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GLUCOSE METABOLISM OF THE SWIMBLADDER TISSUE OF THE EUROPEAN EEL *ANGUILLA ANGUILLA*

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Summary

Glucose uptake from, and lactate release into, the blood have been analysed in the active gas-depositing swimbladder of the immobilized European eel *Anguilla anguilla*. Under normoxic conditions, 0.72 μmolmin^{-1} glucose was removed from the blood supply, while lactate was released into it at a rate of 1.16 μmolmin^{-1}. The rate of gas deposition into the swimbladder was significantly correlated with the rate of lactate production. Under hypoxic conditions, glucose consumption by, and lactate production of, the swimbladder tissue were reduced, as was the rate of gas deposition. Compared with normoxic conditions, lactate concentration in the swimbladder tissue was elevated after 1h of hypoxia, indicating a decrease in lactate release. No difference in the osmolality of arterial and venous blood could be detected in these experiments.

Combining the data for glucose uptake and lactate release measured under normoxic conditions with the values for O_2 uptake and CO_2 production of the swimbladder tissue measured under similar conditions in a previous study, a quantitative evaluation of glucose catabolism was performed. According to the O_2 uptake of the tissue, only about 1% of the glucose was oxidized, while about 80% was fermented to lactic acid. The remaining 0.14 μmolmin^{-1} glucose was presumably catabolized through the pentose phosphate shunt, as indicated by the CO_2 production of 0.16 μmolmin^{-1} that cannot be explained by aerobic metabolism.

Introduction

Gas gland cells of the swimbladder tissue are known to produce lactic acid, even under hyperoxic conditions (D'Aoust, 1970; cf. Pelster, 1993). The release of lactic acid into the blood results in an increase in the partial pressure of all gases through the salting-out effect, the Root effect, and by conversion of HCO_3^- to CO_2. In the hairpin countercurrent system of the swimbladder rete mirabile, this initial increase in gas partial pressures (the 'single concentrating effect') is elevated further by back-diffusion and countercurrent concentration (Fänge, 1983; Pelster and Scheid, 1992*a*).

The high CO_2 content of freshly deposited gas (Wittenberg *et al.* 1964) and the presence of enzymes of the pentose phosphate shunt in cod gas gland (Boström *et al.*

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1972) shed some doubt on the hypothesis that CO_2 originates exclusively from the conversion of HCO_3^- during blood acidification (D'Aoust, 1970). Recent studies have established the presence of high activities of key enzymes of the pentose phosphate shunt in gas gland tissue of various fishes (Pelster and Scheid, 1991; Walsh and Milligan, 1993). We have, furthermore, observed CO_2 formation without equivalent consumption of O_2 in a Ringer-perfused swimbladder preparation as well as in the active gas-depositing swimbladder of the European eel *in situ*, suggesting that this CO_2 was formed *via* the pentose phosphate shunt (Pelster *et al.* 1989; Pelster and Scheid, 1992*b*). By incubating gas gland tissue of the toadfish *Opsanus beta in vitro* with labelled glucose, Walsh and Milligan (1993) were able to demonstrate that CO_2 was formed through the pentose phosphate shunt.

The present study analyses glucose and lactate metabolism of the active gas-depositing swimbladder of the European eel *Anguilla anguilla* under normoxic and hypoxic conditions. The experiments were designed to investigate the importance of anaerobic glucose metabolism for the secretory activity of the swimbladder and to provide a data set consistent with that on the gas exchange of swimbladder tissue (Pelster and Scheid, 1992*b*). Aerobic and anaerobic glucose metabolism of swimbladder tissue and the possible contribution of the pentose phosphate shunt were evaluated quantitatively.

Materials and methods

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

Animal preparation and apparatus

Immobilization of the animals and preparation of the swimbladder were as described previously (Pelster and Scheid, 1992*b*). Briefly, the immobilized eels were placed into an eel-holder, and the gills were irrigated with well-aerated tap water (22–24°C) at a flow rate of 1.5–2.0 l min^{-1}. The body wall was opened ventrally, and the swimbladder was carefully exposed. The ductus between the secretory and the resorbing parts of the swimbladder and the blood vessels from other tissues entering the swimbladder vein were ligated.

A cuff-type Doppler flow probe (0.5–1.0mm i.d.) was placed around the artery supplying the retia, after carefully separating it 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). An additional catheter in the swimbladder allowed measurement of the rate of gas deposition. After each removal of swimbladder gas, 500 μl of the gas was returned to the swimbladder to prevent its walls from sticking together.

