The Journal of Experimental Biology **198**, 273–281 (1995)
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REVIEW

NITROGEN EXCRETION: THREE END PRODUCTS, MANY PHYSIOLOGICAL ROLES

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Summary

There are diverse physiological functions of nitrogen end products in different animal groups, including excretion, acid–base regulation, osmoregulation and buoyancy. Animals excrete a variety of nitrogen waste products, but ammonia, urea and uric acid predominate. A major factor in determining the mode of nitrogen excretion is the availability of water in the environment. Generally, aquatic animals excrete mostly ammonia, whereas terrestrial animals excrete either urea or uric acid. Ammonia, urea and uric acid are transported across cell membranes by different mechanisms corresponding to their different chemical properties in solution. Ammonia metabolism and excretion are linked to acid–base regulation in the kidney, but the role of urea and uric acid is less clear. Both invertebrates and vertebrates use nitrogen-containing organic compounds as intracellular osmolytes. In some marine invertebrates, NH_4^+ is sequestered in specific compartments to increase buoyancy.

Key words: ammonia, urea, uric acid, excretion, metabolism, membrane transport, acid–base balance, osmoregulation, buoyancy, nitrogen.

Who excretes what?

Animals excrete three main nitrogen products, ammonia, urea and uric acid (Fig. 1), as well as some minor nitrogen excretory products, including trimethylamine oxide, guanine, creatine, creatinine and amino acids. The term ammonia will be used to indicate the total ammonia, whereas NH_3 and NH_4^+ will refer to non-ionic ammonia and ammonium ion, respectively. Whether an animal excretes predominantly ammonia, urea or uric acid depends upon a number of factors in the animal's environment. But one major problem that all animals face is the relatively toxicity of ammonia when it is concentrated in body tissues.

Aquatic animals are generally more tolerant of elevated blood ammonia levels than terrestrial animals. In teleost fish, plasma total ammonia concentrations may vary between 0.05 and $1\,mmol\,l^{-1}$ (e.g. Wright *et al.* 1993), but when plasma ammonia levels approach $2\,mmol\,l^{-1}$ in arctic char, flaccid paralysis results (Lumsden *et al.* 1993). In contrast, blood ammonia levels greater than $0.05\,mmol\,l^{-1}$ can be toxic to the central nervous system of most mammals (Meijer *et al.* 1990).

Ammonia exerts its toxic effects at many different levels (for a review, see Cooper and Plum, 1987). Elevated levels of ammonia modify the properties of the blood–brain barrier (Sears *et al.* 1985), interfere with amino acid transport (Mans *et al.* 1983), disrupt cerebral blood flow (Andersson *et al.* 1981), impede excitatory amino acid neurotransmitter metabolism, particularly that of glutamate and aspartate (Hindfelt *et al.* 1977), and cause morphological changes in astrocytes and neurons (Gregorios *et al.* 1985). NH_4^+ interrupts nerve conduction by directly substituting for K^+ in ion-exchange mechanisms (Binstock and Lecar, 1969). Furthermore, ammonia has been shown to alter carbohydrate and fat metabolism and ATP levels, not only in the brain, but in other tissues as well (Wiechetek *et al.* 1979). Which of these toxic effects is most detrimental is not clear but, taken together, they can result in convulsions, coma and eventually death. Therefore, nitrogen excretory strategies must be designed to avoid the toxic accumulation of ammonia.

Aquatic versus *terrestrial animals*

Aquatic animals, including invertebrates, fish and larval amphibians, excrete mostly ammonia. Ammonia is highly soluble in water and permeates cell membranes relatively easily. Despite its high solubility, an animal must use 400 ml of water to dilute every gram of ammonia to maintain ammonia concentrations below toxic levels. Only animals that respire in water, therefore, excrete ammonia as their major nitrogen waste product.

