

THE DISTRIBUTION OF AMMONIA AND H^+ BETWEEN TISSUE COMPARTMENTS IN LEMON SOLE (*PAROPHRYS VETULUS*) AT REST, DURING HYPERCAPNIA AND FOLLOWING EXERCISE

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Summary

The distribution of ammonia and [^{14}C]DMO was compared in white muscle, heart, brain, red cells and plasma of lemon sole (*Parophrys vetulus* Girard) at rest, during hypercapnia and following strenuous exercise. In red cells at rest, measured intracellular ammonia levels were equal to those predicted by the plasma to red cell pH gradient. Red cells are unusual in that hydrogen ions are passively distributed according to membrane potential (E_M), whereas in other tissues this is not the case. In white muscle, heart and brain under all experimental conditions, intracellular ammonia levels far exceed those predicted by transmembrane pH gradients. Calculated $E_{NH_4^+}$ values in these tissues are very close to published resting values of E_M . We conclude that, in lemon sole, NH_4^+ permeates cell membranes and that intracellular ammonia stores are not determined by transmembrane pH gradients.

Introduction

There is a substantial body of knowledge on the mechanisms of ammonia excretion across the gills of various fish (e.g. Maetz & Garcia-Romeu, 1964; Maetz, 1973; Evans, 1977; Payan, 1978; Cameron & Heisler, 1983; Wright & Wood, 1985), but little is known about ammonia stores in body tissues of fish. Ammonia is a weak base that exists in solution as ammonia (NH_3) and ammonium ions (NH_4^+). (The term ammonia or T_{amm} will be used to indicate the total ammonia concentration, while NH_4^+ and NH_3 will refer to ammonium ion and non-ionic ammonia, respectively.) With a pK of 9.7 at 10°C, the majority of

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ammonia in body fluids ($\text{pH} = 6.5\text{--}8.0$) will be in the ionized form, as NH_4^+ . Movement of ammonia between tissue compartments is thought to be primarily dependent on NH_3 partial pressure gradients (P_{NH_3}), because biological membranes are highly permeable to NH_3 (Castell & Moore, 1971; Klocke, Andersson, Rotman & Forster, 1972; Boron, 1980; Lockwood, Finn, Campbell & Richman, 1980; see also Good & Knepper, 1985). Despite the greater permeability of NH_3 , there is evidence that NH_4^+ electrochemical gradients also play a role in ammonia transfer across cell membranes (Thomas, 1974; Boron & DeWeer, 1976; Aickin & Thomas, 1977). Transfer of ammonium ions will depend on the diffusive permeability of the membrane for NH_4^+ , the availability of ion carriers in the membrane, and their affinity for NH_4^+ . A significant diffusive permeability to NH_4^+ is now recognized in many biological membranes (Schwartz & Tripolone, 1983; Arruda, Dytko & Withers, 1984; Evans & Cameron, 1986). Ammonium ions can substitute for K^+ in the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter (Kinne, Kinne-Saffran, Schutz & Scholermann, 1986) and in the Na^+,K^+ -ATPase pump (e.g. Post & Jolly, 1957; Skou, 1960; Robinson, 1970; Aickin & Thomas, 1977; Sorensen, 1981; Kurtz & Balaban, 1986), and for H^+ in the Na^+/H^+ ion exchange mechanism (e.g. Kinsella & Aronson, 1981; Aronson, 1983). Also, NH_4^+ can replace K^+ in nerve K^+ channels (Binstock & Lecar, 1969; Hille, 1973). Thus, it appears that transfer of ammonia between tissue compartments may involve both NH_3 and NH_4^+ movements.

In a closed system at equilibrium, the distribution of ammonia across biological membranes will be determined by the pH gradient across the membrane, as long as the effective permeability to NH_3 is much greater than that to NH_4^+ , and there is no chemical binding of the species. However, the greater the effective NH_4^+ permeability, the greater will be the influence of the electrical gradient on the equilibrium distribution. Living animals, especially ammoniotelic fish, are certainly not closed systems at equilibrium. Nevertheless, the assumption has often been made that the distribution of ammonia is largely a function of the intracellular to extracellular pH gradient, in both mammals (e.g. Visek, 1968; Pitts, 1973; Meyer, Dudley & Terjung, 1980; Mutch & Banister, 1983; Remesy, Demigne & Fafournoux, 1986) and fish (Randall & Wright, 1987; Dobson & Hochachka, 1987).

In a recent study, Wright, Wood & Randall (1988) investigated the distribution of ammonia between plasma and red cells of rainbow trout, *Salmo gairdneri*, and found that ammonia was passively distributed according to transmembrane pH gradients under normal conditions both *in vitro* and *in vivo*. Red cells are unusual compared with many other tissues in that the hydrogen ion distribution is passive and follows the membrane potential over a range of pH values (Heming *et al.* 1986; Heming, Randall & Mazeaud, 1987; Nikinmaa, Steffensen, Tufts & Randall, 1987; Lassen, 1977). Thus, it was impossible with the trout data to determine whether the distribution of ammonia was solely dependent on pH gradients or if electrical gradients were also important. To distinguish between the two it is necessary to look at tissues other than red cells, where H^+ is not passively distributed (see Roos

& Boron, 1981). If ammonia movements across cell membranes are solely dependent on P_{NH_3} gradients, that is, NH_4^+ movements are negligible, then one would expect the distribution of ammonia to follow transmembrane pH gradients. However, if NH_4^+ is permeant, then one would expect the ammonia distribution to follow electrochemical NH_4^+ gradients. In the present study, we investigated the distribution of ammonia and H^+ across cell membranes in white muscle, heart and brain, as well as red blood cells. Measurements were made at rest, to establish the situation under conditions as close to steady state as possible, and under two treatments designed to perturb the normal acid-base status by very different mechanisms – respiratory acidosis (hypercapnia) and metabolic acidosis (exhaustive exercise). Lemon sole, *Parophrys vetulus*, were chosen for these experiments as flatfish have a discrete white muscle mass uncontaminated by pink or red fibres, in contrast to salmonids (Mosse, 1979), and because their blood pH is somewhat lower (by about 0.3 units) than in trout. Instructive comparisons could therefore be made with our previous study on ammonia distribution in trout blood (Wright *et al.* 1988), as well as with a previous theoretical analysis of ammonia distribution in trout muscle (Randall & Wright, 1987).

Materials and methods

Experimental animals and surgical techniques

Lemon sole (*Parophrys vetulus*) weighing 292 ± 14 g (mean \pm 1 s.e.m.) were collected by two 15-min otter trawls in Barkley Sound, British Columbia. Fish were held at the Bamfield Marine Station in a sandy bottomed, outdoor, fibreglass tank supplied with flow-through sea water (pH approx. 7.8, salinity approx. 31‰, temperature 11°C) for at least 7 days before experimentation. Sole were not fed during captivity, so that variations in body ammonia levels due to feeding history would be eliminated (Fromm, 1963; Brett & Zala, 1975).

Sole were fitted with caudal artery cannulae (PE 50) after anaesthetization in MS 222 (1:15 000), as described by Watters & Smith (1973). A small amount of oxytetracycline-HCl, a general antibiotic, was placed in the caudal incision before the wound was closed with surgical silk. After surgery, the sole were placed in small plastic chambers (approx. 8 l), covered with sand, and allowed to recover in flowing sea water for 72 h prior to experimentation. To re-establish resting conditions, flatfish require relatively long recovery periods and the availability of sand in which to bury (Wood, McMahon & McDonald, 1979).