After the last blood samples had been taken (t=150min), a PE 20 catheter was inserted

occlusively into the artery supplying the swimbladder tissue (see above) and tied in place. Arterial blood of the animal was perfused through this catheter with a peristaltic pump for calibration of the signal of the cuff-type Doppler flow probe.

At the end of the experiment, the epithelium of the secretory bladder was quickly separated from the connective tissue, rinsed in Ringer's solution to remove most of the blood, blotted dry, and frozen in liquid nitrogen for later analysis of metabolite concentrations. The tissue was stored in liquid nitrogen until analysis.

Analytical procedures

The transducer crystal of the cuff-type flow probe was connected to a Doppler flowmeter (Bioengineering, Iowa, USA). The velocity signal and arterial blood pressure were recorded on line at a sampling rate of 20Hz with a microcomputer using a BrainWave Systems (Broomfield, Colorado) software package. Gas deposition was measured volumetrically by repeated gas sampling using a gas-tight syringe.

Blood samples were analysed for pH using Radiometer electrodes (G299 contained in a BMS III, Radiometer, Copenhagen, Denmark). Osmolality was determined on 10 μl samples with a vapour pressure osmometer (model 5500, Wescor, Logan, Utah). Haematocrit (Hct) was determined using a haematocrit centrifuge (model M1100, Compur, München, Germany).

For determination of metabolite concentrations, the swimbladder epithelium was extracted with perchloric acid (Beis and Newsholme, 1975). Blood was similarly deproteinized with perchloric acid and the neutralized supernatants were used for enzymatic determination of glucose 6-phosphate, glucose and lactate concentrations, as outlined by Bergmeyer (1974).

Experimental protocol

To obtain a consistent set of data for the analysis of the aerobic and anaerobic glucose catabolism, all experiments were performed at 22–24°C as described in a previous study, in which the gas exchange of the swimbladder was analysed (Pelster and Scheid, 1992*b*).

Series I

A possible dependence of swimbladder gas deposition on glucose metabolism and lactate release was tested in eight animals by measuring the variables under normoxic conditions (P_{O_2} in inspiratory water 17–20kPa) for 150min without further experimental manipulation. Blood samples were collected every 30min. The rate of gas deposition was measured every 30min by withdrawing all gas and returning 0.5ml into the swimbladder. Thus, blood and gas were collected five times in each experiment. Swimbladder epithelial tissue sampled at the end of these experiments served as the normoxic control.

Series II

In six 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 90min, the inspiratory water P_{O_2} was lowered to about 4.9kPa by bubbling the water reservoir with nitrogen. 30 and 60min after the onset of hypoxia, gas and blood samples

Table 1. *Glucose and lactate concentrations in arterial and swimbladder venous blood and the rate of glucose removal from, and lactate addition to, the blood ($\dot{M}$) of an active, gas-depositing swimbladder (series I)*

	Glucose	Lactate
Dorsal artery ($mmol\,l^{-1}$)	7.49±2.43	0.65±0.59
Swimbladder vein ($mmol\,l^{-1}$)	6.07±2.31	2.97±0.53
$\dot{M}$ ($\mu mol\,min^{-1}$)	0.72±0.38	1.16±0.73

Overall mean values (±S.D.) obtained in eight animals.

were taken, as in the normoxic series. Swimbladder epithelial tissue, sampled at the end of these experiments, was used as hypoxic tissue.

Data analysis

The net rates of glucose removal from blood, $\dot{M}_{glu}$, and lactate addition to it, $\dot{M}_{lac}$, were calculated from swimbladder blood flow, $\dot{Q}$, and the glucose and lactate concentration differences in arterial and swimbladder venous blood.

Overall mean values for $\dot{M}_{lac}$ and $\dot{M}_{glu}$ in series I were calculated by averaging the values obtained during the five measurements in each experiment. In series II (hypoxia), the values obtained at t=60min and t=90min (the last two samples collected under normoxic conditions) were averaged and used as the control value.

Statistical analysis

Data are presented as mean ± S.D. Statistical differences between control values and values obtained after 30 or 60min of hypoxia were tested by analysis of variance (ANOVA; StatGraphics; STSC Inc., Rockville, Maryland). Multiple regression analysis was performed to describe the relationship between two variables. Significance of differences was accepted when $P<0.05$.

