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THE INFLUENCE OF GAS GLAND METABOLISM AND BLOOD FLOW ON GAS DEPOSITION INTO THE SWIMBLADDER OF THE EUROPEAN EEL *ANGUILLA ANGUILLA*

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Summary

The effects of blood flow through, and metabolic activity in, the swimbladder epithelium on gas deposition into the swimbladder have been analysed in the European eel, *Anguilla anguilla*. Blood flow in the artery supplying the retia was measured by Doppler flow probes; measurement of O_2 and CO_2 content in arterial and venous blood samples from the swimbladder allowed calculation of the rates of O_2 removal from, and CO_2 addition to, swimbladder blood. 83 % of the O_2 removed from the blood was transferred into the swimbladder lumen and only 17 % was metabolized in the tissue. In spite of the deposition of CO_2 into the swimbladder lumen, the CO_2 content in rete venous blood was higher than that in arterial blood, indicating production of CO_2 in the swimbladder tissue. The respiratory exchange ratio, calculated from O_2 consumption and CO_2 production of the swimbladder tissue, was significantly greater than one. Gas deposition into the swimbladder increased with increasing swimbladder arteriovenous pH difference, indicating acid release from gas gland cells, and thus their metabolic activity. The rate of gas deposition into the swimbladder increased with increasing blood perfusion of the swimbladder tissue.

Under hypoxic conditions, gas deposition was significantly reduced, as was blood flow through the swimbladder tissue. The decrease in gas deposition during hypoxia coincided with a reduction in the swimbladder arteriovenous pH difference. The results therefore demonstrate that the rate of gas deposition is dependent on blood perfusion of the swimbladder tissue and on metabolic activity of the swimbladder tissue, both of which are reduced under hypoxic conditions.

Introduction

Since the first analysis by Kuhn and Kuhn (1961) many studies have attempted to investigate the mechanisms by which gases are deposited in the fish swimbladder (Fänge, 1973; Pelster and Weber, 1991; Pelster and Scheid, 1992). It is now generally accepted that lactic acid formation in the swimbladder epithelium, even when P_{O_2} is high (D'Aoust, 1970), results in an increase in partial pressure of all gases through the salting-out effect, the Root effect and by conversion of HCO_3^- to CO_2 and that the hairpin

Key words: swimbladder, blood perfusion, metabolism, gas exchange, hypoxia, *Anguilla anguilla*.

counter-current system of the swimbladder rete further elevates the partial pressures of all gases which drive the passive deposition of gas into the swimbladder lumen. We have recently shown that CO_2 is also formed anoxidatively, probably in the pentose phosphate cycle, and that this can markedly aid in the effectiveness of gas deposition (Pelster *et al.* 1989; Pelster and Scheid, 1991).

One of the major questions that has not yet been answered is how the rate of gas deposition might be regulated and adjusted to the animal's functional needs. Stretch receptors have been suggested to be involved because sampling gas from the swimbladder often results in increased rates of gas deposition (Fänge, 1953; Tytler and Blaxter, 1973). Any regulatory activity may act on one or more of at least two possible mechanisms: gas gland metabolism and swimbladder blood flow.

McLean and Nilsson (1981) suggested that gas gland metabolism is under vagal control because vagotomy and cholinergic ganglionic blockers have been shown to inhibit gas deposition (Bohr, 1894; Fänge, 1973; Fänge *et al.* 1976; Lundin and Holmgren, 1991). However, this evidence is indirect because many swimbladder functions may be under vagal control.

Vasoconstriction in swimbladder vessels has been observed during stimulation of the splanchnic and vagus nerves, mediated by α-adrenergic receptors (Stray-Pedersen, 1970; Nilsson, 1972). Injection of catecholamines did not, however, affect the rate of gas deposition. Recently, the vasoactive intestinal peptide (VIP), a potent vasodilator, has been shown to reduce the rate of gas deposition in the cod (Lundin and Holmgren, 1991). These studies, however, do not answer the question of whether metabolic activity of the gas gland cells and swimbladder perfusion are involved in the regulation of gas deposition in the fish swimbladder.

The rate of swimbladder blood perfusion has not been measured in these or other studies. We have developed an anaesthetized eel preparation in which we can measure swimbladder blood flow and sample blood from the swimbladder circulation to calculate the total O_2 removal from and CO_2 addition to the blood. We have correlated the measured rates of swimbladder gas deposition, metabolic activity and blood flow to investigate whether gas deposition depends on either blood flow or metabolic activity. We have performed these experiments in both normoxic and hypoxic conditions.

Materials and methods

Specimens of the European eel, *Anguilla anguilla* L. (body mass 400–500 g), were obtained from a local supplier and kept for at least 1 or 2 weeks in a freshwater aquarium with aerated tap water at 12–16 °C. During this period the fish were not fed and remained mostly in the dark.

