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Ammonia distribution and excretion in fish

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Abstract

This paper reviews the literature concerning ammonia production, storage and excretion in fish. Ammonia is the end product of protein catabolism and is stored in the body of fish in high concentrations relative to basal excretion rates. Ammonia, if allowed to accumulate, is toxic and is converted to less toxic compounds or excreted. Like other weak acids and bases, ammonia is distributed between tissue compartments in relation to transmembrane pH gradients. NH_3 is generally equilibrated between compartments but NH_4^+ is distributed according to pH. Ammonia is eliminated from the blood upon passage through the gills. The mechanisms of branchial ammonia excretion vary between different species of fish and different environments, and primarily involves NH_3 passive diffusion and NH_4^+/Na^+ exchange. Water chemistry near the gill surface may also be important to ammonia excretion, but a more accurate measurement of the NH_3 gradient across the gill epithelium is required before a more detailed analysis of NH_3 and NH_4^+ excretion can be made.

Introduction

Ammonia can be extremely toxic to fish if allowed to accumulate in the body. Small increases in ambient ammonia levels result in an elevation of body ammonia levels which can have many deleterious (chronic) effects on fish. Chronic levels in the water are approximately 2.4 μmol/l NH_3 (Thurston *et al.* 1984) and the effects on the fish include biochemical and structural changes. Higher levels (acute) of ambient ammonia, between 9.4–64.7 μmol/l NH_3 (Thurston and Russo 1983), as in other vertebrates, cause convulsions and death. Ammonia production, therefore, must be balanced by excretion, or ammonia must be converted to less toxic compounds such as urea (Olson and Fromm 1971) or glutamine. Fishes may be subjected to increased ammonia levels in the environment due, for instance, to sewage waste in freshwater systems. Elevated enviromental ammonia levels will reduce excretion and result in a net uptake of ammonia by the fish and this accumulation of ammonia in the tissues is deleterious to the fish.

Ammonia production and utilization

In fishes, ammonia and urea are the major nitrogenous endproducts, with ammonia comprising at least 80% of nitrogen excretion in most teleosts (Smith 1929; Wood 1958). The major pathway for the production of ammonia is through the transamination of various amino acids (Forster and Goldstein 1969; Watts and Watts 1974). The primary site for ammonia production is probably the liver (Pequin and Serfaty 1963), but the necessary en-