Results

Overall mean values for arterial and swimbladder venous glucose and lactate concentrations as well as for net rates of glucose removal from blood, $\dot{M}_{glu}$, and lactate addition to it, $\dot{M}_{lac}$, under control (normoxia; series I) conditions are presented in Table 1. Lactate addition to the blood exceeded the rate of glucose removal from it, but the ratio of $\dot{M}_{lac}/\dot{M}_{glu}$ was less than 2. In this series, gas was deposited into the bladder at a rate of $0.56\pm0.31 ml\,h^{-1}$ and blood flow through the swimbladder tissue averaged $0.48\pm0.19 ml\,min^{-1}$ (mean ± S.D.).

The data revealed a significant positive correlation between lactate release from the swimbladder tissue and gas deposition (Fig. 1A). In preparations with a high rate of gas deposition, the amount of glucose removed from the blood was also higher than in preparations with a low rate of gas deposition (Fig. 1B).

Hypoxia in series II resulted in an increase in arterial blood lactate concentration, while the glucose concentration decreased slightly. The arterio-venous concentration difference

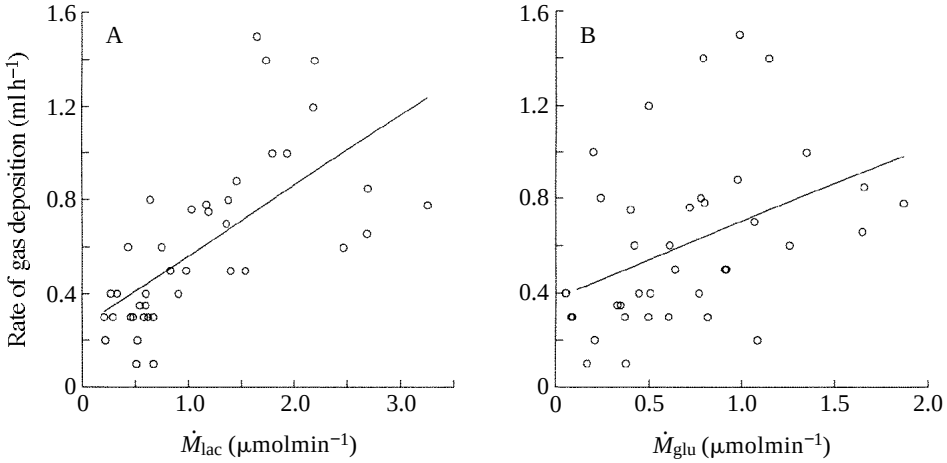

Fig. 1. (A) The relationship between lactate release from the swimbladder tissue ($\dot{M}_{lac}$) and the rate of gas deposition into the swimbladder. The solid line was obtained by linear regression analysis (y=0.30x+0.26, r=0.667, P<0.05). (B) The relationship between glucose uptake by the swimbladder tissue ($\dot{M}_{glu}$) and the rate of gas deposition into the swimbladder. The solid line was obtained by linear regression analysis (y=0.32x+0.37, r=0.423, P<0.05).

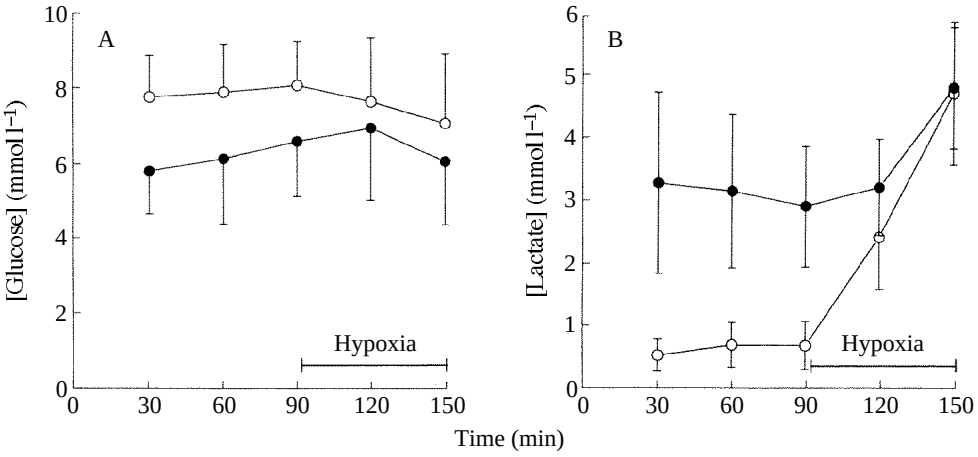

Fig. 2. Arterial (○) and swimbladder venous (●) glucose (A) and lactate (B) concentrations under normoxia and after 30 and 60min of hypoxia (inspiratory P_{O_2} 4.9kPa; mean ± S.D., N=6).