Experimental protocol

Three experimental conditions were studied. (i) A control in which fish were subjected to unaltered, aerated sea water (pH 7.8) prior to sampling. (ii) A second group of fish were placed in a seawater bath equilibrated to approximately 1% CO_2 (pH 6.7) for 30 min before sampling to alter rapidly the extracellular and intracellular pH *via* high P_{CO_2} levels (respiratory acidosis). (iii) The third group of fish were transferred to a large plastic tank and chased for 20 min. The aim of

subjecting fish to exhaustive exercise was to induce a metabolic acidosis in both extracellular and intracellular compartments. These fish were allowed to recover for 30 min prior to sampling.

To determine intracellular pH (pHi) by the DMO (5,5-dimethyl-2,4-oxazolidinedione) distribution technique (Waddell & Butler, 1959), fish were injected with 1 ml kg^{-1} of $5 \mu\text{Ci ml}^{-1}$ [^{14}C]DMO (New England Nuclear, specific activity 50 mCi mmol^{-1}) and $20 \mu\text{Ci ml}^{-1}$ of the extracellular marker, [^3H]mannitol (New England Nuclear, specific activity $27.4 \text{ mCi mmol}^{-1}$) in Cortland saline (adjusted to 160 mmol l^{-1} NaCl) approximately 12 h prior to sampling (Milligan & Wood, 1986a,b).

At sampling, 2 ml of blood was withdrawn from each fish and replaced with an equal volume of saline. Blood was immediately analysed for pHe, pHi, plasma and whole blood total carbon dioxide content (C_{CO_2}), and haematocrit (Hct). Samples were appropriately fixed and stored for later determination of whole blood and plasma ammonia concentration (T_{amm}), whole blood lactate ([lactate]) and haemoglobin ([Hb]) concentrations and plasma and red cell water content. Less than 5 min after blood withdrawal, fish were removed from the water and the spinal cord was quickly severed with a scalpel. Epaxial white muscle samples were excised and a thin slice of tissue was immediately frozen with freeze-clamp tongs, cooled by immersion in dry ice chips. The frozen samples were stored on dry ice for later determination of intracellular T_{amm} and [lactate], whereas the unfrozen tissue samples were later analysed for [^{14}C]DMO and [^3H]mannitol. The heart ventricle was then quickly removed. Part of the ventricle was freeze-clamped for subsequent analysis of intracellular T_{amm} , while the remaining tissue was analysed for [^{14}C]DMO, [^3H]mannitol and water content. Brain tissue was then collected after cutting through the cranium and, as before, part of the tissue was immediately frozen and later assayed for intracellular T_{amm} , whereas the rest of the tissue was reserved for determination of [^{14}C]DMO, [^3H]mannitol and water content. The entire tissue excision and freeze-clamping procedure took approximately 1–2 min. At the end of this procedure, large samples of white muscle tissue were taken for water content analysis.

Analytical techniques and calculations

Arterial pHe was measured immediately upon collection using a Radiometer microelectrode (type E5021) maintained at 11°C and linked to a Radiometer PHM71 acid–base analyser. Plasma and whole blood C_{CO_2} were measured on $50\text{-}\mu\text{l}$ samples by the technique of Cameron (1971), using a Radiometer E5036 CO_2 electrode and the same acid–base analyser. Plasma P_{CO_2} and $[\text{HCO}_3^-]$ were calculated by standard manipulations of the Henderson–Hasselbalch equation and tabulated values for pK' and α_{CO_2} (Boutilier, Heming & Iwama, 1984). Red cell pHi was directly measured by the freeze–thaw technique of Zeidler & Kim (1977) using the same microelectrode assembly. The freeze–thaw method was used instead of the DMO method to measure red cell pHi, because the former technique is a more direct method for pHi determination of isolated cells, it is a

simpler method, and there are no significant differences between red cell pHi values from the two techniques in fish blood (Milligan & Wood, 1985). Plasma and red cell water content were calculated by drying to constant weight in an oven at 100°C.

Lactate levels were assayed enzymatically (L-lactic dehydrogenase/NADH method, Sigma reagents) after deproteinizing 100 µl of whole blood in 200 µl of 8% HClO₄, or homogenizing 100 mg of white muscle in 1 ml of 8% HClO₄ with a glass homogenizer (see Turner, Wood & Clark, 1983). Muscle intracellular [lactate] was expressed per litre of intracellular fluid (ICF) as outlined for muscle intracellular T_{amm} below (equation 9). Haematocrit was determined by centrifugation (5000g for 5 min) and [Hb] using the cyanmethaemoglobin method of Blaxhall & Daisley (1973) using Sigma reagents.

Whole blood and plasma samples (250 µl) were assayed for T_{amm} after deproteinization in 200 µl of iced 8% HClO₄. Samples were then centrifuged and the supernatant was neutralized with saturated Tris buffer. T_{amm} of this neutralized supernatant was measured by the glutamate dehydrogenase enzymatic assay (Kun & Kearney, 1971). Internal ammonia standards were routinely made in whole blood because it was found that distilled water standards gave different results from internal standards. Whole blood internal standards were found to give the same results as plasma internal standards and, therefore, whole blood was used as the reference medium. Plasma T_{amm} was corrected for water content and red cell T_{amm} was calculated using the following formula:

$$\text{red cell } T_{\text{amm}} = \frac{\text{whole blood } T_{\text{amm}} - [(1 - \text{Hct}/100) \times \text{plasma } T_{\text{amm}}]}{\text{Hct}/100} \quad (1)$$

Red cell T_{amm} was then corrected for red cell water content and the final concentration was termed 'measured' red cell T_{amm} to differentiate it from the 'predicted' red cell T_{amm} calculated below.

Plasma NH₃ concentration ([NH₃]) was calculated by the following manipulation of the Henderson-Hasselbalch equation, using pK values given by Cameron & Heisler (1983):

$$\text{plasma } [\text{NH}_3] = \frac{\text{plasma } T_{\text{amm}} \times \text{antilog}(\text{pHe} - \text{pK})}{1 + \text{antilog}(\text{pHe} - \text{pK})} \quad (2)$$

Intracellular [NH₃] could also be calculated from red cell T_{amm} and pHi using a similar equation:

$$\text{intracellular } [\text{NH}_3] = \frac{\text{intracellular } T_{\text{amm}} \times \text{antilog}(\text{pHi} - \text{pK})}{1 + \text{antilog}(\text{pHi} - \text{pK})} \quad (3)$$

Ammonium ion concentration ([NH₄⁺]) in either compartment could be calculated as:

$$[\text{NH}_4^+] = T_{\text{amm}} - [\text{NH}_3] \quad (4)$$

Henry's law was applied to calculate the partial pressure of NH_3 in plasma and red cells (P_{NH_3} in μTorr : $1\text{ Torr} = 133.32\text{ Pa}$), using the appropriate solubility coefficient (α_{NH_3} ; Cameron & Heisler, 1983):

$$P_{\text{NH}_3} = \frac{[\text{NH}_3]}{\alpha_{\text{NH}_3}}. \quad (5)$$

If ammonia is distributed between plasma and red cells entirely according to the pH gradient then plasma $[\text{NH}_3]$ would equal red cell $[\text{NH}_3]$. To determine if this was the case, red cell pHi was predicted from measured plasma and red cell T_{amm} using the Henderson-Hasselbalch equation:

$$\text{predicted intracellular pHi} = \text{pK} + \log \frac{\text{intracellular } [\text{NH}_3]}{\text{intracellular } [\text{NH}_4^+]}, \quad (6)$$

where intracellular $[\text{NH}_3]$ was assumed to be equal to plasma $[\text{NH}_3]$. A predicted red cell T_{amm} could also be calculated by a further manipulation of the Henderson-Hasselbalch equation, employing the measured intracellular pH and again assuming that plasma $[\text{NH}_3]$ was equal to red cell $[\text{NH}_3]$:

$$\text{predicted red cell } T_{\text{amm}} = [\text{NH}_3] + \frac{[\text{NH}_3]}{\text{antilog}(\text{measured pHi} - \text{pK})}. \quad (7)$$

This predicted red cell T_{amm} was then compared with measured red cell T_{amm} determined from the direct measurement of plasma and whole blood ammonia (equation 1).