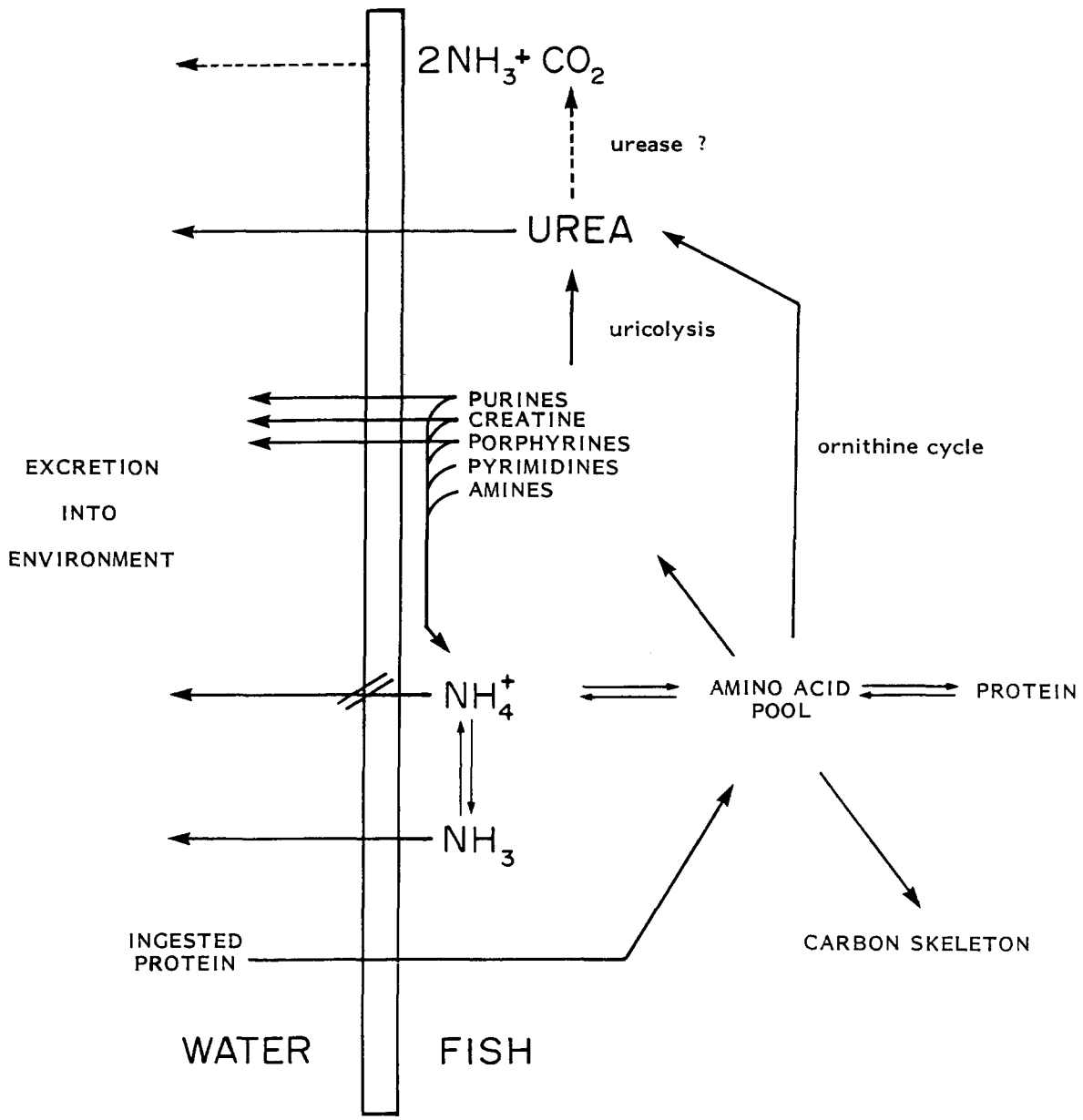

Fig. 1. Metabolic pathways of ammonia production and utilization in fish. It is not clear whether urease is present in fish, but some ammonia may be formed via this pathway.

zymes have also been located in the kidneys, gills, and skeletal muscle tissue (Goldstein and Forster 1961; Walton and Cowey 1977; McBean *et al.* 1966).

Amino acids in excess of those required for protein sythesis are converted to ammonia in the liver. Figure 1 illustrates the sources and fate of ammonia in fishes. Transaminases in the liver convert amino acids and alpha-ketoglutarate to form glutamate, and the accompanying alpha-keto acid. In the subsequent step, glutamate is oxidized to yield ammonia, NADH, and alpha-ketoglutarate (Forster and Goldstein 1969; Watts and Watts 1974). Ammonia is also produced by the deamination of adenylates

in fish muscle (Driedzic and Hochachka 1978) and gill tissue (Payan 1978). The quantitative importance of muscle ammoniogenesis to total ammonia excretion depends on the activity level of the animal, increasing with increasing workload (Suyama *et al.* 1960; Fraser *et al.* 1966; Driedzic and Hochachka 1976). Furthermore, the capacity for anaerobic ammonia production varies among species, with goldfish (*Carassius auratus*) showing remarkable ability to withstand hours of anoxia with no change in ammonia excretion rates (van den Thillart and Kesbeke 1978).

Under aerobic conditions in resting fish, most of the ammonia is produced in the liver, but during anoxia (Mathur 1967; van Waarde *et al.* 1982; van Waarde and De Wilde-Van Berge Hennegouwen 1982) liver production is reduced and ammonia production is maintained by muscle proteolysis. During exhaustive exercise deamination of adenylates in fish muscle becomes a major source of ammonia production (T. Mommsen and P. Hochachka unpublished data) with most of the ammonia being utilized rather than excreted during the recovery period. Mommsen and Hochachka (unpublished data) could account for the decrease in the muscle adenylate pool by the increase in IMP and NH_4^+; they suggested that the accumulated IMP was subsequently utilized to form AMP, using aspartate synthesized within the muscle from ammonia and fumarate/malate. The elevation of muscle ammonia, along with a reduction in ATP, stimulates phosphofructokinase and maintains glycolytic flux in the face of a reduction in muscle pH during exhaustive exercise (Dobson 1986).

Ammonia toxicity can be ameliorated by the formation of less toxic compounds, namely glutamine and urea. Long term exposure to elevated ambient ammonia levels had little effect on urea excretion by rainbow trout but caused a rapid and significant increase in urea excretion by goldfish (Olson and Fromm 1971). Levi *et al.* (1974) recorded high levels of glutamine in the brain of goldfish and found that brain glutamine levels increased with ambient ammonia concentrations. Webb and Brown (1976) found high glutamine synthetase activity in the brains of teleosts and elasmobranchs, and this may be important in protecting the brain from sudden surges in ammonia concentration. Walton and Cowey (1977) were able to detect glutaminase activity in the gills of rainbow trout (*Salmo gairdneri*), but were unable to measure any *in vivo* utilization of glutamine by the gills.

Ammonia can be converted, through carbamyl phosphate, to urea either via purines (uricolysis) or via the ornithine cycle. The enzymes required for uricolysis have been found in most fishes (Forster and Goldstein 1969; Watts and Watts 1974), but Florkin and Duchateau (1943) were unable to detect any activity of uricolytic enzymes in the cyclostome, *Lampetra*. The ratio of urea production via the ornithine cycle to production via uricolysis is about 100 to 1 in elasmobranchs and dipnoi, whereas in teleosts most of the urea is formed via uricolysis (Gregory 1977).

Ammonia distribution

NH_3 is a polar substance which binds a proton to form ammonium ion in aqueous solution, as described by the reaction:

$$NH_3 + nH_2O \rightleftharpoons NH_3 \cdot nH_2O \rightleftharpoons$$
$$NH_4^+ + OH^- + (n-1)H_2O$$

The ammonia reaction rate in water is extremely rapid, with the interconversion of NH_4^+ to NH_3 having a half time of less than 50 msec (Stumm and Morgan 1981). The NH_3 fraction increases with increasing pH and temperature but decreases with increases in ionic strength of the solution (Thurston *et al.* 1979). The combined concentrations of ammonia gas (NH_3) and ammonium ions (NH_4^+) in solution will be referred to as total ammonia.