was reduced under hypoxia (Fig. 2). Table 2 summarizes the changes in blood variables, gas deposition, blood perfusion, $\dot{M}_{lac}$ and $\dot{M}_{glu}$ measured during the last 30min of the control period and after 30 and 60min of hypoxia. The nearly unchanged haematocrit of the blood samples taken in series indicates that the experimental protocol did not impair the gas-transport capacity of the blood. Osmolality of venous blood was not significantly different from that in arterial blood, and the difference between two samples collected simultaneously on average did not exceed 3mosmol l^{-1}. Hypoxia caused a significant

Table 2. *Measured and calculated variables during normoxia and hypoxia (series II)*

		Hypoxia	
	Control	30min	60min
$P_{W_{O_2}}$ (kPa)	17.7±1.0	4.3±1.0*	4.9±1.4*
pHa	7.968±0.160	8.040±0.173	7.961±0.177
pHv	7.780±0.113	7.864±0.141	7.757±0.118
Hct_a (%)	29.4±3.0	28.3±3.2	27.3±4.2
Hct_v (%)	28.6±3.6	26.8±4.1	27.8±4.8
$\dot{V}_g$ (ml h^{-1})	0.71±0.27	0.33±0.09*	0.15±0.05*
$\dot{Q}$ (ml min^{-1})	0.47±0.34	0.14±0.09*	0.11±0.05*
$\dot{M}_{lac}$ (μmol min^{-1})	1.21±0.98	0.12±0.08*	0.04±0.09*
$\dot{M}_{glu}$ (μmol min^{-1})	0.75±0.52	0.06±0.04*	0.07±0.04*
[G-6-P] (μmol g^{-1} wetmass)	3.8±1.2	ND	3.7±0.9
[Lactate] (μmol g^{-1} wetmass)	8.5±2.9	ND	14.9±2.2*
Osm_a (mosmol l^{-1})	283.7±28.9	276.8±27.4	277.7±26.7
Osm_v (mosmol l^{-1})	280.8±22.7	269.9±34.7	269.8±36.7

Values are mean ± S.D., N=6.

$P_{W_{O_2}}$, P_{O_2} of water; a, v, arterial and swimbladder venous blood; Hct, haematocrit; $\dot{M}_{lac}$, rate of addition of lactate to the blood; $\dot{M}_{glu}$, rate of removal of glucose from the blood; $\dot{V}_g$, gas deposition rate; $\dot{Q}$, swimbladder blood flow; G-6-P, glucose 6-phosphate; Osm, osmolality; ND, not determined.

*Significantly different from the control value (P<0.05).

reduction in the rate of gas deposition and of blood flow through the swimbladder tissue. The rates of lactate production and glucose consumption, $\dot{M}_{lac}$ and $\dot{M}_{glu}$, were greatly decreased. The glucose 6-phosphate contents of swimbladder epithelial cells under normoxia (tissue sampled in series I) and under hypoxia (series II) were similar, but the tissue lactate content was significantly elevated during hypoxia.

Discussion

Glucose metabolism of the gas-depositing swimbladder

We have previously observed that glucose metabolism of a saline-perfused swimbladder preparation of the European eel *Anguilla anguilla* was not completely balanced by lactate formation (Pelster *et al.* 1989), and the same was observed in the present study with an active gas-depositing swimbladder perfused with blood. Only about 80% of the glucose taken up was converted to lactic acid, calling for other metabolic pathways for glucose metabolism. Thus, the present results qualitatively confirm the previous study, although there are distinct quantitative differences. The rates of glucose uptake by, and of lactate release from, the swimbladder tissue measured in these experiments far exceed those measured with the saline-perfused swimbladder preparation. In the saline-perfused swimbladder preparations, gas deposition was nearly absent, while gas was deposited in this study at a rate of 0.56ml h^{-1}, suggesting that the secretory activity of the bladder is associated with an increase in metabolic activity of the

Table 3. *The contribution of lactate formation (anaerobic glycolysis) and aerobic metabolism (glucose oxidation) to total glucose metabolism and CO_2 production of swimbladder tissue of the European eel*

	Total	Lactate formation	Aerobic metabolism	Pentose phosphate shunt
$\dot{M}_{glu}$ (μmolmin^{-1})	0.72	0.58	0.007	0.14
$\dot{M}_{CO_2}$*(μmolmin^{-1})	0.20	–	0.041	0.16

*Pelster and Scheid (1992*b*).