During the evolution of terrestrial animals, water conservation became an important concern. Most terrestrial animals convert ammonia to either urea or uric acid, compounds that can be concentrated in body fluids to a greater extent than ammonia with no toxic effect. Urea requires about 10 times less water than ammonia for excretion, whereas uric acid is highly insoluble and requires about 50 times less water. Terrestrial animals, therefore, can simultaneously conserve water and eliminate nitrogenous wastes. A classic example of

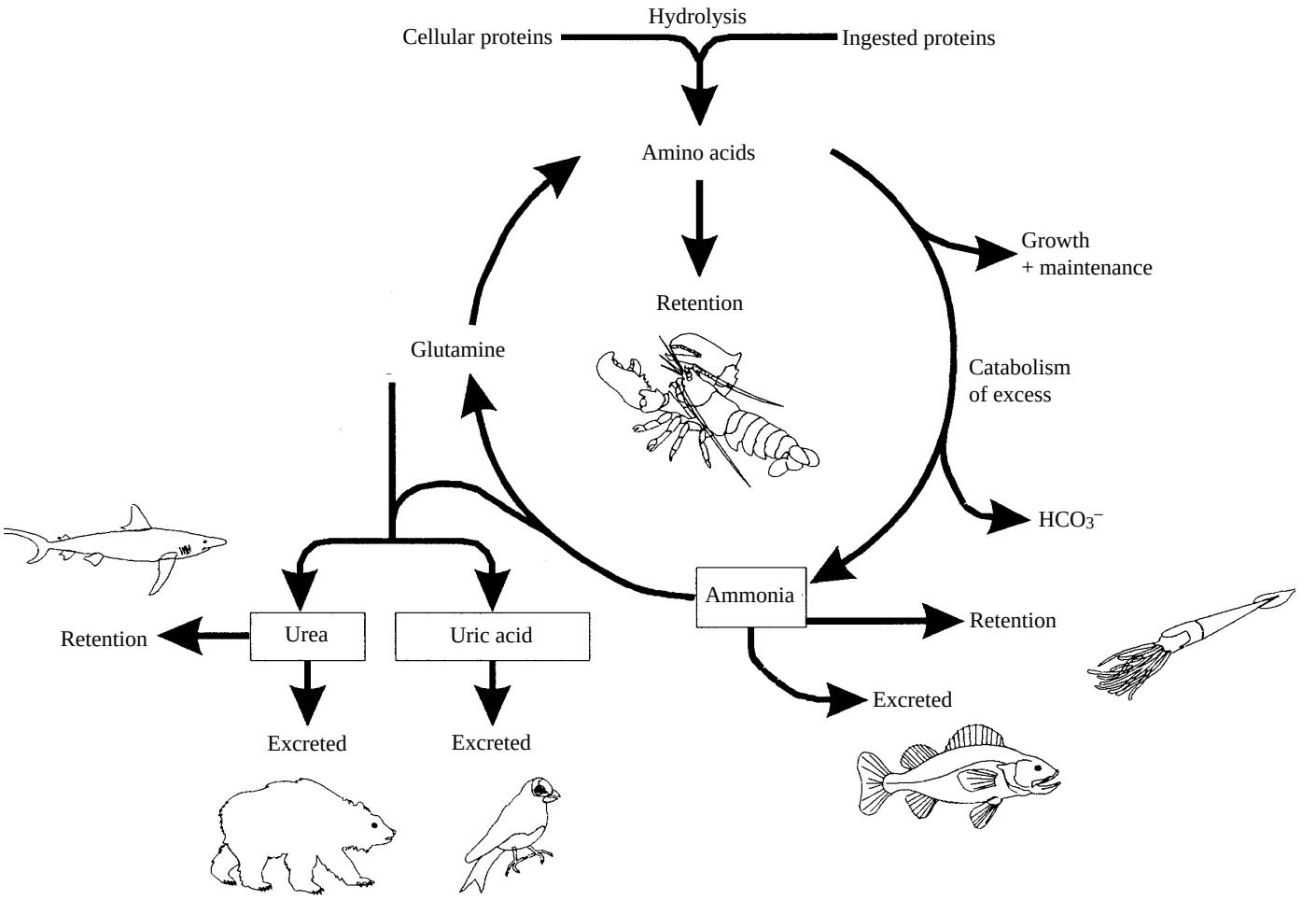

Fig. 1. General overview of nitrogen metabolism and excretion in animals. The three main nitrogen excretory products are highlighted in boxes.

the influence of water availability on nitrogenous excretion is seen in amphibians during development. As tadpoles, *Rana catesbeiana* live in water and excrete about 80 % of their nitrogen wastes as ammonia. But during metamorphosis, enzymes involved in urea synthesis are induced and a gradual transformation occurs from ammonia to urea excretion (Brown *et al.* 1959; Atkinson, 1994).