Cameron and Heisler (1983) found that ammonia was slightly more soluble in fish plasma than in water, and they also constructed a nomogram to describe the effects of ionic strength and temperature on the pK of the NH_3/NH_4^+ reaction (see also Kormanik and Cameron 1981a; Boutilier *et al.* 1984). At the pH of fish tissues nearly all of the ammonia will be as NH_4^+ because the pK is 9.75 at 10°C. The Henderson-Hasselbalch equation describes this relationship:

$$pH = pK + \log([NH_3]/[NH_4^+])$$

According to the pK of ammonia, acidification of a given compartment lowers the NH_3 concentration relative to that of NH_4^+, resulting in the passive influx of NH_3. NH_3 diffuses rapidly across biological membranes down its partial pressure gradient (Klocke *et al.* 1972) at about the same rate as CO_2 (Cameron and Heisler 1983). Biological membranes are generally less permeable to ionized compounds, such as NH_4^+, which has a large hydrated diameter and net charge (Jacobs 1940). Total ammonia concentration will increase in a low pH compartment as NH_3 diffuses into that compartment, where it will be immediately protonated and then trapped as NH_4^+. If the membrane is semipermeable (i.e., NH_4^+ flux is negligible compared to NH_3), then NH_4^+ will be partitioned between compartments in proportion to pH, as the system approaches equilibrium. Thus, the distribution of ammonia in various body compartments should be pH-dependent, and can be described by the equation:

$$\frac{[\text{intracellular}]}{[\text{extracellular}]} = \frac{1 + 10^{(\text{pKa} - \text{pH intracellular})}}{1 + 10^{(\text{pKa} - \text{pH extracellular})}}$$

Furthermore, because the pH of intracellular compartments is considerably lower than extracellular fluid, one would expect intracellular ammonia stores to be relatively high (Fig. 2). For example, Wright (unpublished data) found that the distribution of ammonia in resting rainbow trout blood was determined by the pH gradient between plasma and erythrocytes. Moreover, total ammonia in the erythrocyte was about three times more concentrated relative to the plasma. In general, however, very little is known about the differences in total ammonia content in various tissues in fish and how the distribution of ammonia is effected by changing physiological conditions.

Holeton *et al.* (1983) have determined changes in blood pH and ammonia excretion, Milligan and Wood (1986) in muscle and blood pH, whereas Mommsen and Hochachka (unpublished data) have measured blood and whole muscle total ammonia levels, following exhaustive exercise in rainbow trout. The studies of Holeton *et al.* (1983) and

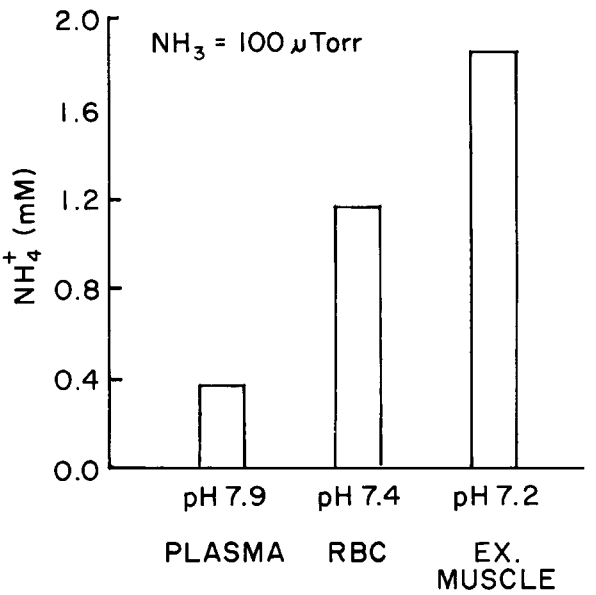

Fig. 2. Calculated values for $[NH_4^+]$ in plasma, erythrocytes (RBC) and exercising muscle (EX.MUSCLE), assuming that blood plasma NH_3 (100 μ Torr) is in equilibrium with erythrocytes and muscle. Blood data typical of rainbow trout at 10°C, muscle pH assumed to be 7.2.

Milligan and Wood (1986) were carried out at 15°C whereas the Mommsen and Hochachka study was at 8°C. If we assume that similar blood and muscle ammonia and pH changes occur at 8 and 15°C, then combining the data allows the calculation of blood NH_3 levels using the Henderson-Hasselbalch equation. Mommsen and Hochachka reported ammonia levels in plasma and in muscle homogenates. We first calculated whole blood ammonia levels from the plasma values reported by Mommsen and Hochachka, using plasma and red blood cell relationships for ammonia established for trout blood (Wright unpublished data). We calculated musle ammonia concentrations from the data of Mommsen and Hochachka and intracellular fluid volumes reported by Milligan and Wood (1986) and assuming extracellular fluid had the same ammonia concentration as whole blood (Table 1). If we then assume various NH_3 gradients between muscle and blood, it is possible to calculate the muscle pH from the NH_3/NH_4^+ ratio within muscle (Table 1). The estimated muscle pH at rest is similar to that reported by Milligan and Wood

Table 1. Changes in muscle and blood pH, NH_3, and total ammonia levels in rainbow trout at rest and following exhaustive exercise