Intracellular muscle, heart and brain T_{amm} were determined in the following manner. Frozen tissue samples were weighed, deproteinized in iced 8% HClO_4 (1:20 dilution), and homogenized (Tekmar Tissumizer with microprobe head). Samples were centrifuged and the supernatant was extracted and neutralized with saturated Tris buffer. The enzymatic assay technique was identical to that described above for blood, except that muscle tissue extract provided the reference medium for internal standards. (There were no differences between internal standards made up in muscle, heart and brain tissue extracts.)

Muscle, heart and brain levels of $[\text{}^3\text{H}]$ and $[\text{}^{14}\text{C}]$ radioactivity were measured by digesting 50–150 mg of tissue in 2 ml of NCS tissue solubilizer (Amersham) for 1–2 days until the solution was clear. Extracellular fluid $[\text{}^3\text{H}]$ mannitol and $[\text{}^{14}\text{C}]$ DMO activity were determined in the same manner, except 100 μl of plasma was added to 2 ml of tissue solubilizer. The tissue digests were neutralized with 60 μl of glacial acetic acid and 10 ml of fluor (OCS; Amersham) was added. To decrease chemiluminescence, all samples were kept in the dark overnight before being counted on a liquid scintillation counter. Dual-label quench correction was performed using quench standards prepared from each type of tissue and the external standard ratio method (Kobayashi & Maudsley, 1974), as used by Milligan & Wood (1985, 1986a,b).

Tissue extracellular fluid volume (ECFV, ml g⁻¹) was calculated according to the equation:

$$\text{ECFV} = \frac{\text{tissue } [^3\text{H}]\text{mannitol (disints min}^{-1}\text{ g}^{-1})}{\text{plasma } [^3\text{H}]\text{mannitol (disints min}^{-1}\text{ g}^{-1})/\text{plasma H}_2\text{O (ml g}^{-1})} \cdot \quad (8)$$

Total tissue water was determined as previously described for plasma and whole blood samples. Intracellular fluid volume (ICFV, ml g⁻¹) was calculated as the difference between total tissue water and ECFV.

Intracellular T_{amm} was determined by first accounting for ammonia trapped in the extracellular fluid and then correcting for intracellular water, as follows:

$$\text{intracellular T}_{\text{amm}} = \frac{\text{tissue T}_{\text{amm}} - (\text{plasma T}_{\text{amm}} \times \text{ECFV})}{\text{ICFV}} \cdot \quad (9)$$

Tissue pHi was calculated according to the equation:

$$\text{pHi} = \text{pK}_{\text{DMO}} + \log \left\{ \frac{[\text{DMO}]_i}{[\text{DMO}]_e} \times (10^{\text{pHe} - \text{pK}_{\text{DMO}}} + 1) - 1 \right\}, \quad (10)$$

where pK_{DMO} was taken from Malan, Wilson & Reeves (1976) and [DMO]_e and [DMO]_i represent extracellular and intracellular [DMO], respectively. These two values were calculated as:

$$[\text{DMO}]_e \text{ (disints min}^{-1}\text{ ml}^{-1}) = \frac{\text{plasma } [^{14}\text{C}]\text{DMO (disints min}^{-1}\text{ g}^{-1})}{\text{plasma H}_2\text{O (ml g}^{-1})} \quad (11)$$

and

$$[\text{DMO}]_i \text{ (disints min}^{-1}\text{ ml}^{-1}) = \frac{\text{tissue } [^{14}\text{C}]\text{DMO (disints min}^{-1}\text{ g}^{-1}) - (\text{ECFV} \times [\text{DMO}]_e)}{\text{ICFV (ml g}^{-1})} \cdot \quad (12)$$

Tissue [NH₃], [NH₄⁺] and P_{NH₃} levels were calculated as described previously using equations 3, 4 and 5, respectively.

To determine whether the distribution of ammonia between extracellular and intracellular compartments in various tissues was according to the pH gradient, tissue pHi was predicted from the measured ammonia distribution using equation 6, assuming intracellular [NH₃] = plasma [NH₃]. These predicted pHi values were compared with the measured values determined from the DMO distributions. A predicted intracellular T_{amm} could also be calculated using equation 7, again assuming intracellular [NH₃] = plasma [NH₃].

To determine whether the distribution of ammonia between extracellular and intracellular compartments in various tissues could alternatively be a function of the membrane potential, E_{NH₄⁺} was calculated from the Nernst equation:

$$E_{\text{NH}_4^+} = \frac{\text{RT}}{\text{ZF}} \ln \frac{[\text{NH}_4^+]_e}{[\text{NH}_4^+]_i}, \quad (13)$$

where R, T, Z and F have their usual values, and [NH₄⁺]_e and [NH₄⁺]_i represent

plasma and intracellular levels based on measured extracellular and intracellular pH and T_{amm} values (equations 3, 4, 9 and 10).

Data are presented as means \pm 1 s.e.m. (N). Student's unpaired t -test was employed to evaluate the significance of differences between mean values ($P < 0.05$).

Results

Acid-base and fluid volume changes

The acid-base status and haematological variables of the three experimental groups are given in Tables 1 and 2. pHi was by far the highest in brain with a value approximately equal to pHe (Table 1B). Red cells, white muscle and heart ventricle all maintained a pHe-pHi gradient of 0.3-0.4 units. Hypercapnia resulted in lower pHi values in all tissues compared with the values in control fish, with greater decreases in brain and white muscle (approx. 0.3 units) than in red cells or heart (approx. 0.2 units). The acidifying effect of exhaustive exercise was much more selective, with pHi significantly lower only in the working muscle, whereas the acid-base status of the other tissues was unaffected.

Table 1. *Acid-base status in sole during control, hypercapnia and exercise regimes*

A					
Treatment	pHe	Plasma P_{CO_2} (Torr)	Plasma [HCO ₃ ⁻] (mmol l ⁻¹)	Whole blood [lactate] (mmol l ⁻¹)	Muscle [lactate] (mmol l ⁻¹)
Control ($N = 10$)	7.73 \pm 0.01	1.94 \pm 0.08	4.64 \pm 0.19	0.11 \pm 0.03 ($N = 6$)	3.22 \pm 0.09 ($N = 6$)
Hypercapnia ($N = 9$)	7.32 \pm 0.04*	7.44 \pm 0.74*	5.92 \pm 0.14*	0.19 \pm 0.05 ($N = 7$)	6.00 \pm 2.16 ($N = 7$)
Exercise ($N = 9$)	7.48 \pm 0.04*	1.99 \pm 0.35	2.40 \pm 0.23*	0.98 \pm 0.16* ($N = 10$)	19.57 \pm 1.55* ($N = 10$)
B					
Treatment	Red cell pHi	White muscle pHi	Heart pHi	Brain pHi	
Control ($N = 10$)	7.25 \pm 0.02	7.29 \pm 0.03	7.32 \pm 0.06	7.75 \pm 0.03	
Hypercapnia ($N = 9$)	7.01 \pm 0.02*	6.95 \pm 0.03*	7.10 \pm 0.03*	7.44 \pm 0.02*	
Exercise ($N = 9$)	7.22 \pm 0.02	7.12 \pm 0.03*	7.27 \pm 0.07	7.71 \pm 0.05	

* Significantly different from control value, t -test, $P < 0.05$.
Values are means \pm s.e.m.