Unusual patterns of nitrogen excretion

Water availability is clearly an important factor in the mode of nitrogen excretion; however, it is not the whole story. For instance, some terrestrial snails (Speeg and Campbell, 1968), crabs (Greenaway and Nakamura, 1991; De Vries and Wolcott, 1993) and isopods (Wieser and Schweizer, 1969; Wright and O'Donnell, 1993) excrete a significant portion of their nitrogen wastes by ammonia volatilization. In contrast, a completely aquatic teleost fish, *Oreochromis alcalicus grahami*, living in Lake Magadi (pH 10), excretes no ammonia, only urea (Randall *et al.* 1989; Wood *et al.* 1989). Elasmobranch fish that retain urea as an osmolyte (see below), such as the marine dogfish (*Squalus acanthias*), also excrete most of their nitrogen wastes as urea (urea 98 %, ammonia 2 %; C. M. Wood, P. Part and P. A. Wright, in preparation).

How are nitrogen end products formed?

Ammonia is mostly formed from the catabolism of proteins (Fig. 1). Both ingested and cellular proteins are hydrolysed to form a pool of amino acids that can be used to form new proteins for growth and basic protein turnover. Unlike carbohydrates and lipids, amino acids cannot be stored to any great extent in animal tissues (although they are retained as osmolytes in some marine animals, see below). The excess amino acids, not used in protein production, are catabolised to ammonia, which is either excreted or converted to urea or uric acid in the liver. In animals that are very sensitive to ammonia, such as mammals, ammonia is transported in the blood as glutamine, before it is converted to urea for excretion. In addition to amino acid catabolism, nitrogenous end products may also result from purine, methylamine and creatine metabolism.

Ammonia metabolism

As stated above, the major route for ammonia formation is through amino acid catabolism, usually in the liver. Most L-amino acids are first transaminated to form glutamate, catalysed by a group of transaminase enzymes. Glutamate is then deaminated to form NH_4^+ and α-ketoglutarate, catalysed

Table 1. *Enzymes concerned with nitrogen metabolism for which a cDNA clone is available*

Pathway	Animal group	Enzyme	Reference
Ornithine–urea cycle	Mammals	Carbamoyl phosphate synthetase I	Adcock and O'Brien (1984)
		Ornithine transcarbamylase	Takiguchi *et al.* (1984)
		Argininosuccinate synthetase	Freytag *et al.* (1984)
		Argininosuccinate lyase	O'Brien *et al.* (1986)
		Arginase	Ohtake *et al.* (1988)
	Amphibians	Carbamoyl phosphate synthetase I	Helbing *et al.* (1992)
		Ornithine transcarbamylase	Helbing *et al.* (1992)
		Arginase	Xu *et al.* (1993)
	Elasmobranchs	Carbamoyl phosphate synthetase III	Hong *et al.* (1994)
Uricolysis	Mammals	Uricase	Wu *et al.* (1989)
Ammonia synthesis	Mammals	Glutamate dehydrogenase	Amuro *et al.* (1989)
			Das *et al.* (1987)
		Phosphate-dependent glutaminase	Shapiro *et al.* (1991)
		AMP deaminase	Debatisse *et al.* (1988)
Uric acid synthesis	Mammals	Glutamine synthetase	deGroot *et al.* (1987)
	Birds	Glutamine synthetase	Campbell and Smith (1992)

by glutamate dehydrogenase (GDH). Transdeamination is the term given to this two-step process. Ammonia can also be released from urea by the action of urease, present in some mollusc (Speeg and Campbell, 1969) and coral (Barnes and Crossland, 1976) tissues and contained in microorganisms in the liver tissue of two shark species (Knight *et al.* 1988), but more importantly in the digestive tract of most animals.

The best illustration of digestive microbial activity is in ruminants; urea produced in the liver is recycled through the rumen, where it is degraded to ammonia by microbial urease (Church, 1975). The ammonia produced supports microbial protein synthesis, an especially important process for animals on low-protein diets (Kay *et al.* 1980).