Glucose metabolism and CO_2 production not accounted for by aerobic and anaerobic glycolysis are listed under pentose phosphate shunt (see text for further explanations).

tissue. Indeed, we observed a significant correlation between the rates of lactate formation and of gas deposition. Glucose metabolism also increased significantly with increasing secretory activity of the swimbladder.

A comparison of the rates of glucose uptake and lactate release in these two preparations shows an 8.2-fold increase in lactate release in the active swimbladder and the rate of glucose uptake also increased 4.3-fold. In the active swimbladder *in situ*, O_2 uptake and CO_2 production were about 3.5 times higher than in the inactive saline-perfused swimbladder preparation (Pelster and Scheid, 1992*b*). Thus, during periods of gas deposition, the fraction of glucose that is converted into lactic acid increases slightly compared with the fraction going through other metabolic pathways, suggesting an important role of lactate formation for swimbladder function.

Using rates of O_2 uptake (0.041 μmolmin^{-1}) and CO_2 formation (0.20 μmolmin^{-1}) measured previously in the same preparation (Pelster and Scheid, 1992*b*) and rates of glucose uptake by and lactate release from the swimbladder tissue measured in this study during periods of gas deposition, a quantitative analysis of glucose metabolism can be performed (Table 3). The fraction of glucose converted into lactate can be calculated from lactate formation (2mol of lactate per mol glucose) and the amount metabolized aerobically from O_2 uptake (6mol of O_2 per mol glucose). This calculation reveals that about 80% of the glucose removed from the blood is converted into lactate, while the O_2 uptake data indicate that less than 1% of glucose is oxidized completely, leaving about 20% for other pathways. Based on the O_2 uptake of the preparation, most of the CO_2 formed must originate from other metabolic pathways, and we suggest that it originates from the pentose phosphate shunt. This is supported by the presence of enzymes of the pentose phosphate shunt in swimbladder tissue (Pelster and Scheid, 1991), and also by the fact that, during *in vitro* incubation with radiolabelled glucose, gas gland tissue does produce CO_2 through the pentose phosphate shunt (Walsh and Milligan, 1993). In the shunt, glucose is decarboxylated once, giving one molecule of CO_2. The rate at which glucose is presumably shifted into the pentose phosphate shunt is almost equal to the rate of evolution of CO_2 from it, suggesting that pentose is not recirculated into the glycolytic pathway. This is supported by the very low activity of the enzymes transaldolase and transketolase measured in gas gland tissue of the toadfish (Walsh and Milligan, 1993).

The influence of hypoxia

To analyse the influence of hypoxia, the gills were ventilated with hypoxic water (P_{O_2} about 4.9kPa). Although this P_{O_2} is not uncommon in the natural habitat of the eel, acute exposure has been shown to provoke marked changes in cardiac activity of the eel (Peyraud-Waitzenegger and Soulier, 1989), and in the present study resulted in an increase in dorsal arterial lactate concentration, indicating that, at least in some tissues, energy metabolism was shifted towards anaerobic glycolysis with formation of lactic acid. In the swimbladder, however, the arterio-venous concentration difference for lactate decreased in spite of a reduction in blood flow. The decrease in glucose uptake and lactate formation during hypoxia far exceeded the concomitant decrease in blood flow, and similar results have been obtained for the oxygen consumption (Pelster and Scheid, 1992*b*). Therefore, the decrease in metabolic activity is not just a consequence of the decrease in perfusion, and the results thus provide strong evidence for a down-regulation of metabolic activity in the swimbladder tissue during hypoxia. Taken together with the positive correlation between the rate of lactate formation and the rate of gas deposition observed under control conditions, the results illustrate the importance of lactate formation by the gas gland tissue for swimbladder function.