A. Calculation of muscle pH assuming no NH_3 gradients between muscle and blood.

	Blood plasma			Muscle	
Time (h)	pHe[a]	ΣNH_3[b] (μmol/l)	NH_3 (μmol/l)	ΣNH_3[b] (μmol/l)	pH_i (calculated)
Rest	7.76	210	3.13	672	7.25
0	7.45	520	3.83	9616	6.18
0.5 – 0.58	7.55	610	5.64	6832	6.50
1	7.50	650	5.36	6439	6.50
2	7.55	550	5.09	4689	6.62
4	7.80	290	4.73	4742	6.58

B. Calculation of muscle pH assuming NH_3 gradients between muscle and blood of between 0 and 15 μM NH_3.

	NH_3 gradient muscle-to-blood				
Time (h)	0	5	10	15	μmol/l NH_3
Rest	7.25	–	–	–	
0	6.18	6.54*	6.74	6.87	
0.5 – 0.58	6.50	6.77	6.94	7.06	
1	6.50	6.79	6.96	7.08	
2	6.62	6.91	7.09	7.21	
4	6.58	6.89	7.07	7.20	

pK of ammonia at 15°C = 9.58 (Boutilier *et al.* 1984)
[a]Data from Milligan and Wood (1986)
[b]Data from T. Mommsen and P. Hochachka (unpublished)
*Corresponds to pH_i data from Dobson (1986)

(1986) measured by DMO distribution. This indicates that, at least under resting conditions, the assumption of equilibration of NH_3 between muscle and blood may be reasonable.

Holeton *et al.* (1983) observed over a four-fold increase in ammonia excretion in the first few hours following exhaustive activity and then a sharp reduction in excretion below control levels at 4 h after exhaustive exercise. Mommsen and Hochachka (unpublished data) observed a 14-fold increase in white muscle ammonia levels which rapidly decreased during the next hour. Muscle ammoniogenesis results in an elevation in blood ammonia levels and is undoubtedly the cause of the increased excretion (Mommsen and Hochachka unpublished data; Turner *et al.* 1983). The high rate of ammonia production during, and excretion following, exhaustive exercise, however, may result in NH_3 gradients between muscle and blood. If we assume a 5 μM gradient for NH_3 between muscle and blood immediately after exhaustive exercise, then muscle pH will be 6.54 (Table 1), a value similar to that reported by Dobson (1986) for rainbow trout white muscle after exercise. Thus the development of an ammonia gradient between muscle and blood will have the effect of ameliorating the pH change seen in muscle immediately following exhaustive exercise. The rate of loss of ammonia from muscle (Mommsen and Hochachka unpublished data) and the fish (Holeton *et al.* 1983) is much reduced 2 h after exhaustive exercise and NH_3 levels may equilibrate once more between muscle and blood. If so, then the change in muscle pH, following exhaustive exercise, may have the profile seen in Fig. 3. These

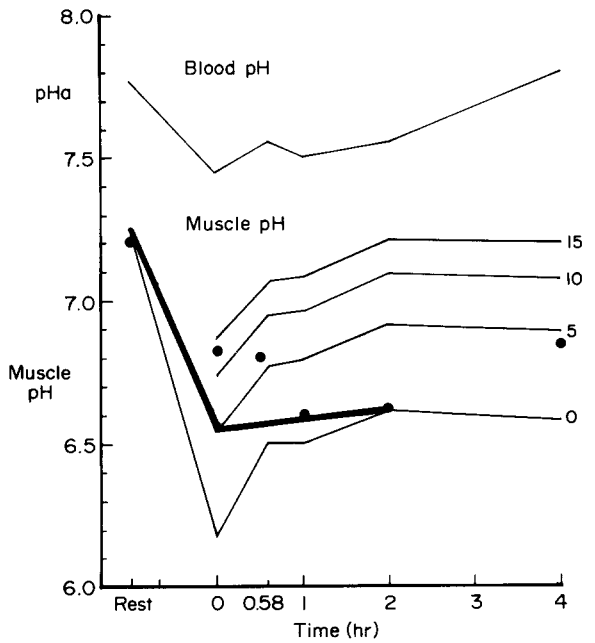

Fig. 3. Measured changes in blood pH (Milligan and Wood 1986) and calculated changes in muscle pH following exhaustive exercise in rainbow trout (data presented in Table 1); the thick line represents possible changes in pH_i in muscle (see text for further explanation). The dots are the pH_i values for muscle reported by Milligan and Wood (1986).

calculated pH_i values for muscle immediately after exercise are lower than those reported by Milligan and Wood (1986). Their measurements depend on complete equilibration of DMO between the intracellular and extracellular compartments, which is unlikely immediately following exercise when pH_i is changing rapidly. The pH_i value for muscle at 4 h (Fig. 3) reported by Milligan and Wood (1986) when combined with the data of Mommsen and Hochachka, would indicate that NH_3 gradients are developing between muscle and blood during this post exercise period. The rate of decrease in muscle ammonia following exhaustive exercise observed by Mommsen and Hochachka was in excess of the excretion rate reported by Holeton *et al.* (1983); therefore, much of the ammonia must be utilized by the muscle, as suggested by Mommsen and Hochachka.