Animal preparation and apparatus

Under anaesthesia with MS 222 (0.2 g l^{-1}, neutralized with NaOH), the animals were quickly immobilized by penetrating the skull with a thick needle and by spinal pithing using a long wire. This method of immobilization has been compared with that of continuous anaesthesia using MS 222 (B. Pelster and P. Scheid, unpublished results), and

no difference between these treatments was observed with respect to swimbladder blood perfusion, gas deposition, dorsal aortic blood pressure or arterial blood gases. Even the circulatory response to injection of catecholamines was independent of the method of immobilization. Under MS 222 anaesthesia, however, contractions of the body wall musculature are not eliminated, which renders delicate preparations of the swimbladder vessels, such as separation of the artery and vein supplying the swimbladder tissue, or catheterization of the vein leaving the retia mirabilia, very difficult.

The animals were placed into an eel-holder and the gills were irrigated with well-aerated tap water (22–24 °C) at a flow rate of about 2.0 l min^{-1}. The body wall was opened ventrally and the swimbladder was carefully exposed and freed of connective tissue. The connection between the secretory and the resorbing part of the swimbladder was ligated between the two retia mirabilia. Blood vessels from other tissues were also ligated before they entered the vein leaving the retia.

A cuff-type Doppler flow probe (0.5–1.0 mm i.d.) was placed around the artery supplying the retia, after careful separation of the artery from the rete effluent vein, which was non-occlusively cannulated (PE 20) for blood collection. Separation of both vessels more centrally, towards the dorsal artery, allowed later occlusive cannulation of the artery for flow calibration. The dorsal artery was cannulated (PE 50) for arterial blood collection and blood pressure recording (Gould, Statham, BD 23 ID). The general anatomy of the swimbladder and the localization of the flow probe and of the various catheters is depicted in Fig. 1.

The bulbus arteriosus was exposed and a Doppler transducer crystal was implanted for measurement of cardiac output (uncalibrated recording). A catheter was inserted into the

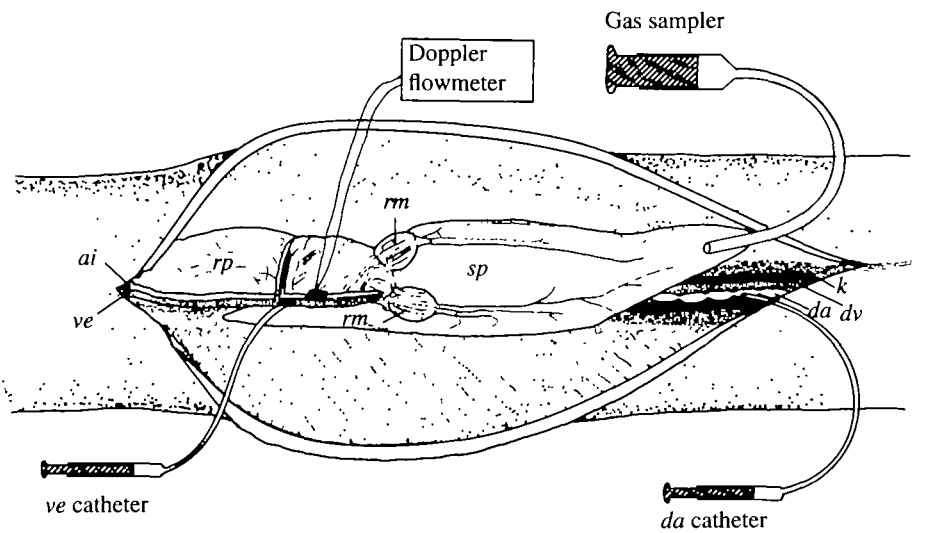

Fig. 1. Schematic drawing of the eel swimbladder preparation showing the location of the various catheters. *ai*, arterial influx; *da*, dorsal artery; *dv*, dorsal vein; *k*, kidney; *rm*, rete mirabile; *rp*, resorbing part of the swimbladder; *sp*, secretory part of the swimbladder; *ve*, venous efflux.

swimbladder for gas sampling. After removal of swimbladder gas, the bladder was reinflated with 150 or 200 μl of air to prevent the swimbladder walls from sticking together.

Analytical procedures

The transducer crystal and the cuff-type flow probe were connected to a Doppler flowmeter (Bioengineering, Iowa, USA). The velocity signals and the arterial blood pressure were continuously recorded on a pen recorder. Gas deposition was measured volumetrically by repeated gas sampling using a gas-tight syringe.