The mechanism of lactate extrusion from gas gland cells is unknown, but the increased lactate content of the swimbladder tissue after 1h of hypoxia suggests that not only the production of lactate, but also the release of lactate from the cells, is modified under these conditions. The permeability of the rete mirabile for lactate (Kobayashi *et al.* 1989) and the resulting countercurrent concentration prevent direct evaluation of the exact magnitude of the lactate gradient between gas gland tissue and swimbladder blood (distal to the rete) in the present study. Lactate concentrations in blood collected by blood vessel puncture proximal and distal to the rete mirabile increased by about 2.5–3.5 $\mu mol ml^{-1}$ during arterial passage of the rete and there was a concomitant decrease during venous passage (Steen, 1963; Kobayashi *et al.* 1989). Assuming similar values for the present study, we can expect lactate concentrations of 3–4 $\mu mol ml^{-1}$ in arterial and 6–7 $\mu mol ml^{-1}$ in venous blood distal to the rete under normoxic conditions, with an intracellular content of 8.5 $\mu mol\,g^{-1}$ wetmass. After 1h of hypoxia, the arterial lactate concentration increased by about 4 $\mu mol ml^{-1}$, but tissue lactate content increased by about 6.5 $\mu mol\,g^{-1}$ wetmass. The reduced rate of lactate release from the swimbladder tissue will cause a reduction in the countercurrent exchange of lactate and thus a smaller increase in arterial lactate concentration during passage through the rete (Kobayashi *et al.* 1989). Therefore, the lactate gradient between gas gland cells and blood is expected to increase during hypoxia, indicating a diminished release of lactate from gas gland cells under hypoxic conditions and, in spite of the decreased rate of production, part of the lactate is accumulated within the cells.

Physiological significance of the pentose phosphate shunt

The balance of the glucose metabolism reveals that about 20% of the glucose removed from the blood has been metabolized through the pentose phosphate shunt (see above). The formation of CO_2 appears to be of special importance for the acidification of the

blood, in that it produces an increase in P_{O_2} *via* the Root effect (Root, 1931; cf. Pelster and Weber, 1991). The reduction of the haemoglobin oxygen-carrying capacity will be achieved almost instantaneously during passage of the gas gland cells due to the high diffusability of CO_2 and to the intraerythrocytic carbonic anhydrase reaction. Protons, however, generated by anaerobic glycolysis, do not easily cross membranes and need special transport systems, for example the carbonic anhydrase reaction and anion exchange *via* the band III protein (Nikinmaa, 1992). Plasma is devoid of carbonic anhydrase. The enzyme is present in gas gland cells (Skinazi, 1953; Dorn, 1961), but it is not yet known whether a membrane-bound carbonic anhydrase is available to the plasma. If the dehydration of HCO_3^- is restricted to the uncatalyzed rate, the transfer of the acid into the red cells may be too slow and a disequilibrium in the acid load of the plasma and the erythrocytes may evolve during passage through the gas gland cells. As a consequence, the onset of the Root effect and thus the increase in P_{O_2} will be delayed and may occur after the blood has left the swimbladder epithelium.

Even if we assume a rapid equilibrium of the HCO_3^-/CO_2 reaction in plasma, another problem affects lactic acid release for the single concentrating effect for oxygen. Proton release and the subsequent dehydration of HCO_3^- in plasma will result in an increase in P_{CO_2}, providing a diffusion gradient towards the swimbladder lumen. Part of the CO_2 will disappear into the swimbladder and will no longer be available for the acidification of the red cell, which is necessary to initiate the Root effect. With CO_2 being produced in the gas gland cells, the highest P_{CO_2} must be expected in the cells; from there CO_2 will diffuse into the swimbladder as well as into the blood, preventing a HCO_3^- wash-out due to the extrusion of lactic acid and facilitating the initiation of the Root effect.

Besides the CO_2, NADPH is formed in the pentose phosphate shunt, and it must be reoxidized in order not to stop the shunt. Some enzymes involved in the detoxification of oxygen radicals, such as glutathione reductase, need NADPH as a coenzyme. Swimbladder tissue certainly faces the highest P_{O_2} values in nature. Therefore, it is quite possible that the pentose phosphate shunt serves the dual role of providing CO_2 for the initiation of the Root effect and the deposition of gases, and of providing the reducing equivalents necessary to prevent the tissue from oxygen damage. Walsh and Milligan (1993) indeed reported an increased rate of CO_2 formation in the pentose phosphate shunt under hyperoxic conditions in gas gland tissue of the toadfish.

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