Ammonia is produced in the mitochondria, which have a higher pH than the cytosol. Given the very high surface-to-volume ratio of mitochondria, it is difficult to envision any substantial NH_3 gradients between the mitochondria and the cytosol. The NH_4^+ levels within the mitochondria, however, will be below those in the cytosol because of the much higher mitochondrial pH. Thus ammonia production probably occurs in somewhat lower concentrations of NH_4^+ than seen in the more acidic cytosol.

Ammonia excretion

The gills are the major site of ammonia excretion in fishes, but smaller quantities of ammonia may also be eliminated by the kidneys (Edwards and Condorelli 1928; Grollman 1929; Fromm 1963; Maetz 1972) and skin (Morii *et al.* 1978). Although the majority of branchial ammonia excretion represents clearance from the blood, gill metabolism may contribute between 20% (Payan and Matty 1975) and 5–8% (Cameron and Heisler 1983) to net ammonia excretion.

The excretion of ammonia by fishes is variable, depending on the state of the animal, the environmental conditions and the species. Ammonia excretion tripled in sockeye salmon (*Oncorhynchus nerka*) following daily feeding (Brett and Zala 1975), but remained low and unchanging during starvation (Brett and Zala 1975; Guerin-Ancey 1976a). In freshwater fishes ammonia excretion increases in response to exercise (Sukumaran and Kutty 1977; Holeton *et al.* 1983), long-term acid exposure (McDonald and Wood 1981; Ultsch *et al.* 1981), hypercapnia (Claiborne and Heisler 1984), and NH_4Cl infusion (Hillaby and Randall 1979). In contrast, increased levels of environmental ammonia (Guerin-Ancey 1976b) and short-term exposure to acid or alkaline water (Wright and Wood 1985) cause a decrease in ammonia excretion. It is not known if these changes in excretion reflect changes in the rate of total ammonia production or in the total ammonia content of the body. The total ammonia content of a fish is likely to be the equivalent of the ammonia excreted in about 2 h, most of the ammonia being in the tissues with lower pH, like muscle. Blood levels are around 0.2–0.3 mmol/l, but muscle at a lower pH may have con-

centrations up to 1 mmol/l, thus a 1-kg fish may contain about 0.5–0.7 mmol/l of ammonia and have an excretion rate of about 0.3 mMol/l per hour.

Ammonia excretion by the dogfish in seawater is unaffected by temperature change, exercise, hyperoxia, hypercapnia, or the infusion of either HCl or $NaHCO_3$ or anything that induces acid-base stress (see Heisler 1984, for review). This is surprising because many of these changes affect pH and therefore would be expected to alter the ammonia content of body compartments and consequently ammonia excretion.

It is perhaps surprising that ammonia excretion declines (Brett and Zala 1975) in the face of an elevation in blood ammonia during starvation (Hillaby and Randall 1979; Morii *et al.* 1978). Blood ammonia concentrations also rise both with increases in temperature (Fauconneau and Luquet 1979) and with higher ammonia concentrations in the water (Fromm and Gillette 1968; Thurston *et al.* 1984). Exposure of fishes either to air (Gordon 1970) or to increased ammonia levels in water (Fromm 1970; Guerin-Ancey 1976b) raises blood ammonia levels and reduces ammonia excretion. Unlike the above studies, Buckley *et al.* (1979) found no change in blood total ammonia when coho salmon (*Oncorhynchus kisutch*) were exposed to elevated ammonia concentrations in the environment. They did observe, however, a signficant rise in plasma sodium, indicating some coupling between sodium uptake and ammonia excretion (see below).

Mechanisms of ammonia excretion

The study of ammonia movement is complicated by the fact that, with present analytical techniques, it is impossible to distinguish between the transfer of a molecule of NH_3 plus a H^+ ion from the transfer of a NH_4^+ ion. Only indirect evidence, therefore, can be obtained as to the relative NH_3 and NH_4^+ movements across the gill epithelium.

Three possible mechanisms of ammonia excretion have received the most attention: passive NH_3 flux, ionic exchange of NH_4^+ for Na^+, and passive NH_4^+ flux. There seems to be little doubt that a significant pathway for branchial ammonia excretion is by the passive diffusion of NH_3 down its partial pressure gradient. Changes in the NH_3 partial pressure gradient are positively correlated with changes in net ammonia excretion in freshwater catfish (Kormanik and Cameron 1981b), and rainbow trout (Cameron and Heisler 1983; Wright and Wood 1985). Ammonia entry into the fish has also been shown to be dependent on the NH_3 gradient (Wuhrmann *et al.* 1947; Wuhrmann and Woker 1948; Fromm and Gillette 1968).

The excretion of NH_4^+ is strongly coupled to the movement of other ions. Many studies have attempted to link the transepithelial exchange of Na^+ uptake to NH_4^+ efflux. Although there is considerable indirect evidence for the presence of a coupled ionic exchange mechanism under certain conditions (Maetz and Garcia-Romeu 1964; Maetz 1973; Payan and Maetz 1973; Evans 1977, 1980; Payan 1978; Girard and Payan 1980; Wright and Wood 1985), the ubiquity and stoichiometry of this exchange remain controversial. While Na^+ influx can be monitored with isotopes, it is difficult to determine NH_4^+ efflux. Investigators have attempted to quantify the relationship between Na^+ uptake and NH_4^+ excretion by manipulating Na^+ levels in the environmental water, by pharmaceutical inhibition of the Na^+ influx mechanism, or by loading the fish with ammonia.