Blood samples were analysed for pH, P_{O_2} and P_{CO_2} using Radiometer electrodes (G 299; E 5047 and E 5037) regulated to 22 °C (BMS III; Radiometer, Copenhagen, Denmark). Oxygen content (C_{O_2}) and CO_2 content (C_{CO_2}) were measured in 10 μl samples according to Tucker (1967) and Cameron (1971), respectively. O_2 and CO_2 electrodes were calibrated using gas mixtures provided by precision gas-mixing pumps (Wösthoff, Bochum, FRG). Haematocrit (Hct) was determined using a haematocrit centrifuge (model M1100, Compur, München, FRG).

Experimental protocol

All experiments were performed at room temperature (22–24 °C). After inflating the swimbladder with 150 or 200 μl of air, a control period of 30–60 min was allowed for attainment of a steady state. A possible dependence of swimbladder gas deposition on blood perfusion was tested in 15 animals by measuring gas deposition and blood perfusion for up to 3 h without further experimental manipulation. In 12 of these preparations blood samples were also collected for determination of blood gases and pH. P_{O_2} in inspiratory water was kept at atmospheric values in this series. The rate of gas deposition was measured every 30 min by withdrawing all gas and returning 0.15 or 0.2 ml into the swimbladder. Arterial and swimbladder venous blood samples (0.2 ml) were taken at the same time, but in some preparations only once every hour. The preparation was stable for at least 4–5 h.

In eight preparations the influence of a reduced inspiratory P_{O_2} on gas deposition, swimbladder perfusion and metabolism was analysed. After a normoxic control period of 90–120 min, the inspiratory water P_{O_2} was lowered to about 5.5 kPa by bubbling the water reservoir with nitrogen. 30 min and 60 min after onset of hypoxia, gas and blood samples were taken, as in the normoxic series.

At the end of an experiment, a PE 20 catheter was inserted occlusively into the artery supplying the swimbladder tissue (see above) and tied in place. Blood of the animal was perfused through this catheter with a peristaltic pump for calibration of the signal of the cuff-type flow probe. The velocity signal obtained from the bulbus arteriosus, displayed in kHz Doppler shift (Fd), could not be calibrated.

Data analysis

The net rates of O_2 removal from blood, $\dot{M}b_{O_2}$ and of CO_2 addition to it, $\dot{M}b_{CO_2}$, were calculated from the swimbladder blood flow, $\dot{Q}_{Sb}$, and the O_2 and CO_2 concentration

differences in arterial and swimbladder venous blood, $Ca_{O_2}-Cv_{O_2}$ and $Cv_{CO_2}-Ca_{CO_2}$. For each animal mean values of two to four single determinations of each variable, obtained after attainment of steady state during a period with stable arterial blood gases and a measurable rate of gas deposition (> 0.1 ml h^{-1}), are given. The O_2 removal from blood includes both the metabolic O_2 consumption by the swimbladder tissue, $\dot{M}m_{O_2}$, and the rate of O_2 deposition into the swimbladder gas, $\dot{M}g_{O_2}$:

$$\dot{M}b_{O_2}=\dot{M}m_{O_2}+\dot{M}g_{O_2}. \quad (1)$$

The same holds true for CO_2, except that the CO_2 addition to the blood is the difference between the production rate and the rate of CO_2 deposition into the swimbladder gas:

$$\dot{M}b_{CO_2}=\dot{M}m_{CO_2}-\dot{M}g_{CO_2}. \quad (2)$$

The respiratory exchange ratio (RE) of the swimbladder tissue was calculated as:

$$RE = \dot{M}m_{CO_2}/\dot{M}m_{O_2}. \quad (3)$$

The O_2 and CO_2 deposition rates were calculated from the total rate of gas deposition, assuming 60 % of the newly deposited gas to be O_2 and 25 % to be CO_2 (Kobayashi *et al.* 1990).

Statistical analysis

Data are presented as mean ± S.E. Statistical differences between control values and values obtained after 30 or 60 min of hypoxia were established by applying the Student's *t*-test. To describe a relationship between two variables, multiple regression analysis was performed. Significance of differences was accepted when $P<0.05$.

Results

Overall mean values for total O_2 removal ($\dot{M}b_{O_2}$) from, and CO_2 addition ($\dot{M}b_{CO_2}$) to, the blood together with gas deposition rates obtained from 12 preparations are listed in Table 1. On average, total O_2 removal from blood was twice the mean value of CO_2 addition to it. Using these values and the amount of O_2 and CO_2 deposited into the swimbladder estimated from the total rate of gas deposition, the metabolic gas exchange

Table 1. *Average rates of gas deposition ($\dot{M}g$), O_2 removal ($\dot{M}b_{O_2}$) from, and CO_2 addition ($\dot{M}b_{CO_2}$) to, swimbladder blood; O_2 and CO_2 deposition rates into swimbladder gas (calculated from total gas deposition; see Materials and methods); and metabolic O_2 consumption and CO_2 production rates ($\dot{M}m_{O_2}$, $\dot{M}m_{CO_2}$; equations 1 and 2)*

