hibernating garter snakes (*Thamnophis sirtalis*). This retrograde movement of urine from cloaca into the intestine is consistent with the observation of antiperistalsis described in the turtle (*Chinemys reevesii*) and the adult green iguana (*Iguana iguana*) (Bjorndal, 1997). Studies of the intestinal microflora have been done generally in herbivorous reptiles (turtles, tortoises and lizards). Fermentation end products have been quantified in four species of turtles and two species of lizards (Bjorndal, 1997) and their concentration appears to be highest in the large intestine. The end products generated from fermentation in reptiles are the same as those that have been identified in the gastrointestinal tract of birds and mammals. In a few studies, the actual gut microbes have been identified in herbivorous reptiles (Bjorndal, 1997; McBee, 1989). However, there are no data as to whether these microbes are capable of degrading uric acid or urea.

Unlike birds and mammals, reptiles are ectotherms and, in general, resting metabolic rate is approximately 5% of the value observed in mammals. Schmidt-Nielsen (1984) has recalculated the resting metabolic rate of lizards for a body temperature as high as that of mammals (38 °C). Even at this temperature, these reptiles would have a

metabolic rate of only one-quarter of the mammalian value. Coulson and Herbert (1981) have measured the disappearance rate of injected amino acids in a variety of animals (alligators, caimans, turtles, lizards, dogs and rats). The average rate of removal of single amino acids was proportioned to the metabolic rate of the experimental animal. Turtles and alligators catabolize amino acids very slowly (Coulson et al., 1977) with half-lives of injected amino acids being measured in days (Coulson and Herbert, 1981). If turtles and alligators are representative of reptiles in general then, the time requirements of a nitrogen conservation system in reptiles would be very different than those of mammals or birds.

As already discussed, the intestinal microflora has been most extensively studied in herbivorous reptiles (Bjorndal, 1997). The principal fermentation region is the large intestine, but fermentation end products (chiefly short chain fatty acids) are found not only in the colon, but also in the small intestine albeit at a lower concentration (Bjorndal, 1997). In these same species (tortoise and turtle), amino acid absorption appears to occur chiefly in the small intestine and to a lesser extent in the large intestine (Skoczylas, 1978).

In summary, the data are sparse, but tantalizing. The elements of a nitrogen conserving mechanism similar to that of mammals and birds appear to be present in reptiles. Reptiles excrete urine containing uric acid and urea directly into the cloaca. Antiperistalsis has been described in the iguana and turtle and urine has been detected in the large and small intestine of the garter snake. Thus, like the bird, reptiles appear to move urine retrogradely from the cloaca into the colon and small intestine. The intestinal tract contains microbes that generate fermentation end products similar to those identified in the guts of birds and mammals. However, the crucial experiments have not been done. Can the intestinal microflora break down uric acid and urea and use the generated ammonia as a nitrogen source for amino acid/protein synthesis? If so, are microbial synthesized amino acids transferred back to the host, and is this process 'upregulated' during times of reduced nitrogen intake? Finally, like birds, the intestinal tract of reptiles (such as lizards, snakes and crocodiles) is capable of modifying the volume and composition of urine moved retrogradely up from the cloaca (Skadhauge, 1977; Beyenbach, personal communication; Bentley, 1976) by reabsorbing sodium and water. Hence, the intestinal tract also plays an important role in osmoregulation.

5. Fish

Most fish excrete ammonia as the chief nitrogenous end product. However, ureotely is observed in a few teleosts and in marine elasmobranchs (Wood, 1993). Obviously, a nitrogen conserving mechanism similar to that of mammals or birds would only be observed in ureotelic fish. The gulf toadfish, *Opsanus beta*, a carnivore, is one of the teleosts expressing a full complement of ornithine–urea cycle enzymes in the liver (Mommsen and Walsh, 1989). This toadfish becomes facultatively ureotelic under a variety of circumstances, such as when subjected to crowding or close confinement in the laboratory (Wood et al., 1997). Urea excretion in this teleost is pulsatile, whereas metabolic production of urea is continuous (Wood et al., 1997). Walsh and Milligan (1995) have studied the effects of feeding and confinement on nitrogen excretion in *O. beta*. In one series of experiments, toadfish, 72 h after feeding were subjected to confined conditions. After an additional 72 h, half the toadfish were fed and half were not. Toadfish adapted to confined conditions reduced the excretion rate of total nitrogen and excreted the bulk of this nitrogen (~80%) as urea. For the unfed toadfish kept in continued confinement the rate of nitrogen excretion (with most of it being urea) remained unchanged. In the fed group (also kept confined), the rate of total nitrogen excretion increased significantly but the pattern changed in that, about equal portions of nitrogen were excreted as urea and ammonia. The excess nitrogen load from the feed was cleared within 24 h. After 24 h, the rate of total nitrogen excretion and the pattern (~80% urea) returned to that of the unfed state. Walsh (1997) has speculated that the shift to ureotely and reduced nitrogen excretion rate during confinement may represent a nitrogen conservation mechanism. The observation that fish when fed while confined, excrete about half of the nitrogen load as ammonia would be consistent with the hypothesis that confinement-induced ureotely is a nitrogen conservation process.