Uric acid metabolism

Uric acid is formed from the metabolism of adenine- and guanine-based purines. In animals that lack the uric acid degradation enzyme uricase, birds, reptiles (except chelonians from mesic, semi-aquatic and aquatic habitats; Campbell *et al.* 1987), some amphibians (e.g. *Phyllomedusa sauvegei*, *Chiromantis xerampelina*; Dantzler, 1989) and most insects (Cochran, 1985), uric acid constitutes the major nitrogenous end product. Uricase is also not expressed in hominoid primates (Varela-Echavarria *et al.* 1988) and uric acid has proved to be a powerful radical scavenger and antioxidant in many human tissues (Becker, 1993). However, elevated blood uric acid levels can lead to the painful condition termed gouty arthritis.

The final enzyme in the uric acid formation pathway, xanthine dehydrogenase, is missing in arachnids and, consequently, guanine is the major excretory product in these animals (Anderson, 1965).

Urea metabolism

Urea is synthesized from NH_4^+ and HCO_3^- in the liver *via* the ornithine–urea cycle (OUC; for a review, see Meijer *et al.* 1990). Urea may also be formed from the degradation of uric acid or arginine (for a review, see Campbell, 1991). Elasmobranchs, the coelacanth, amphibians and mammals utilize the OUC, whereas most invertebrates and teleosts synthesize urea by uricolysis or argininolysis. As mentioned above, a few teleostean species synthesize significant amounts of urea in response to environmental conditions that limit ammonia excretion (*O. a. grahami*, Randall *et al.* 1989; *Opsanus beta*, Walsh *et al.* 1990; *Heteropneustes fossilis*, Saha and Ratha, 1989). These species are unique in expressing the full complement of OUC enzymes, but in other adult teleosts, some of the genes for OUC enzymes appear to be repressed (e.g. Wright, 1993; for reviews, see Campbell and Anderson, 1991; Mommsen and Walsh, 1991). Key OUC enzymes are absent in adult rainbow trout, but are expressed around the time of hatching in larval trout (Dépêche *et al.* 1979; Wright *et al.* 1995). Hence, OUC genes may be retained and expressed during the early life stages in all teleosts, but a functional OUC persists in mature fish only in cases where unusual environmental conditions predicate ureotelism. The question remains as to why larval trout express OUC enzymes; possibly the OUC is used as a 'safeguard' mechanism to prevent ammonia toxicity during a particularly sensitive stage of neural development.

How is synthesis regulated?

Regulation of enzyme synthesis

Metabolic control can be separated into the regulation of *de novo* protein synthesis and the regulation of 'pre-existing' enzymes. Table 1 is a partial list of enzymes related to nitrogen metabolism for which a cDNA clone is available. Most of the cloning studies have concentrated on mammalian species. Recently, genes for OUC enzymes have been cloned in amphibians (Helbing *et al.* 1992: Xu *et al.* 1993) and elasmobranchs (Hong *et al.* 1994). A single glutamine synthetase gene has also been identified in elasmobranch liver that codes for two isoenzymes expressed in different dogfish

(*Squalus acanthias*) tissues (Campbell and Anderson, 1991). In addition, Campbell and Smith (1992) have cloned the gene for glutamine synthetase in the chicken. As the same or similar genes are cloned in various animals, evolutionary relationships can be determined. For instance, glutamine synthetase has been cloned in various plant and animal species and has proved to be a good molecular clock, i.e. the rate of gene evolution is regular between phylogenetically diverse species (Pesole *et al.* 1991).

Regulation of 'pre-existing' enzyme

Modification of pre-existing enzymes, may involve (1) allosteric regulation, (2) covalent modification, (3) changes in the rate of enzyme turnover or degradation and/or (4) regulation of the assembly states of proteins or enzyme complexes. The first three regulatory mechanisms are described in any biochemistry textbook, so I will only discuss the regulation of enzyme complexes, a relatively new development. It has been proposed that the major function of enzyme complexes is to provide a means of direct transfer of substrates or modulators from one enzyme to another (for reviews, see Srere, 1987; Somero and Hand, 1990). Channelling of substrates from enzyme to enzyme in the OUC has been demonstrated in mammals (Wanders *et al.* 1984; Meijer, 1985; Cheung *et al.* 1989). Watford (1989) described the OUC as a 'metabolon', spanning both the mitochondrial and cytosolic compartments. To my knowledge, OUC channelling has not been investigated in animals other than mammals. The enzyme responsible for the first step in the pathway, carbamoyl phosphate synthetase, and the mitochondrial/cytosolic distribution of OUC enzymes are quite different in fish and mammals (Mommsen and Walsh, 1989).