	Total	O_2	CO_2
$\dot{M}b$ (ml h^{-1})		0.35±0.06	0.17±0.07
$\dot{M}g$ (ml h^{-1})*	0.48±0.08	0.29±0.06	0.12±0.02
$\dot{M}m$ (ml h^{-1})		0.06±0.04	0.29±0.06

*$\dot{M}g_{O_2}$ and $\dot{M}g_{CO_2}$ were calculated by assuming 60 % of the deposited gas to be O_2 and 25 % to be CO_2.

of the swimbladder tissue can be calculated using equations 1 and 2. Only 17 % of the oxygen taken up from the blood was metabolized in the swimbladder tissue and the production of CO_2 in the swimbladder tissue ($\dot{M}m_{CO_2}$) exceeded the rate of oxygen

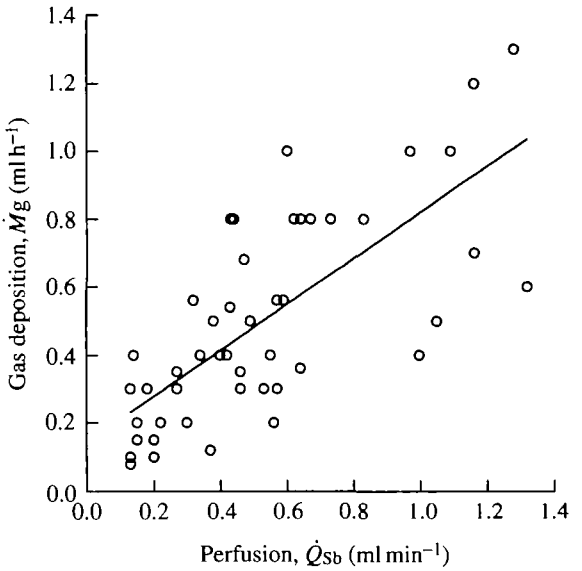

Fig. 2. The relationship between swimbladder perfusion and the rate of gas deposition into the swimbladder. The solid line was obtained by linear regression analysis (y=0.677x+0.142, r=0.721, P<0.0001).

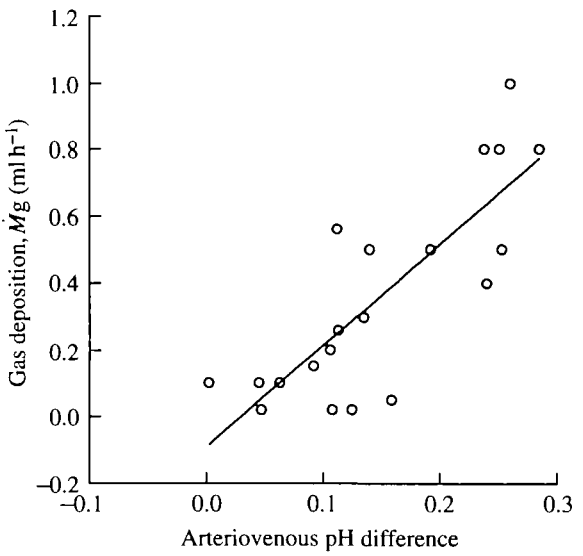

Fig. 3. The relationship between arteriovenous pH difference measured in the dorsal aorta and the swimbladder vein and the rate of gas deposition into the swimbladder. The solid line was obtained by linear regression analysis (y=3.022x−0.089, r=0.810, P<0.0001).

consumption ($\dot{M}m_{O_2}$). In these preparations swimbladder perfusion ($\dot{Q}_{Sb}$) averaged 0.54±0.08 ml min^{-1}.

Gas deposition in all preparations varied between 0.08 and 1.3 ml h^{-1} and there was a highly significant positive correlation between gas deposition and blood perfusion of the swimbladder (Fig. 2). Gas deposition was also correlated to the pH difference between dorsal arterial blood and venous blood leaving the swimbladder tissue, so that high gas deposition rates coincided with a pH difference of about 0.2 units or even more, while a difference of about 0.1 pH units was measured in preparations with a low rate of gas deposition (Fig. 3).