Table 2. Blood haemoglobin [Hb], haematocrit (Hct), mean cellular haemoglobin concentration (MCHC) and red cell and plasma water content in three groups of lemon sole; control fish, hypercapnic fish and fish swum to exhaustion

Treatment	Hb (g 100 ml ⁻¹ blood)	Hct (%)	MCHC (g ml ⁻¹ cells)	Red cell water (%)	Plasma water (%)
Control (N = 10)	3.0 ± 0.2	11.0 ± 1.0	0.28 ± 0.01	76.3 ± 1.4	97.4 ± 0.3
Hypercapnia (N = 9)	3.7 ± 0.2	15.9 ± 1.1*	0.24 ± 0.01*	73.6 ± 0.5	96.6 ± 0.0*
Exercise (N = 9)	3.7 ± 0.6	17.2 ± 2.2*	0.24 ± 0.00*	73.9 ± 1.4	95.9 ± 0.2*

* Significantly different from control, unpaired *t*-test, *P* < 0.05.
Values are means ± s.e.m.

Muscle was the only tissue in which there were changes in fluid volume distribution between treatments. Following exhaustive exercise, ECFV was significantly lower (control, 0.0997 ± 0.0113, exercise, 0.0599 ± 0.0090 ml g⁻¹) and ICFV (control, 0.7174 ± 0.0129, exercise, 0.7552 ± 0.0097 ml g⁻¹) higher in white muscle, suggesting a net water flux into the cells, probably in response to the osmotic effect of the increased intracellular [lactate] (Table 1A).

Ammonia distribution

The distribution of ammonia between plasma and tissues under control, hypercapnia and exercise conditions is presented for red cells in Table 3, white muscle in Table 4, heart ventricle in Table 5, and brain in Table 6. In hypercapnic fish, plasma ammonia levels were significantly lower than in the control group by about 25 %, although tissues showed generally smaller, non-significant reductions. In exercised fish, intracellular ammonia levels were elevated by about 55 % over control levels, an effect which was significant in all tissues except brain. Plasma ammonia levels were not significantly altered.

Red cell ammonia levels were consistently higher than plasma levels, resulting in ammonia concentration ratios of 0.22–0.29 (plasma-to-red cell, Table 3). Red cell pHi predicted from the plasma-to-red cell ammonia distribution was not significantly different from measured pHi in the control experiment, but during hypercapnia and exercise, predicted pHi was significantly less than measured pHi (Table 3). NH₃ was in equilibrium between plasma and red cells at rest (Fig. 1), but not during either of the extracellular acidoses (hypercapnia and exercise, Fig. 1). The P_{NH₃} gradient from red cell-to-plasma during hypercapnia was 10 μTorr and following exercise about 30 μTorr. Our calculations of P_{NH₃} levels assume an equilibrium between NH₃ and NH₄⁺ in the red cell as well as the plasma, because the NH₃ → NH₄⁺ reaction is considered to be instantaneous

Table 3. Measured pHe, red cell pH_i, plasma and red cell T_{amm} and the ratio of plasma:red cell T_{amm} at rest, during hypercapnia and following exercise in lemon sole

Treatment	Measured				Predicted			Calculated	
	pHe	pH _i (freeze-thaw method)	Plasma T _{amm} (μmol l ⁻¹)	Red cell T _{amm} (μmol l ⁻¹)	Ammonia concentration ratio	pH _i	Red cell T _{amm} (μmol l ⁻¹)	E _{NH₄⁺} (mV)	E _{H⁺} (mV)
Control (N = 10)	7.73 ± 0.01	7.25 ± 0.02	192 ± 13	657 ± 93	0.29	7.23 ± 0.07	588 ± 52	-29 ± 4	-28 ± 1
Hypercapnia (N = 9)	7.32 ± 0.04*	7.01 ± 0.02*	142 ± 11*	573 ± 96	0.25	6.79 ± 0.08†	302 ± 24‡	-31 ± 5	-18 ± 2*
Exercise (N = 9)	7.48 ± 0.04*	7.22 ± 0.02	228 ± 31	1029 ± 213*	0.22	6.89 ± 0.05†	453 ± 90‡	-38 ± 4	-16 ± 1*

* Significantly different from control, $P < 0.05$.

† Significantly different from measured pH_i, $P < 0.05$.

‡ Significantly different from measured red cell T_{amm}, $P < 0.05$.

Predicted red cell pH_i calculated from the ratio of NH₃:NH₄⁺ in the red cell using equation 6 is compared with measured pH_i (freeze-thaw method).

Predicted red cell T_{amm} (equation 7) is compared with measured red cell T_{amm}.

E_{NH₄⁺} and E_{H⁺} were calculated from equation 13.

Means ± S.E.M.

Table 4. Measured pHe, muscle pHi, plasma and muscle T_{amm} and the ratio of plasma: muscle T_{amm} at rest, during hypercapnia and following exercise in lemon sole

Treatment	Measured				Ammonia		Predicted		Calculated
	pHe	pHi (DMO)	Plasma T_{amm} ($\mu\text{mol l}^{-1}$)	Muscle T_{amm} ($\mu\text{mol l}^{-1}$)	concentration ratio	pHi	Muscle T_{amm} ($\mu\text{mol l}^{-1}$)	$E_{\text{NH}_4^+}$ (mV)	
Control (N = 9)	7.73 ± 0.01	7.29 ± 0.03	192 ± 13	6005 ± 577	0.03	6.24 ± 0.05†	550 ± 35‡	-83 ± 3	
Hypercapnia (N = 9)	7.32 ± 0.04*	6.95 ± 0.03*	142 ± 11*	4623 ± 516	0.03	5.83 ± 0.06†	343 ± 26‡	-83 ± 3	
Exercise (N = 9)	7.48 ± 0.04*	7.12 ± 0.03*	228 ± 31	9344 ± 1256*	0.02	5.84 ± 0.12†	560 ± 112‡	-88 ± 4	

* , †, ‡ see Table 3.

Predicted muscle pHi calculated from the ratio of $\text{NH}_3:\text{NH}_4^+$ in muscle using equation 6 is compared with measured pHi (DMO method).

Predicted muscle T_{amm} (equation 7) is compared with measured muscle T_{amm} .

$E_{\text{NH}_4^+}$ was calculated from equation 13.

Means ± S.E.M.

Table 5. Measured pHe, heart pHi, plasma and heart T_{amm} and the ratio of plasma: heart T_{amm} at rest, during hypercapnia and following exercise in lemon sole

Treatment	Measured				Predicted			Calculated $E_{NH_4^+}$ (mV)
	pHe	pHi (DMO)	Plasma T_{amm} ($\mu\text{mol l}^{-1}$)	Heart T_{amm} ($\mu\text{mol l}^{-1}$)	Ammonia concentration ratio	pHi	Heart T_{amm} ($\mu\text{mol l}^{-1}$)	
Control (N = 9)	7.73 ± 0.01	7.32 ± 0.06	192 ± 13	7891 ± 598	0.02	6.11 ± 0.05†	543 ± 78‡	-90 ± 3
Hypercapnia (N = 9)	7.32 ± 0.04*	7.10 ± 0.03*	142 ± 11*	6941 ± 998	0.02	5.67 ± 0.08†	254 ± 33‡	-94 ± 4
Exercise (N = 9)	7.48 ± 0.04*	7.27 ± 0.07	228 ± 31	12355 ± 1071*	0.02	5.70 ± 0.10†	403 ± 81‡	-96 ± 2

* , †, ‡ see Table 3.