Transport of nitrogen end products

Ammonia

Ammonia exists in solution as both NH_3 and NH_4^+. The NH_3/NH_4^+ ratio varies with pH, the pK of the reaction being about 9.5. NH_3 is a small molecule (molecular mass 17), is moderately lipid-soluble and penetrates cell membranes mainly by lipid-phase permeation (Fig. 2). Although most cell membranes are highly permeable to NH_3, recent studies indicate that gastric gland cells (Waisbren *et al.* 1994), the renal thick ascending limb tubule cells (Garvin *et al.* 1988; Kikeri *et al.* 1989; Flessner and Knepper, 1993) and *Xenopus* oocyte plasma membranes (Burckhardt and Frömter, 1992) are relatively impermeable to NH_3. NH_3 levels are higher in more alkaline compartments (e.g. extracellular fluid), but total ammonia levels are higher in more acidic compartments (e.g. intracellular fluid), because NH_3 diffuses across the cell membrane, picks up H^+ and is 'trapped' as NH_4^+ (Wright *et al.* 1988*a*,*b*). Thus, in the kidney (Knepper *et al.* 1989) and gills (Wright *et al.* 1989), an acidic disequilibrium pH in the tubule lumen or in the water next to the gill surface facilitates NH_3 secretion.

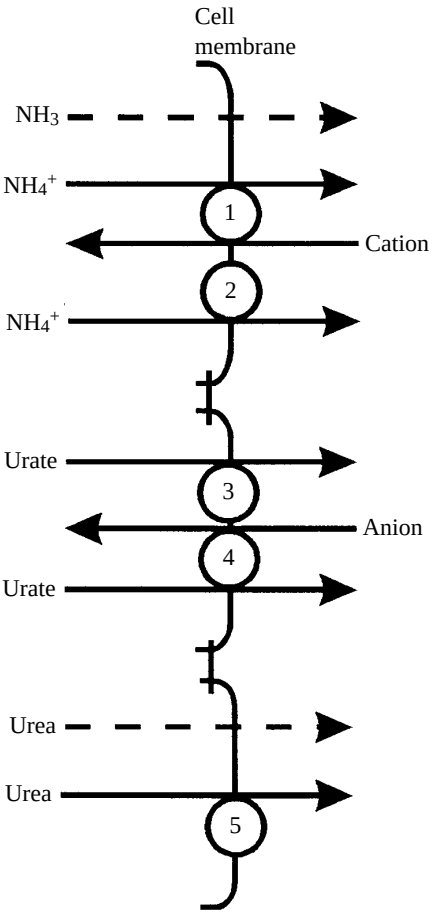

Fig. 2. Transport mechanisms for ammonia, uric acid and urea. Dashed lines indicate non-specific permeation pathways through the lipid bilayer, whereas solid lines refer to transport through specialized protein carriers or channels. Ammonia can substitute for K^+ or Na^+ in the Na^+/K^+-ATPase pump, in $Na^+/2Cl^-/K^+$ cotransport, in Na^+/H^+ exchanger (1) or in the K^+ channel (2). Urate may be transported by either a urate uniporter (3) or a urate/anion exchanger (4). There may be several types of urea transporters (5), some requiring the presence of ions, some of which may be active and some of which are vasopressin-sensitive, but in many tissues the evidence supports a facilitated transporter that is sensitive to inhibition by phloretin and urea analogs.

NH_4^+ transport across cell membranes is dependent on both the concentration and the electrical potential gradient. Owing to its charge, NH_4^+ cannot easily penetrate the lipid bilayer and requires specialized transport pathways (Fig. 2). NH_4^+ has the same hydrated ionic radius as K^+ and can substitute for K^+ in the Na^+/K^+-ATPase transporter (Towle and Holleland, 1987) and the $Na^+/2Cl^-/K^+$ cotransporter (Good *et al.* 1984). NH_4^+ also has a limited ability to penetrate K^+ channels (for a review, see Knepper *et al.* 1989). In many epithelial tissues, NH_4^+ also interacts with the H^+/Na^+ exchanger, substituting for Na^+ (Kinsella and Aronson, 1981).