In a second series of experiments, animals were exposed to 60 min of hypoxia (P_{O_2} in inspired water, 5.5 kPa) after an initial control period of 90–120 min. Table 2 summarizes the changes in blood variables, blood pressure and cardiac output measured during the control period and after 30 and 60 min of hypoxia. In these experiments several blood samples had to be taken in series, but the sample volume was kept small enough to prevent a significant decrease in haematocrit. Hypoxia resulted in a decrease in arterial and venous blood P_{O_2} and P_{CO_2} as well as in oxygen and total CO_2 content (C_{O_2}, C_{CO_2}). Oxygen removal from the blood, $\dot{M}b_{O_2}$, was also reduced under these conditions, but there were no significant changes in $\dot{M}b_{CO_2}$. Dorsal arterial systolic blood pressure (Pa_{sys})

Table 2. *Mean values (±S.E.) of measured and calculated variables during normoxia and hypoxia*

		Hypoxia		
	Control	30 min	60 min	*N*
Pw_{O_2} (kPa)	15.6±0.5	5.2±0.7*	5.5±0.5*	(8)
Pa_{O_2} (kPa)	7.11±0.90	1.52±0.21*	1.49±0.15*	(8)
Pv_{O_2} (kPa)	5.23±0.61	1.73±0.22*	1.48±0.17*	(7)
Pa_{CO_2} (kPa)	0.27±0.03	0.17±0.01*	0.14±0.01*	(8)
Pv_{CO_2} (kPa)	0.45±0.05	0.28±0.03*	0.24±0.04*	(8)
Ca_{O_2} (mmol l^{-1})	3.52±0.31	1.36±0.22*	1.65±0.45*	(8)
Cv_{O_2} (mmol l^{-1})	2.93±0.22	1.25±0.16*	1.63±0.48*	(7)
Ca_{CO_2} (mmol l^{-1})	10.12±0.76	8.62±0.72	8.19±0.77*	(8)
Cv_{CO_2} (mmol l^{-1})	10.73±0.93	8.96±1.20	8.71±0.93	(7)
pHa	8.042±0.034	8.097±0.071	8.139±0.051*	(8)
pHv	7.840±0.029	8.047±0.064*	8.034±0.056*	(7)
Hcta (%)	23.6±2.7	22.0±1.9	22.1±1.9	(8)
Hctv (%)	24.2±2.9	22.9±1.9	21.6±1.9	(7)
Pa_{dia} (kPa)	3.96±0.44	2.24±0.15	2.29±0.04	(4)
Pa_{sys} (kPa)	4.56±0.49	2.84±0.13	2.73±0.07*	(4)
$\dot{Q}_{tot}$ (Fd, ΔkHz)	0.96±0.18	0.58±0.14*	0.48±0.14*	(6)
$\dot{M}b_{CO_2}$ (ml h^{-1})	0.11±0.12	0.25±0.14	0.20±0.05	(6)
$\dot{M}b_{O_2}$ (ml h^{-1})	0.35±0.04	0.10±0.06	0.09±0.05*	(6)

*Denotes a value significantly different from the normoxia value, $P<0.05$; N=number of experiments.

Fd, Doppler shift.

The abbreviations are explained in the text.

was reduced and hypoxia also resulted in a decrease in cardiac output ($\dot{Q}_{tot}$) as well as in swimbladder perfusion ($\dot{Q}_{Sb}$, see Fig. 4), but $\dot{Q}_{Sb}$ declined less rapidly than $\dot{Q}_{tot}$.

Mean rates of gas deposition into the swimbladder, of swimbladder perfusion and of the magnitude of the arteriovenous blood pH difference during hypoxia are shown in Fig. 4, revealing a significant decrease in all three variables after 30 and 60 min of hypoxia.

Discussion

Metabolism of the swimbladder tissue

Studies with a saline-perfused swimbladder preparation indicated that CO_2 is not just released from the blood but is produced by the anaerobic metabolism of the swimbladder tissue and from there released into the swimbladder lumen and into the bloodstream (Pelster *et al.* 1989). The results of the present study clearly support these results under normal conditions with blood perfusion. Most of the oxygen liberated from the haemoglobin was transferred into the swimbladder and only 17 % was consumed in the tissue. The rate of CO_2 production by the swimbladder tissue, evaluated by adding the amount of CO_2 deposited into the swimbladder lumen to that added to the bloodstream, greatly exceeded the rate of O_2 consumption of the tissue, so the respiratory exchange ratio was significantly greater than one. Although most of the 'swimbladder tissue' is probably swimbladder epithelium (gas gland), we should keep in mind that it also includes thin muscle fibres (Dorn, 1961) and the retia mirabilia. *In situ* the contribution of