In goldfish (Maetz 1973) and in irrigated gills of rainbow trout (Kirschner *et al.* 1973), Na^+ influx was best correlated with the sum of H^+ and NH_4^+ ion efflux. The possibility of a Na^+ uptake carrier coupled to either NH_4^+ or H^+ appears likely in other fishes as well (Kerstetter *et al.* 1970; Payan and Maetz 1973; Evans 1977). In perfused heads of trout, Na^+ uptake was tightly coupled to NH_4^+ efflux (Payan *et al.* 1975; Payan 1978). Wright and Wood (1985) demonstrated that in intact trout the rate of ion exchange was influenced by external water pH, increasing from no exchange at pH 4 to maximal rates at pH 8. The relationship between Na^+ and NH_4^+ was one-to-one at water pH less than 8. Wright and Wood found, however, that a significant amount of ammonia was eliminated by diffusion, and only when NH_3 was subtracted

from total ammonia efflux was the NH_4^+/Na^+ exchange evident. In carp (de Vooys 1968), and skate (*Raja erinacea*) (Evans *et al.* 1979), however, ammonia excretion was unaffected by a reduction in environmental Na^+ levels. Cameron and Heisler (1983) found that under resting conditions diffusive movement of NH_3 could account for ammonia excretion in trout, but when the ammonia gradient was reserved and directed inwards, a NH_4^+/Na^+ exchange could be operating to counterbalance the diffusive uptake of NH_3 from the water. If Cameron and Heisler's theory is correct, then it would explain the unchanging blood ammonia but increased Na^+ levels in coho salmon exposed to elevated water ammonia (Buckley *et al.* 1979).

The $Na^+/NH_4^+(H^+)$ exchange is probably located on the epithelial apical membrane. Either acid conditions or amiloride in the water inhibit Na^+ influx across the gills and both these conditions result in a reduction of ammonia excretion (Wright and Wood 1985).

Ammonium ion can displace potassium in many membrane processes, for example in squid giant axon (Binstock and Lecar 1969), and this is the probable reason that elevated ammonia causes convulsions in so many vertebrates. In various aquatic animals it is possible that NH_4^+ can substitute for potassium in ouabain-sensitive sodium/potassium exchange (cf. Payan *et al.* 1975; Girard and Payan 1980; Towle and Taylor 1976; Towle *et al.* 1976; Mallery 1979). NH_4^+ ions will substitute for K^+ ions across the epithelial basolateral border (Karnaky *et al.* 1976; Richards and Fromm 1970; Shuttleworth and Freeman 1974), but the importance to net ammonia transfer in freshwater fishes is unknown.

The passive movement of NH_4^+ down its electrochemical gradient may also contribute to net ammonia excretion (Claiborne *et al.* 1982; Goldstein *et al.* 1982). Lipid membranes are relatively impermeable to cations (Jacobs 1940) and because respiratory epithelial cells of freshwater fishes are joined by tight junctions (cf. Girard and Payan 1980), it appears unlikely that NH_4^+ diffusion is of quantitative importance (cf. Kormanik and Cameron 1981a). Indeed, Wright and Wood (1985) found a negative correlation between ammonia excretion and the NH_4^+ concentration gradient in rainbow trout exposed to five different water pH regimes. Although it appears that NH_4^+ diffusion may be of minor importance, simultaneous measurements of the electrical and chemical gradient have not been made and are necessary before conclusions can be drawn.

Branchial excretion and water pH changes

Much of the research on the mechanisms of ammonia excretion by aquatic animals involves the calculation of blood-to-water ammonia gradients across the gill based on blood and water pH and total ammonia concentration measurements (Maetz 1972, 1973; Cameron and Heisler 1983; Wright and Wood 1985). The difficulty with this approach is that the exact pH of water and blood on either side of the gill epithelium is not known. The pH of afferent and efferent blood can be measured and the mean of these two may be used as an approximation of the mean pH of blood in the gills. Water pH is equally difficult to determine. Water flow is laminar through the mouth and over the gills. Boundary layers are undoubtedly present next to the mucous layer covering the gill surface since water flow through the gill channels in relation to their dimensions results in Reynolds numbers that have been reported by Hughes (1984) to be very small (>10). Sampling within this unstirred layer is virtually impossible with present techniques and is confounded by the possibility that boundary layers may disappear and reform with intermittent movements of the gill filaments and/or cyclic ventilatory movements. Concentrations gradients will exist across the boudary layer as material is added by the fish to the gill water. Moreover, concentration gradients will exist in the longitudinal plane, that is, as water proceeds along the respiratory epithelium branchially excreted molecules will become more concentrated in the water. Fishes excrete CO_2, NH_3, NH_4^+, H^+, and HCO_3^- across their gills. All of these molecules will affect the pH of water near the gill surface, and the magnitude of the effect will depend on the relative rates of excretion, the rates of chemical equilibrium, the water buffering capa-

city, the water pH, and the rate of water flow past the gills.