Predicted heart pHi calculated from the ratio of $\text{NH}_3:\text{NH}_4^+$ in heart using equation 6 is compared with measured pHi (DMO method). Predicted heart T_{amm} (equation 7) is compared with measured heart T_{amm} .

$E_{NH_4^+}$ was calculated from equation 13.

Means ± S.E.M.

Table 6. Measured pHe, brain pHi, plasma and brain T_{amm} and the ratio of plasma: brain T_{amm} at rest, during hypercapnia and following exercise in lemon sole

Treatment	Measured				Ammonia		Predicted		Calculated $E_{NH_4^+}$ (mV)
	pHe	pHi (DMO)	Plasma T_{amm} ($\mu\text{mol l}^{-1}$)	Brain T_{amm} ($\mu\text{mol l}^{-1}$)	concentration ratio	pHi	Brain T_{amm} ($\mu\text{mol l}^{-1}$)		
Control (N = 9)	7.73 ± 0.01	7.75 ± 0.03	192 ± 13	3419 ± 434	0.06	6.48 ± 0.06†	195 ± 21‡	-69 ± 3	
Hypercapnia (N = 9)	7.32 ± 0.04*	7.44 ± 0.02*	142 ± 11*	2787 ± 948	0.05	6.19 ± 0.11†	115 ± 11‡	-65 ± 6	
Exercise (N = 9)	7.48 ± 0.04*	7.71 ± 0.05	228 ± 31	4970 ± 864	0.05	6.12 ± 0.12†	144 ± 2‡	-72 ± 4	

* , †, ‡ see Table 3.

Predicted brain pHi calculated from the ratio of NH_3 : NH_4^+ in brain using equation 6 is compared with measured pHi (DMO method).
Predicted brain T_{amm} (equation 7) is compared with measured brain T_{amm} .

$E_{NH_4^+}$ was calculated from equation 13.

Means ± s. e. M.

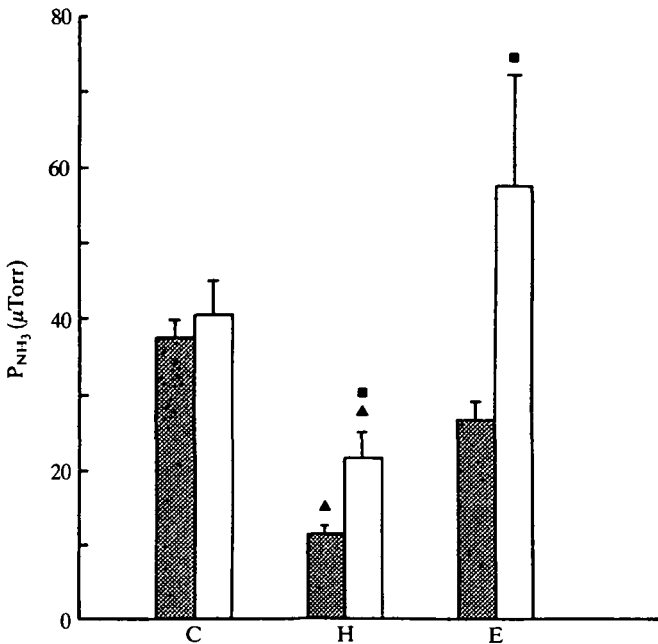


Fig. 1. Plasma P_{NH_3} (stippled bars) is compared with red cell P_{NH_3} (open bars) in control fish (C), fish exposed to hypercapnia (H) and exercised fish (E). ■ denotes significant difference from plasma P_{NH_3} ; ▲ denotes significant difference from control P_{NH_3} . Means ± 1 s.e.m. ($N = 9-10$).

(<50 ms, Stumm & Morgan, 1981). If there is a NH_3 gradient from red cell to plasma and if H^+ is passively distributed across red cell membranes, as commonly believed (Lassen, 1977; Heming *et al.* 1986; Nikinmaa *et al.* 1987), then there must also be an electrochemical gradient for NH_4^+ . As red cell NH_3 levels are elevated, NH_4^+ will also be elevated as NH_3 molecules combine with H^+ to form NH_4^+ . In hypercapnia and exercise regimes, therefore, there was a net diffusional gradient for both NH_3 and NH_4^+ out of the red cell. This is illustrated by the values of $E_{\text{NH}_4^+}$ and E_{H^+} calculated from the Nernst equation (equation 13) (Table 3). $E_{\text{NH}_4^+}$ and E_{H^+} were similar under control conditions; however, during both acidotic treatments, E_{H^+} became less negative as expected (e.g. Lassen, 1977) whereas $E_{\text{NH}_4^+}$ became more negative.

Ammonia distribution ratios between muscle and plasma (control ratio = 0.03, Table 4), heart and plasma (control ratio = 0.02, Table 5) and brain and plasma (control ratio = 0.06, Table 6) were considerably lower than those between red cells and plasma (ratios >0.20, Table 4). Ammonia was clearly not distributed among tissue compartments according to the H^+ distribution. In all tissues and treatments, intracellular pHi calculated from the plasma-to-tissue ammonia distribution was significantly less (by 1.0–2.0 units) than pHi calculated from the [^{14}C]DMO distribution (Tables 4–6). Also, predicted tissue T_{amm} was significantly

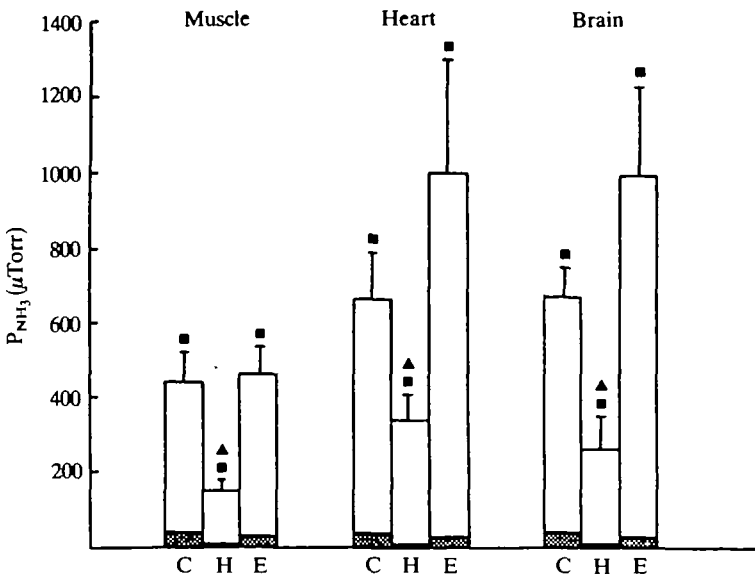


Fig. 2. White muscle, ventricular heart muscle and brain P_{NH_3} levels (open bars) are compared with plasma P_{NH_3} levels (stippled bars) in control fish (C), fish exposed to hypercapnia (H) and exercised fish (E). ■ denotes significant difference from plasma P_{NH_3} ; ▲ denotes significant difference from control P_{NH_3} . Means \pm 1 s.e.m. ($N = 9-10$).