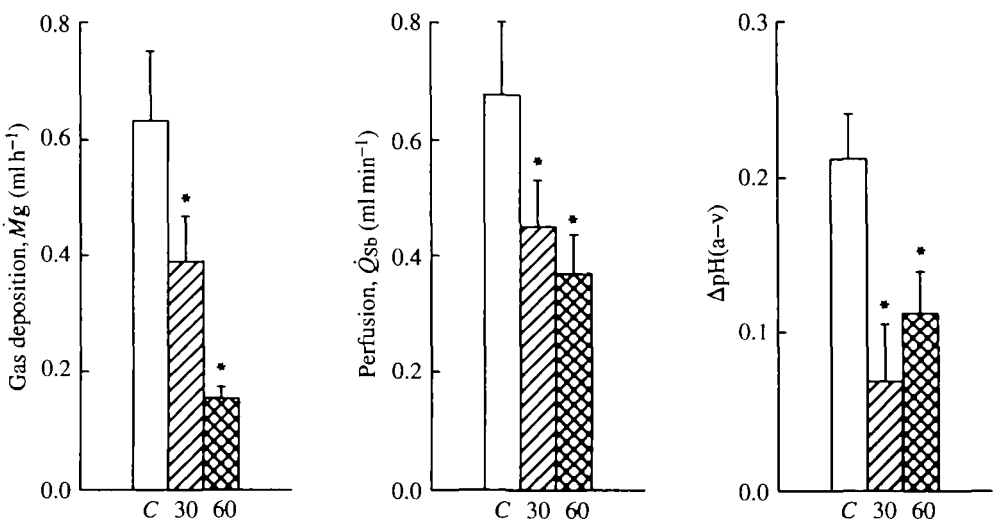

Fig. 4. Mean (± S.E.) values of gas deposition, swimbladder blood flow and arteriovenous pH difference, ΔpH(a−v), measured between the dorsal aorta and the swimbladder vein under normoxia and after 30 and 60 min of hypoxia [inspiratory P_{O_2} (P_{IO_2})=5.5 kPa; N=8]. Asterisks indicate significant differences from control (P<0.05). *C*, control; 30, 30 min of hypoxia; 60, 60 min of hypoxia.

these components to the total O_2 consumption and CO_2 release measured in this study cannot really be quantified because of the anatomical arrangement and the small amount of tissue.

To evaluate the possible contribution to swimbladder metabolism made by the nucleated red blood cells during their passage through the swimbladder tissue we need to know their transit time. Based on morphometric information (Stray-Pedersen and Nicolaysen, 1975) and on our flow measurements, we estimate the transit time for each limb in the rete to be about 1–2 s. Because of the lack of morphometric information on the circulatory system of the swimbladder epithelium, the transit time for this tissue cannot be calculated, but it seems reasonable to assume a value of the order of several seconds for the whole swimbladder. Tufts and Boutilier (1991) reported an oxygen uptake of trout red cells of about 100 nmol g^{-1} Hb min^{-1} (=2.24 μl g^{-1} Hb min^{-1}) at 15 °C. With a haemoglobin concentration of 120 g l^{-1}, a blood flow ($\dot{Q}_{Sb}$) of 0.54 ml min^{-1} and a transit time of 30 s we obtain an oxygen consumption of about 0.07 μl min^{-1} for the red cells during passage through the swimbladder, compared to 1 μl min^{-1} measured for the swimbladder tissue.

The rates of O_2 consumption and CO_2 production in our *in situ* preparations were about 3.5 times higher than those measured in a saline-perfused preparation (Pelster *et al.* 1989). In saline-perfused preparations gas deposition was virtually zero, which, as shown in this study (see below), reduces the metabolic rate of the swimbladder epithelium.

Dependence of gas deposition on perfusion and metabolism

There is a positive correlation between the rate of gas deposition and the rate of perfusion of the swimbladder tissue under normoxic conditions. This may indicate a predominant perfusion-limitation, rather than diffusion-limitation, of gas transfer from the blood to the swimbladder lumen under these conditions, which seems reasonable given the extended capillary network of the swimbladder and the small diffusion distance between the blood and the swimbladder lumen.

The results do not, however, confirm the study of Sund (1977), whose model for counter-current concentration in the swimbladder predicted a bell-shaped curve with decreasing gas deposition at blood flow rates above 0.5 ml min^{-1} for the eel. This author also predicted 'a negligible rate' of oxygen deposition under atmospheric pressure and when the swimbladder gas composition resembled that of the atmosphere, while Kobayashi *et al.* (1990) found the oxygen fraction in freshly deposited gas to be 0.6 in the European eel under these conditions. These discrepancies could be explained by the effect of CO_2 back-diffusion in the rete mirabile on the oxygen binding of the haemoglobin, i.e. the Root effect. CO_2 entering the arterial rete capillaries acidifies the blood and, therefore, decreases the haemoglobin's oxygen-carrying capacity (Root effect). As a consequence, a significant increase in rete arterial P_{O_2} can be observed without back-diffusion of oxygen (Kobayashi *et al.* 1990).