The elimination rate of CO_2 far exceeds that of other branchially excreted molecules, and if the CO_2-hydration reaction is rapid relative to water transit time of the gills (100–400 msec; Randall 1982a, 1982b), water will be acidified near the gill surface. The uncatalysed rate of CO_2 hydration is on the order of minutes (Kern 1960) at typical fish water pH and temperatures. Carbonic anhydrase (CA), the enzyme responsible for catalysing CO_2 hydration and dehydration reactions, is concentrated in the apical regions of branchial epithelial cells (Dimberg *et al.* 1981) and the cytoplasm and mucous granules of gill goblet cells (Lacy 1983) in teleosts. The thin layer of mucous associated with the external surface of the gill epithelium, therefore, may contain CA. Wright *et al.* (1986) investigated the pH of interlamellar water of trout and concluded that acidification of expired water was due to the catalysed conversion of excreted CO_2 to HCO_3^- and H^+ at the gill surface. They found that fish mucus contained CA activity, thus the mucous boundary layer will be acidified due to the hydration of CO_2 excreted across the gills. This boundary layer is probably more acid than the interlamellar water (Wright *et al.* 1986).

In starved fish, branchial ammonia excretion is approximately 10% of O_2 uptake (Heisler 1984; Brett and Zala 1975; van Waarde 1983). The reaction of NH_3 with water is considered instantaneous (Eigen 1964), and NH_3 excretion will result in the immediate elevation of water pH at the gill surface. Alternatively, NH_4^+ excretion will have a negligible effect on water pH, inasmuch as water is rarely as alkaline as pH 9.5, the pK of the NH_3/NH_4^+ reaction. One would predict that branchial ammonia excretion would be influenced by alterations in carbon dioxide excretion across the gills of fish in certain freshwater habitats. In poorly buffered freshwaters, CO_2 excreted by the fish acidifies intralamellar water (Wright *et al.* 1986) and any change in the CO_2 excretion pattern would alter the blood to water pH gradient and, thus, ammonia gradients across the gills (Fig. 4).

The gill epithelium is permeable to protons (McWilliams and Potts 1978) and H^+ efflux (in exchange for Na^+ or NH_4^+) will alter the pH of water instantaneously, but net excretion or uptake of H^+ will be very small under most conditions. It should be noted that H^+ efflux cannot be distinguished from OH^- or HCO_3^- uptake, although the effect on water pH will be the same.

Wright and Wood (1985) measured ammonia excretion in rainbow trout exposed to a variety of water pH conditions, and they found that Na^+ influx was zero at pH 4.1. If it is assumed that under these conditions NH_4^+/Na^+ exchange is also zero, then all ammonia excreted must have been due to NH_3 diffusion. The NH_3 gradient was calculated from measurements of pH and total ammonia content in water and blood. The calculated permeation coefficient for ammonia diffusion across the trout gill was 65% of that calculated by Cameron and Heisler (1983). The NH_3 excretion was then calculated for trout under other conditions using this permeation coefficient and the estimated NH_3 gradients. The NH_4^+ excretion was then determined by subtracting the NH_3 excretion from the measured total ammonia excretion. Wright and Wood (1985) concluded that NH_4^+ excretion was strongly coupled to Na^+ uptake, and the ammonia was excreted both as NH_3 and NH_4^+. Randall and Wright (1986) reviewed the subject and based on a further analysis of the data of Wright and Wood (1985) concluded that NH_4^+ excretion must be pH dependent if coupled to Na^+ influx because Na^+ uptake was maximal between pH 7 and 8 but declined at higher and lower pH levels in the water, approaching zero at pH 4 and 11. Randall and Wright (1986) also concluded that in higher pH waters, water pH in the gill boundary layer was 0.1 pH units less than in the bulk medium. The acidification of water at the gills below that in the bulk phase is due to the catalysed hydration of CO_2, which will be dissociated to a greater extent at higher pH (Wright *et al.* 1986) (pK = 6).

Carbon dioxide in the water affects ammonia toxicity; if CO_2 levels are raised, total ammonia toxicity is decreased (Alabaster and Herbert 1954). Carbon dioxide causes a fall in pH and decreases the proportion of NH_3 in solution. In the environment, the un-ionized form has a greater toxic effect because ammonia must enter the fish to exert its