less than measured tissue T_{amm} in each treatment (Tables 4-6). The tissue intracellular-to-extracellular P_{NH_3} gradients (Fig. 2) were an order of magnitude greater than red cell-to-plasma gradients (Fig. 1). As stated above for red cells, our calculations of P_{NH_3} assume an equilibrium between NH_3 and NH_4^+ in each tissue compartment. The difference between red cells and other intracellular compartments, however, is that H^+ distribution is not passive (Roos & Boron, 1981). Hence, NH_3 gradients can exist in the absence of NH_4^+ electrochemical gradients, in a situation where NH_4^+ is passively distributed across the membrane, but H^+ is not (see Discussion). Control P_{NH_3} gradients were smaller between muscle and plasma ($410 \mu\text{Torr}$) than between heart ($630 \mu\text{Torr}$) or brain ($640 \mu\text{Torr}$) and plasma. In all tissues, P_{NH_3} was significantly reduced during hypercapnia, largely because of intracellular acidosis, although intracellular T_{amm} did decrease slightly in all tissues. There were no significant changes in P_{NH_3} gradients after exercise (Fig. 2), although T_{amm} gradients from tissue to plasma were greatly increased in all tissues (Tables 4-6).

The equilibrium potentials for NH_4^+ ($E_{\text{NH}_4^+}$) across muscle, heart and brain cell membranes were calculated from the Nernst equation and are presented in Tables 4, 5 and 6, respectively. These values were 2-3 times larger than those in red blood cells (see Table 3). There were no significant differences in $E_{\text{NH}_4^+}$ between treatments.

Discussion

Evaluation of methods

The accuracy of red cell T_{amm} calculations depends largely on Hct, increasing with increasing Hct (equation 1). For instance, in control fish, the error in red cell T_{amm} may be as great as 7% if Hct is out by 1%, at the mean Hct value of 11%. A 7% error in measured red cell T_{amm} would not alter the conclusion that ammonia is distributed according to the H^+ distribution across red cell membranes at rest. The significance of this problem diminishes in the hypercapnia (Hct = 16%) and exercise (Hct = 17%) treatments, where the error in red cell T_{amm} is at most 4% for a 1% digression in Hct. The potential for error in measured red cell T_{amm} calculations in the hypercapnia and exercise experiments is small relative to the large discrepancy between measured and predicted red cell T_{amm} . The potential for error in measured intracellular T_{amm} for muscle, heart and brain tissue (equation 9) is even smaller; for instance, for a 1% digression in ICFV, the error in tissue T_{amm} is less than 2%. Thus, ICFV is not a critical variable in the calculation of intracellular T_{amm} .

The accuracy and limitations of the [^{14}C]DMO/[^3H]mannitol technique for intracellular pH measurements in fish have been assessed in detail previously (Milligan & Wood, 1985, 1986a,b) and will not be discussed in the present paper.

Acid–base changes: comparisons with other studies

Resting blood acid–base parameters in lemon sole were very similar to those measured by McDonald, Walker, Wilkes & Wood (1982) on the same species. Hypercapnia resulted in a plasma acidosis that was typical of other saltwater species (*Conger conger*, Toews, Holeyton & Heisler, 1983; *Scyliorhinus stellaris*, Heisler, Weitz & Weitz, 1976) when compared over the same period of time. The metabolic acidosis incurred after exhaustive exercise in lemon sole was typical of other benthic, sluggish fish swimming to exhaustion, with very low blood [lactate] despite high muscle [lactate] and a considerable blood metabolic acid load (Wood & Perry, 1985). In other fish, however, a respiratory component (P_{CO_2} elevation) often persists for 1–2 h following exercise, which was not seen here. Hence, if lemon sole did suffer a respiratory acidosis in addition to the metabolic acidosis following exercise, full recovery must have occurred within 30 min.

White muscle, brain and red cell pH_i in resting lemon sole were identical to the values reported for another flatfish, the starry flounder (*Platichthys stellatus*), using the same DMO and freeze–thaw techniques (Wood & Milligan, 1987), although heart pH_i was approximately 0.2 units lower. Exhaustive exercise in lemon sole resulted in a less marked intracellular acidosis in white muscle, and no acidosis in heart or brain tissue, in contrast to the effects on the starry flounder. This difference undoubtedly reflects the lack of a respiratory component in lemon sole relative to the large P_{CO_2} elevation in flounder at a comparable time after exercise. Thus, in the sole, the intracellular acidosis was limited to the working

muscle itself and was completely metabolic in nature, as signalled by the large elevation in [lactate] white muscle. We are aware of no comparable studies on the intracellular responses to hypercapnia in fish over this time period.

Red cell ammonia distribution

The results show that ammonia is distributed according to the pH gradient across red cells at resting blood pH values. These findings agree with our earlier study on *in vitro* and *in vivo* trout red cells (Wright *et al.* 1988) and a study on human red cells (Bone, Verth & Lambie, 1976). If NH_4^+ transfer across red cell membranes is negligible, and ammonia distribution is solely dependent on P_{NH_3} gradients, then one would expect the red cell-to-plasma ammonia distribution to follow the H^+ distribution, as for other weak acids and bases with impermeant ion forms. Red cells, however, are unusual in that H^+ is passively distributed across the membrane according to the membrane potential (Lassen, 1977; Heming *et al.* 1986; Nikinmaa *et al.* 1987), unlike the situation in other tissues (see Roos & Boron, 1981). Therefore, *exactly the same* distribution would occur if the membrane was permeable only to NH_4^+ , and the ammonia distribution was set entirely by the membrane potential. We conclude that ammonia is passively distributed between red cells and plasma under control conditions, but we cannot determine whether ammonia flux across red cell membranes depends solely upon P_{NH_3} gradients or if NH_4^+ electrochemical gradients are also important.

Ammonia is not distributed across red cell membranes according to the pH gradient during hypercapnia and exercise in sole, which agrees with data on trout red cells *in vitro* and *in vivo* during hypercapnia (Wright *et al.* 1988) and avian red cells *in vitro* over the pH range 7–8 (Bone *et al.* 1976). Bone *et al.* (1976) proposed that ammonia and DMO would distribute differently between the cytosol and the acidic nucleus, resulting in a discrepancy between pHi calculated from the DMO distribution *versus* the ammonia distribution. Roos & Boron (1981), however, reanalysed their data and found that intracellular compartmentalization could not fully account for the discrepancy between the two calculated pHi values. Wright *et al.* (1988) discussed three possible explanations why ammonia accumulation in red cells might be out of equilibrium with the pH gradient during an acidosis: intracellular ammonia synthesis, nonsteady-state conditions, and active uptake of NH_4^+ by the cell membrane Na^+, K^+ -ATPase pump. All three possibilities were experimentally eliminated in the *in vitro* situation, though it was not possible to perform similar tests *in vivo*. However, because of the similarity between the *in vitro* and *in vivo* data, the authors suggested that other factors must be involved, one of which could be active NH_4^+ uptake by a mechanism other than Na^+, K^+ -ATPase.

We now propose that one other explanation should also be considered. Red cell-to-plasma ammonia gradients during hypercapnia and exercise may be due to binding of ammonia to proteins (Barker, 1968) in the red cell. It seems unlikely, however, that differential binding would occur, that is, binding under acidotic conditions (hypercapnia and exercise) but not at control pH values. Furthermore,

there is evidence that ammonia binds in some mammalian intracellular compartments (see below), but we are unaware of any reports of ammonia binding in red cells.