The rate of gas deposition is also dependent on the metabolism and acid release of the swimbladder tissue. The metabolism comprises not only production of CO_2, but also, and even more importantly, production of lactic acid. Therefore, the arteriovenous pH difference in the blood perfusing the swimbladder tissue appears to be a very good

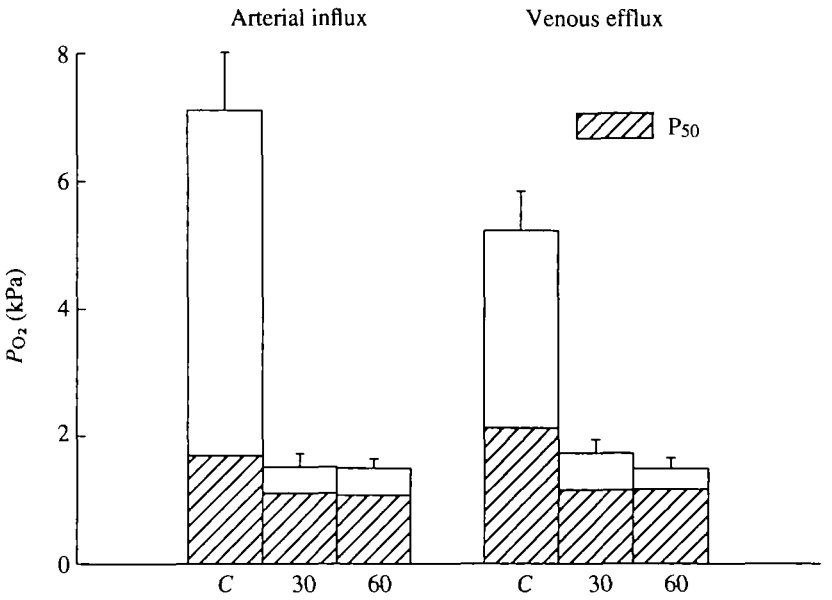

Fig. 5. Mean (±S.E.) P_{O_2} values measured in the dorsal aorta and the swimbladder vein under normoxia and hypoxia (P_{IO_2}, 5.5 kPa; N=8, 7; see Table 2) in comparison with the half-saturation of the haemoglobin (P_{50}, data from Wood and Johansen, 1972). *C*, control; 30, 30 min of hypoxia; 60, 60 min of hypoxia.

measure of the total acid release, and indeed there was a significant positive correlation between this difference and gas deposition. The acid releases oxygen from the haemoglobin by the Root effect (Root, 1931; Pelster and Weber, 1991), and thus increases P_{O_2}. The increases in P_{O_2} and in P_{CO_2} – because of the production of CO_2 and acidification of the blood – enhance the diffusion gradient for these gases towards the swimbladder lumen and thus increase the rate of gas deposition.

Metabolic activity of the swimbladder epithelium appears to be under vagal control, while adrenergic modification of swimbladder perfusion has been found (Nilsson, 1983). The decreases in gas deposition, blood flow and acid release observed under hypoxic conditions indicate that both perfusion and metabolism are regulated. In hypoxia, the arteriovenous pH difference measured in swimbladder vessels was only half that found under control conditions, revealing a reduction in proton release by the swimbladder tissue. This effect is underestimated because the decrease in pH difference has been measured in spite of a 45 % reduction in blood flow. Therefore, the decrease in acid release, and thus in metabolic activity of the swimbladder tissue, is much larger than indicated by the pH alone. It is difficult to calculate the exact difference in the number of protons released, because the buffer capacity of the blood has to be taken into account, which in itself is pH-dependent (Pelster *et al.* 1990), and the pH in the swimbladder vessels could not be measured in this study. Nevertheless, hypoxia induces a significant decrease in acid release by the swimbladder epithelium and the reduced blood acidification diminishes or even abolishes the Root effect. The combined decrease in CO_2 formation and in O_2 release from the haemoglobin results in a significantly lowered gas deposition.

A water P_{O_2} of only 5.5 kPa is rather low but, as shown in Fig. 5, the arterial as well as the venous oxygen partial pressures in the blood are above the P_{50} value. Therefore, the blood still contained oxygen that could have been released on acidification. Nevertheless, in hypoxia the significance of the Root effect is reduced. When the level of hypoxia is deep enough to prevent full O_2 saturation of arterial blood, then even with unchanged acid release from the swimbladder tissue the amount of oxygen that can be released from the haemoglobin will be reduced. Thus, the enhancement by the Root effect will be largely attenuated and O_2 deposition into the swimbladder should be reduced.

The present study demonstrates that gas deposition into the swimbladder depends on both blood perfusion and metabolic activity (acid release) of the swimbladder tissue. Furthermore, the results suggest that both variables can be regulated in order to modify the rate of gas deposition.

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References

BOHR, C. (1894). The influence of section of the vagus nerve on disengagement of gases in the airbladder of fishes. *J. Physiol., Lond.* **15**, 494–500.