In fish, the gills not the kidney are the major site of nitrogen excretion. Teleosts excrete urine into a widened duct commonly termed the urinary

bladder which vents to the outside through the urinary papilla (Curtis and Wood, 1991). Hence, unlike the situation in reptiles and birds, urine is not directly exposed to the intestinal tract. Urease activity has been detected in the intestinal contents of gulf toadfish but not in liver, gill, kidney, skeletal muscle or the gut itself (Mommsen and Walsh, 1989). Urea has a higher concentration in intestinal fluid than in plasma (Walsh et al., 1990). How is urea transported into the intestinal lumen? Although a urea transporter has been identified in the gill of the toadfish, one has not been detected in the intestine (Walsh et al., 2000). When toadfish were treated with antibiotics (Walsh et al., 1990) the number of viable intestinal bacteria was significantly reduced. Compared to controls, plasma urea concentration fell slightly and urea excretion increased slightly in antibiotic treated fish, although these changes were not statistically significant. If antibiotic treatment suppressed microbial mediated urea hydrolysis and if urea production remained unchanged, then a lower plasma urea concentration would necessitate an increase in urea excretion.

However, this interpretation is confounded by the fact that this fish is not in a steady state with respect to urea metabolism. Urea excretion is pulsatile whereas urea production is continuous (Wood et al., 1997). Plasma urea concentration increases steadily between excretory pulses then falls precipitously during a pulse. Assuming the urea volume of distribution does not change, then the urea pool would display cyclical increases and decreases, and hence, never be in a steady state. Using radioactive urea to experimentally measure a difference between production and excretion (i.e. to quantify hydrolysis) would be difficult.

Marine elasmobranchs are another group of ureotelic vertebrates (Wood, 1993). Unfed spiny dogfish (*Squalus acanthias*) excrete approximately 95% of total nitrogen as urea (Wood et al., 1995). In this fish urea serves both as a vehicle for nitrogen excretion as well as a major osmolyte for maintaining water balance. The plasma urea concentration in this fish is approximately 300 mmol/l (Wood et al., 1995). Previously Knight et al. (1988) described the presence of ureolytic bacteria in the intestinal tract of sharks as well as in liver homogenates. The blood of these same sharks was found to contain no bacteria on culture. The importance of urea in both nitrogen metabolism and osmoregulation would make the need for a nitrogen conservation system obvious. Knight et al. (1988) speculated that ureolytic bacteria play a role in controlling shark tissue urea storage and flux.

In summary, the data for a nitrogen conservation system in ureotelic fish are very incomplete, but quite suggestive as they are for reptiles. The contents of the gulf toadfish intestinal tract contains urease activity and ureolytic bacteria are found in the intestinal tract and liver of marine elasmobranchs. However, there are no data on the protein synthesizing capacity of these microbes or whether any microbial synthesized amino acids are transferred back to the fish. Like the mammal, urea transferred to the intestinal tract contents of the gulf toadfish must have crossed the gut wall. However, a urea transporter has so far not been detected.

6. Summary

The comparative approach to animal physiology represents a powerful method for uncovering similar adaptations. Mammals and birds belonging to the omnivorous/granivorous and herbivorous dietary categories appear to possess a nitrogen conservation system with similar features. Unfortunately, the data for reptiles and fish with respect to a comparable nitrogen conservation system are sparse and inconclusive. However, when this same data are viewed against the background of mammals and birds as a reference, a clearer picture emerges for the presence of elements of a similar nitrogen conservation system in these two vertebrate groups as well. The elements of this system consist of a series of steps. Urea and/or uric acid is transferred into the intestine, either directly across the bowel wall (mammals and ureotelic fish?) or by excretion and retrograde movement of urine into the intestine (birds, reptiles). Intestinal microbes degrade urea and uric acid and utilize the generated ammonia as a nitrogen source for amino acid/protein synthesis. These microbial-synthesized amino acids are then transferred back to the host. Ammonia released by microbial activity could also be used directly by the host for amino acid synthesis. The evidence for such a sequence of steps is reasonably strong for mammalian and avian species with an intestinal structure and physiology designed for quantitative-

ly significant microbial mediated activity, but much weaker for reptiles and ureotelic fish. Many crucial experiments need to be performed to confirm the presence of these steps particularly in the latter two vertebrate groups. However, even in mammals, there are many gaps in our knowledge. One specific area in which there is practically no information concerns how these steps are regulated and co-ordinated. For example, in mammals, what are the mechanisms by which reduced nitrogen intake increases the proportion of synthesized urea which is hydrolyzed? Such mechanisms must somehow co-ordinate nitrogen intake with both the rate of urea transport across the intestinal wall and the metabolic activity of the intestinal microflora. In birds and presumably reptiles, how does reduced nitrogen availability regulate the amount of urine that is retrogradely moved into the intestine? Currently, there is no information with respect to these questions. The sequence of steps, as outlined, would define the prevailing nitrogen conservation system for herbivores and omnivores at least. However, it would appear that some animals, in particular the fruit-eating bat and perhaps the fruit-eating bird, may have evolved alternative, as yet uncharacterized, adaptations to a very limited nitrogen intake.

Finally, body plans are economical with biological systems and biological molecules subserving multiple functions. In mammals, urea is recycled as part of the nitrogen conservation system, but this same molecule is retained within the renal medulla as an important component of the concentrating–diluting mechanism. In marine elasmobranchs urea is the vehicle for nitrogen waste removal, but also serves as a major osmolyte in water balance. In birds and reptiles, urine is excreted into the cloaca and moves retrogradely into the intestine exposing uric acid and urea to microbial activity. In these same vertebrates, intestinal modification of the composition and volume of urine (prior to its excretion) functions as an important component of osmoregulation. It is clear, then, since there is considerable overlap between the systems for nitrogen conservation and osmoregulation, we will also need information as to how these two systems are co-ordinated with respect to each other.

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