In summary, ammonia is passively distributed according to the plasma-to-red cell H^+ distribution in blood at resting pH values, but not during an extracellular acidosis. We suggest that red cell T_{amm} levels are maintained above those expected from pH considerations by an active NH_4^+ uptake mechanism other than Na^+, K^+ -ATPase or, possibly, by binding of ammonia to intracellular proteins.

Muscle, heart and brain ammonia distribution

Ammonia was not distributed between plasma and muscle, heart or brain tissue according to the H^+ distribution either at rest, during hypercapnia, or following exercise in lemon sole. These findings are consistent with studies on mammalian hepatocytes where, at physiological pH and ammonia concentrations, intracellular ammonia levels are much higher than predicted from the H^+ distribution (Sainsbury, 1980; Remesy *et al.* 1986). Ammonia also accumulates in mammalian brain tissue (Hindfelt, 1975; Benjamin, 1982) and colon (Bown *et al.* 1975) in concentrations above those expected from extracellular ammonia and pH considerations. These studies and the present results indicate that ammonia distribution across tissue membranes cannot be described by the classic diffusion trapping model (Visek, 1968; Pitts, 1973), which predicts intra- to extracellular ammonia distribution according to pH gradients, assuming that only NH_3 is transferred across cell membranes and that NH_4^+ transfer is negligible. If we assume that NH_4^+ permeability is significant, then the distribution of ammonia at equilibrium will be influenced by the membrane potential (E_M) as well as the pH gradient. The greater the permeability to NH_4^+ , the greater the influence of E_M .

Boron & Roos (1976) and Roos & Boron (1981) derived a general equation describing the equilibrium distribution of any weak base for which there is significant permeability to the charged as well as the uncharged form. Adopting this equation for ammonia, and correcting typographical errors in both original publications, this equation becomes:

$$\frac{[T_{amm}]_e}{[T_{amm}]_i} = \frac{\{[H^+]_e + k\}}{\{[H^+]_i + k\}} \times \frac{(pNH_3/pNH_4^+) - [F \times E_M \times \gamma/R \times T(1 - \gamma)] \times ([H^+]_i/k)}{(pNH_3/pNH_4^+) - [F \times E_M/R \times T(1 - \gamma)] \times ([H^+]_e/k)}, \quad (14)$$

where k is the NH_3/NH_4^+ dissociation constant, pNH_3 is the permeability to NH_3 , pNH_4^+ is the permeability to NH_4^+ and:

$$\gamma = \exp[(E_M \times F)/(R \times T)]. \quad (15)$$

If E_M and the pH_i - pH_e gradient are known, then $[T_{amm}]_e/[T_{amm}]_i$ can be calculated as a function of the pNH_3/pNH_4^+ ratio using these equations. E_M in fish white muscle, as in most vertebrate muscle, is uniformly in the range of -80 to

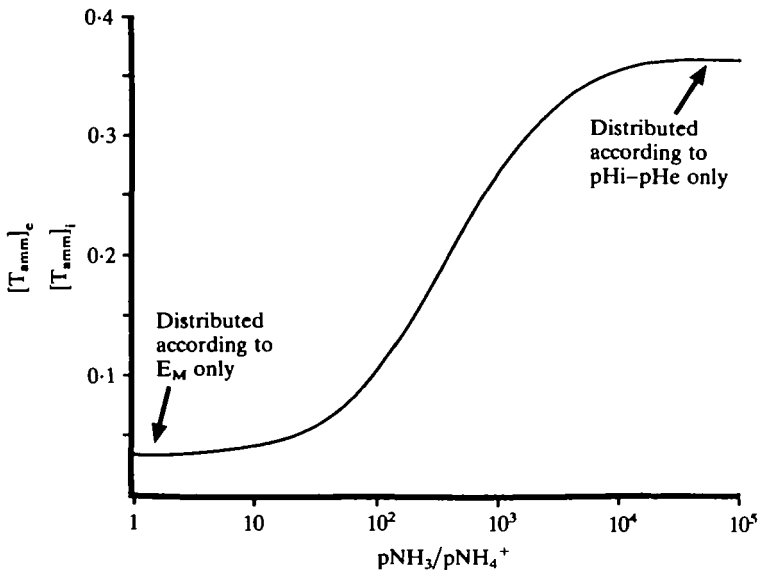


Fig. 3. The relationship between the distribution of ammonia at equilibrium between extracellular and intracellular compartments of resting sole white muscle ($[T_{amm}]_c/[T_{amm}]_i$) and the relative permeability (pNH_3/pNH_4^+) of the cell membrane to NH_3 and NH_4^+ . A membrane potential (E_M) of -83 mV and the measured pHi and pHe values of Table 4 have been assumed. See text for additional details.

-85 mV (Hagiwara & Takahashi, 1967; Hidaka & Toida, 1969; Yamamoto, 1972). In Fig. 3, we have used $E_M = -83$ mV and the measured pHe and pHi values for resting white muscle (Table 4) to calculate $[T_{amm}]_c/[T_{amm}]_i$ as a function of muscle cell membrane permeability ratio (pNH_3/pNH_4^+). The analysis shows that at $pNH_3/pNH_4^+ > 10^4$, the distribution asymptotically approaches the theoretical maximum of approx. 0.36, where it is entirely a function of the pH gradient, while at $pNH_3/pNH_4^+ < 10$, the distribution asymptotically approaches the theoretical minimum of approx. 0.03, where it is entirely a function of the membrane potential (E_M). In between these limits, there is an approximately log/linear relationship between pNH_3/pNH_4^+ and the equilibrium distribution ratio. The measured distribution ratio was 0.03 (Table 4), strongly suggesting that it was entirely a function of E_M , and therefore pNH_3/pNH_4^+ was low, i.e. permeability to NH_4^+ was relatively high. It is probably safe to assume that pNH_3/pNH_4^+ was at least below 25, where the ratio would be < 0.06 . Reversing the analysis, and using the measured distribution to predict E_M by the Nernst equation produced the answer $E_{NH_4^+} = -83 \pm 3$ mV (Table 4) in agreement with literature values for fish white muscle E_M .

Similar analyses have been performed with measured and predicted ammonia distributions in heart ventricle and brain, with the same conclusions, i.e. pNH_3/pNH_4^+ is low and the distribution is almost entirely a function of E_M .

Calculated $E_{\text{NH}_4^+}$ for resting heart ventricle was -90 ± 3 mV (Table 5), in agreement with Jaeger's (1962) measured E_M of -84 mV in *Leuciscus rutilus* ventricle, whereas calculated $E_{\text{NH}_4^+}$ for resting brain tissue was -69 ± 3 mV (Table 6), in close accord with the generally accepted value of -70 mV for nervous tissue (Hille, 1984). The similarity between calculated $E_{\text{NH}_4^+}$ values for muscle, heart and brain tissue and published E_M values implies that ammonia is passively distributed according to E_M in these tissues at rest, and that the permeability of tissue membrane to NH_4^+ is significant.