Uric acid

Uric acid has a pK_1 of 5.4 and, consequently, it is present

as the urate salt of K^+, Na^+ or NH_4^+ under most physiological condition. In insect Malpighian tubules (O'Donnell *et al.* 1983) and vertebrate renal tubules (Dantzler, 1989; Abramson and Lipowitz, 1990), uric acid is actively secreted; however, there appear to be differences in the mechanism of transport between animal groups. Evidence for both a urate/anion exchanger (antiporter) and a urate uniporter exists (Fig. 2).

Urea

Urea crosses cell membranes by two fundamental ways, through specialized membrane transporters or through non-specific aqueous pores (for a review, see Marsh and Knepper, 1992). Urea permeability varies widely; for example, in the mammalian kidney, values range from $0.4\times10^{-5}\,cm\,s^{-1}$ in the cortical collecting duct to $69\times10^{-5}\,cm\,s^{-1}$ in the terminal portion of the inner medullary collecting duct (Knepper and Chou, 1995). Although specific urea transporters are thought to be present in many animal tissues (Schmidt-Nielsen and Rabinowitz, 1964; Kaplan *et al.* 1974; Katz *et al.* 1981; Walsh *et al.* 1994), very little is known about the molecular nature of these transport systems. On the basis of their physiological characteristics, however, it appears that several types of urea-transporting proteins may be present in animal tissues. Recently, the complementary DNA for a urea transporter from rabbit renal medulla has been isolated and characterized (You *et al.* 1993). Using this cDNA as a probe, researchers can now search for similar transporters in other tissues and species.

Regulation of acid–base balance

Ammonia

There has been a large amount of research on the role of ammonia in acid–base regulation. Anaerobic metabolism during exhaustive exercise results in muscle acidification and NH_3 formation from adenylate metabolism. Ammonia formed in exercising muscle is mostly retained during the recovery period and is believed to play a role in intracellular buffering (Dudley and Terjung, 1985; Mommsen and Hochachka, 1988).

Ammonia synthesis and excretion in the kidney play an important role in regulating chronic acidosis. Although most of the research has been done in mammals, there is evidence for a similar response in other animal groups (Fig. 3). The increase in renal ammonia excretion results in an increase in net acid excretion (ammonia excretion + titratable acid excretion − bicarbonate excretion), returning systemic blood pH towards normal values. Glutamine metabolism to NH_4^+ and HCO_3^- is increased during chronic metabolic acidosis (Wright *et al.* 1992; for a review, see Tannen, 1992). The HCO_3^- produced is retained by the kidney and returned to the systemic circulation, while NH_4^+ is excreted in the urine (for a review, see Knepper *et al.* 1989).

Uric acid

The data are sketchy on uric acid excretion and acid–base regulation. It might be predicted that acidosis would result in a decrease in uric acid excretion, thereby retaining base equivalents. However, uric acid excretion is not correlated with the systemic acid–base state in locusts (Harrison and Kennedy, 1994). Alkalosis is accompanied by an increase in urate excretion in snakes (Dantzler, 1968), but whether this response is present in other uricotelic organisms is unknown.

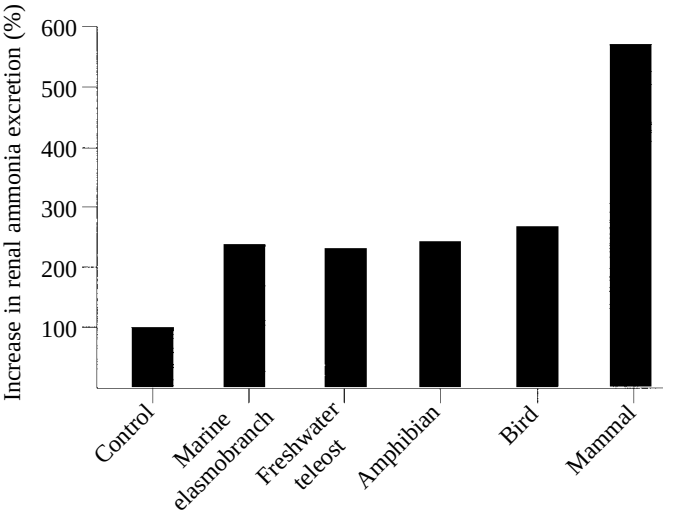

Fig. 3. Percentage increase in renal ammonia excretion during acidosis in various vertebrate groups. Excretion rates have been normalized so that control rates are 100 % in each each study. (Modified from Wolbach, 1955; Yoshimura *et al.* 1961; King and Goldstein, 1983*a*,*b*; Knepper *et al.* 1989.)