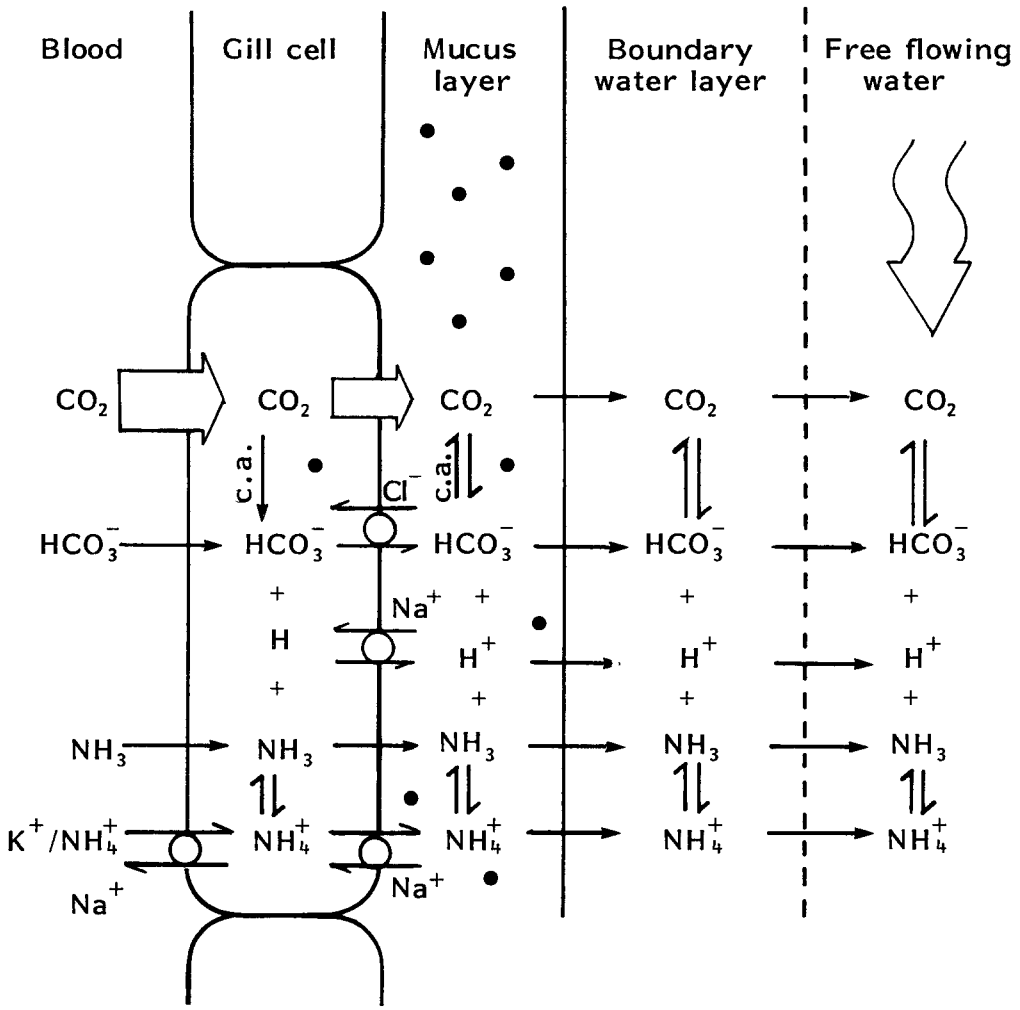

Fig. 4. Possible interactions between carbon dioxide and ammonia in the gills of fish and interlamellar water. The size of the arrows indicates the relative excretion rate of that molecule. •, carbonic anhydrase.

toxic action, and lipid membranes are much more permeable to NH_3 (Wuhrmann *et al.* 1947; Wuhrmann and Woker 1948; Thurston *et al.* 1981). Thus the reduction in NH_3 associated with the fall in water pH caused by the rise in CO_2 decreased total ammonia toxicity. Lloyd and Herbert (1960) found, however, that although total ammonia toxicity was reduced at high CO_2 levels, the inverse was true when considering NH_3 alone. More NH_3 is required in low CO_2-high pH water to exert the same toxic effect as seen in fishes in high CO_2-low pH water. The explanation presented by Lloyd and Herbert (1960) for the decreased toxicity of NH_3 in low CO_2 water was that CO_2 excretion across the gills would reduce pH and therefore the concentration of NH_3 in water flowing over the gills. This is consistent with the conclusions of Randall and Wright (1986) and Wright *et al.* (1986). Another possible explanation is that the blood pH of the fish also varied inversely with the CO_2 content of the water such that the total ammonia content of the blood decreases with the CO_2 content of the water. Elevated CO_2 levels were associated with a reduction in water pH in Lloyd and Herbert's experiments, and both these factors are known to reduce blood pH in fishes (Janssen and Randall 1975;

Randall *et al.* 1976; Heisler 1980). The fish in Lloyd and Herbert's experiments were exposed to water of different pH and CO_2 levels for 18 h. If hypercapnic compensation was incomplete, then the blood pH of these fish was probably inversely related to CO_2 levels in the water at the time of ammonia exposure. Blood pH will be an important determinant of blood total ammonia levels and this in turn is an important factor in its toxic action (Hillaby and Randall 1979). Blood pH probably decreased with increasing CO_2 levels in the water and this causes an increase in the blood total ammonia levels for a given NH_3 concentration. This could account for the differences in NH_3 toxicity observed. What is required is accurate measurements of pH in both blood and water and therefore NH_3 gradients across the gill epithelium.

Changes in gill ventilation and gill ammonia diffusing capacity may also affect ammonia excretion rates, however, there is almost no information on this aspect of ammonia excretion. Elevated ammonia levels cause an increase in gill ventilation in rainbow trout (Smart 1978). Hypercapnia (Wright, unpublished data), exposure to elevated environmental ammonia levels (D. MacKenzie, unpublished data), and exercise (calculated from the data of Holeton *et al.* 1983, and Mommsen and Hochachka unpublished data) all result in an increase in the gill diffusing capacity for ammonia. This increased diffusing capacity may be due to a more acidic boundary layer, which will trap NH_4^+ and enhance the blood to water NH_3 difference. Alternatively, it may be due to an increase in the NH_3 permeation coefficient of the gills, possibly related to an increase in circulating catecholamines. Blood catecholamines are known to be elevated in fish exposed to increased ammonia levels (Jeney *et al.* 1982).

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