It is possible that NH_4^+ permeability is not as great as is suggested by this analysis and that intracellular binding of ammonia accounts for the high intracellular T_{amm} values. Wanders, Hoek & Tager (1980) presented evidence that a large fraction of intracellular ammonia was bound to mitochondrial protein in rat heart tissue, whereas smaller fractions of ammonia were associated with protein in skeletal muscle and brain. Thus, deproteinization of tissues during the extraction method would liberate this protein-bound ammonia and would result in an overestimation of free ammonia in the cytosol. We have calculated mean cellular T_{amm} levels in heart, muscle and brain of the sole, assuming that (1) the values given by Wanders *et al.* (1980) for bound ammonia in mitochondria of rat are valid for fish, (2) the mitochondrial volume is between 9 and 13% (Hoek, Nicholls & Williamson, 1980), (3) the cytosol constitutes the major portion of the remaining volume, and (4) the T_{amm} levels in the cytosol are determined by extra- to intracellular pH gradients. T_{amm} levels calculated with these assumptions are only 8–13% of our measured values. Hence, even if some fraction of cellular ammonia binds to mitochondrial proteins in fish, it cannot account for the high T_{amm} levels measured in the present study.

Resting levels of ammonia in sole white muscle measured in this study are between published values for cod, *Gadus morhua* (Fraser *et al.* 1966) and carp, *Cyprinus carpio* (Driedzic & Hochachka, 1976), but about 4–8 times higher than resting levels in rainbow trout (Dobson & Hochachka, 1987; T. Mommsen & P. Hochachka, unpublished data). In a recent review, Randall & Wright (1987) analysed the distribution of ammonia between trout white muscle and plasma, at rest and following exhaustive exercise (data taken from Mommsen & Hochachka, 1988; Milligan & Wood, 1986b). The analysis was based on the principle that ammonia was distributed across muscle cells according to the H^+ distribution, assuming that muscle cells were essentially impermeable to NH_4^+ . From their calculations, ammonia was distributed according to the H^+ distribution in trout, at rest, but not following exercise, where NH_3 gradients existed between muscle and blood. It seems odd that the ammonia distribution in resting trout white muscle apparently follows the H^+ distribution, while in sole it does not. Possible explanations include the differences in acid–base status and muscle fibre composition alluded to in the Introduction. Another problem with comparing the trout and sole studies is that the trout muscle T_{amm} levels (Mommsen & Hochachka, 1988) and pH_i values (Milligan & Wood, 1986b) were obtained from two separate studies (see Randall & Wright, 1987). Before any firm conclusions can be

drawn, muscle T_{amm} levels and pHi values must be determined on the same group of trout.

Following exercise, muscle ammonia levels increased by approx. 55 % compared with control values. An increase in white muscle ammonia levels with exercise is consistent with other studies on fish (Suyama, Koike & Suzuki, 1960; Fraser *et al.* 1966; Driedzic, 1975; Driedzic & Hochachka, 1976; Dobson & Hochachka, 1987) and is due to deamination of the adenylate pool (Driedzic & Hochachka, 1976; Dobson & Hochachka, 1987). In mammalian muscle, it is recognized that ammonia production and glycolysis may be functionally coupled in several ways. Ammonia may enhance glycolysis by activating phosphofructokinase, H^+ production by glycolysis may in turn enhance ammonia production by activating AMP deaminase, and ammonia production (as NH_3) may be important in buffering the H^+ produced by glycolysis (Lowenstein, 1972; Mutch & Banister, 1983; Dudley & Terjung, 1985). The quantitative importance of the latter has recently been discounted by the detailed study of Katz, Broberg, Sahlin & Wahren (1986) on working human leg muscle, because ammonia production was less than 4 % of lactate production. However, in the lemon sole, 30 min after exhaustive exercise, ammonia accumulation in white muscle (approx. 3.3 mmol l^{-1} , Table 4) was 20 % of lactate accumulation (approx. 16.3 mmol l^{-1} , Table 1). Identical stoichiometry was reported by Dobson & Hochachka (1987) in trout white muscle after exhaustive exercise. Thus, in ammoniotelic teleost fish, deamination/glycolysis coupling may be more important than in mammals, especially in limiting intracellular acidosis.

Ammonia levels also increased in heart (approx. 55 %) and brain (approx. 45 %) tissue following strenuous exercise, although it is unlikely that ammonia production can account for this. Heart is predominantly an aerobic tissue and therefore, unlike white muscle, the deamination of adenylates during exercise would be negligible. In nervous tissue, ammonia is known to be extremely toxic (see Randall & Wright, 1987), and high ammonia production rates are therefore improbable. In addition, it is doubtful that increased body ammonia levels after exercise are related to reduced ammonia excretion, because in other teleosts, ammonia excretion either increases or remains unchanged after strenuous exercise (Holeton, Neumann & Heisler, 1983; Heisler, 1984; Milligan & Wood, 1986a, 1987). We propose that elevated ammonia levels in heart and brain tissue after exercise are simply due to the passive uptake of ammonia from the increased plasma ammonia pool.

With hypercapnia there were no significant changes in muscle, heart and brain ammonia stores, although mean levels decreased in all tissues, as well as in the plasma. A reduction in total body ammonia stores may have been due to a decrease in ammonia production and/or an increase in ammonia excretion rates. The decrease in water pH (7.8 to 6.7) caused by hypercapnia would certainly favour increased diffusive loss of NH_3 across the gills. Ammonia excretion rates have been reported to increase with hypercapnia in some teleosts (trout, Lloyd & Swift, 1976; Perry, Malone & Ewing, 1987; carp, Claiborne & Heisler, 1984) and

according to E_M , intracellular NH_3 and NH_4^+ are in equilibrium (interconversion reaction rate for $\text{NH}_3 \rightarrow \text{NH}_4^+ < 50 \text{ ms}$, Stumm & Morgan, 1981), and ammonia production is insignificant (Fig. 4A). NH_3 gradients will form in the intracellular compartment as NH_4^+ enters the tissue and dissociates to form NH_3 and H^+ . The direction of this reaction will always be towards the formation of NH_3 and H^+ , because intracellular H^+ levels are lower (i.e. pHi higher) than expected for a passive distribution (Roos & Boron, 1981). The rate of NH_3 'back diffusion' out of the tissue will depend on the magnitude of the P_{NH_3} gradient and the NH_3 permeability (pNH_3), but NH_4^+ will diffuse back into the intracellular compartment down the electrochemical gradient. The result of this cycle will be a net transfer of H^+ to the intracellular compartment. The net effect would be an acidification of the intracellular compartment and alkalization of the extracellular compartment, were there not active H^+ extrusion mechanisms which keep $[\text{H}^+]$, low and well out of electrochemical equilibrium under steady-state conditions. Thus, the uptake of NH_4^+ increases the load on any H^+ transporter.

With ammonia production by muscle during exercise, it seems likely that P_{NH_3} and NH_4^+ electrochemical gradients would form between muscle and plasma (Fig. 4B). If ammonia is produced as NH_3 , then intracellular ammonia will raise pHi by trapping H^+ (from glycolysis) with an almost 1:1 stoichiometry. Ammonia may diffuse out of the cell as either NH_3 or NH_4^+ . Ammonia leaving as NH_3 will not affect the H^+ budget of the intracellular compartment. However, ammonia leaving as NH_4^+ would act as a mechanism to export H^+ , which will also help to regulate intracellular pH .

In summary, the results of this study show that the distribution of ammonia between plasma and muscle, heart and brain tissue in lemon sole is not dependent on the H^+ distribution, indicating that the cell membranes of these tissues have a significant permeability to NH_4^+ . The ammonia distribution appears to follow the membrane potential at rest; calculated $E_{\text{NH}_4^+}$ values generally agree with published values for E_M in these tissues. During hypercapnia and following exercise, NH_4^+ as well as NH_3 gradients may exist between tissue and plasma, particularly in tissues where ammonia production is stimulated, such as white muscle after exhaustive exercise.

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