Urea

It has been proposed that hepatic urea synthesis plays a central role in regulating chronic acid–base disturbances (Atkinson, 1992). Clearly, urea production is influenced by acid–base perturbations (Haussinger and Gerok, 1985; Atkinson, 1992), but there is no direct evidence that urea synthesis plays a primary role in regulating systemic pH in mammals and fish (Halperin *et al.* 1986; Barber and Walsh, 1993). It has been suggested, however, that the functional significance of urea recycling in bears during hibernation is to regulate acid–base status by eliminating excess HCO_3^- (Guppy, 1986). The acid–base status of hibernating bears has not been measured, primarily because of the practical difficulties of working with such large carnivores. Bears do not urinate or defecate during hibernation, but if HCO_3^- is released as CO_2 through the lungs (Nelson *et al.* 1975), then no advantage would be gained by incorporating HCO_3^- into urea. Radiotracer studies of urea metabolism along with blood acid–base measurements in hibernating bears are necessary before this interesting question can be resolved.

Osmoregulation and nitrogen metabolism

Many marine invertebrate species (Simpson *et al.* 1959), hagfish (Robertson, 1976) and elasmobranchs (for a review, see Goldstein and Perlman, 1995) accumulate amino acids in intracellular compartments to counterbalance the osmotic pressure of sea water. The most commonly occurring amino

acid osmolytes in these organisms are glycine, alanine, proline, β-alanine and taurine (for a review, see Yancey *et al.* 1982). The use of amino acids for osmoregulation is not limited to aquatic species. Heilig *et al.* (1989) have demonstrated that, in salt-loaded rats, amino acids accumulate along with methylamines and polyols in brain tissue.

Homer Smith (1936) first discovered that marine elasmobranchs retain relatively high levels of urea and trimethylamine oxide (TMAO) as a strategy for osmoregulation. Subsequent studies on the coelacanth revealed a similar scenario (Griffith *et al.* 1974). Urea is known to destabilize macromolecules, but a 2:1 ratio of urea:TMAO counteracts these effects and stabilizes proteins (Yancey *et al.* 1982). Several amphibian species (for a review, see McClanahan *et al.* 1994) and estivating lungfish (Smith, 1930) accumulate urea when dehydrated. During dormancy, high urea levels would inactivate enzymes reversibly and suppress metabolic activity (Hochachka and Somero, 1984).

To counterbalance the effects of elevated urea and NaCl concentrations in the mammalian kidney, cells of the medulla accumulate organic solutes, methylamines (mostly betaine and glycerophosphorylcholine) and sugar alcohols (mostly sorbitol and myoinositol). These four osmolytes protect the medullary cells from the potentially harmful effects of elevated interstitial NaCl and urea concentrations (for a review, see Garcia-Perez and Burg, 1991).

Nitrogen and buoyancy in marine organisms

Some marine invertebrates have solved the problem of neutral or positive buoyancy in the water column by manipulating the ionic composition of their internal environment. One strategy is to substitute NH_4^+ for heavier ions, such as Ca^{2+}, Mg^{2+} and SO_4^{2-}. For example, tunicate embryos (Lambert and Lambert, 1978) and pelagic shrimp (Sanders and Childress, 1988) and squid (Clarke *et al.* 1979) sequester NH_4^+ in float cells or specialized chambers at concentrations between 300 and 500 mmol l^{-1}. Squid blood ammonia concentrations are relatively low, and when large nerves were exposed to the ammonia levels found in the float chamber, conduction was impeded (Clarke *et al.* 1979). Hence, it appears that only selected 'buoyancy' tissues are capable of coping with potentially toxic levels of ammonia.

The author wishes to thank Dr Pat Walsh for helpful comments on the manuscript. This review is based on a keynote address presented at the symposium *Nitrogen Metabolism and Excretion* at the Society for Experimental Biology meeting in Canterbury, UK, in April 1993.

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