CAMERON, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.

D'AOUST, G. (1970). The role of lactic acid in gas secretion in the teleost swimbladder. *Comp. Biochem. Physiol.* **32**, 637–668.

DORN, E. (1961). Über den Feinbau der Schwimmblase von *Anguilla vulgaris* L. Licht- und elektronenmikroskopische Untersuchungen. *Z. Zellforsch. mikrosk. Anat.* **55**, 849–912.

FÄNGE, R. (1953). The mechanisms of gas transport in the euphysoclist swimbladder. *Acta physiol. scand.* **30**, 1–133.

FÄNGE, R. (1973). The physiology of the swimbladder. In *Comparative Physiology* (ed. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell), pp. 135–159. Amsterdam: North Holland.

FÄNGE, R., HOLMGREN, S. AND NILSSON, S. (1976). Autonomic nerve control of the swimbladder of the goldsinny wrasse, *Ctenolabrus rupestris*. *Acta physiol. scand.* **97**, 292–303.

KOBAYASHI, H., PELSTER, B. AND SCHEID, P. (1990). CO_2 back-diffusion in the rete aids O_2 secretion in the swimbladder of the eel. *Respir. Physiol.* **79**, 231–242.

KUHN, W. AND KUHN, H. J. (1961). Multiplikation von Aussalz- und anderen Einzeleffekten für die Bereitung hoher Gasdrucke in der Schwimmblase. *Z. Elektrochem.* **65**, 426–439.

LUNDIN, K. AND HOLMGREN, S. (1991). An X-ray study of the influence of vasoactive intestinal polypeptide and substance P on the secretion of gas into the swimbladder of a teleost *Gadus morhua*. *J. exp. Biol.* **157**, 287–298.

MCLEAN, J. R. AND NILSSON, S. (1981). A histochemical study of the gas gland innervation in the Atlantic cod, *Gadus morhua*. *Acta Zool.* **62**, 187–194.

NILSSON, S. (1972). Autonomic vasomotor innervation in the gas gland of the swimbladder of a teleost (*Gadus morhua*). *Comp. gen. Pharmac.* **3**, 371–375.

NILSSON, S. (1983). Autonomic nerve function in the vertebrates. In *Zoophysiology*, vol. 13. Berlin, Heidelberg, New York: Springer-Verlag.

PELSTER, B., KOBAYASHI, H. AND SCHEID, P. (1989). Metabolism of the perfused swimbladder of European eel: oxygen, carbon dioxide, glucose and lactate balance. *J. exp. Biol.* **144**, 495–506.

PELSTER, B., KOBAYASHI, H. AND SCHEID, P. (1990). Buffering characteristics of eel blood at the extreme conditions in the swimbladder. *Respir. Physiol.* **79**, 219–230.

PELSTER, B. AND SCHEID, P. (1991). Activities of enzymes for glucose catabolism in the swimbladder of the European eel, *Anguilla anguilla*. *J. exp. Biol.* **156**, 207–213.

PELSTER, B. AND SCHEID, P. (1992). Counter-current concentration and gas secretion in the fish swim bladder. *Physiol. Zool.* **65**, 1–16.

PELSTER, B. AND WEBER, R. E. (1991). The physiology of the Root effect. *Adv. comp. environm. Physiol.* **8**, 51–77.

ROOT, R. W. (1931). The respiratory function of the blood of marine fishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **61**, 427–456.

STRAY-PEDERSEN, S. (1970). Vascular responses induced by drugs and by vagal stimulation in the swimbladder of the eel, *Anguilla vulgaris*. *Comp. gen. Pharmac.* **1**, 358–364.

STRAY-PEDERSEN, S. AND NICOLAYSEN, A. (1975). Qualitative and quantitative studies of the capillary structure in the rete mirabile of the eel, *Anguilla vulgaris* L. *Acta physiol. scand.* **94**, 339–357.

SUND, T. (1977). A mathematical model for counter-current multiplication in the swim-bladder. *J. Physiol., Lond.* **267**, 679–696.

TUCKER, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *J. appl. Physiol.* **23**, 410–414.

TUFTS, B. L. AND BOUTILIER, R. G. (1991). Interactions between ion exchange and metabolism in erythrocytes of the rainbow trout *Oncorhynchus mykiss*. *J. exp. Biol.* **156**, 139–151.

TYTLER, P. AND BLAXTER, J. H. S. (1973). Adaptation by cod and saithe to pressure changes. *Neth. J. Sea Res.* **7**, 31–45.

WOOD, S. C. AND JOHANSEN, K. (1972). Adaptation to hypoxia by increased HbO_2 affinity and decreased red cell ATP concentration. *Nature New Biol.* **237**, 278–279.