J. exp. Biol. ***197****, 119–128 (1994)*
Printed in Great Britain © The Company of Biologists Limited 1994

CONTRIBUTION OF THE PENTOSE PHOSPHATE SHUNT TO THE FORMATION OF CO_2 IN SWIMBLADDER TISSUE OF THE EEL

B. PELSTER*, J. HICKS AND W. R. DRIEDZIC
Department of Biology, Mount Allison University, Sackville, Canada

Accepted 19 July 1994

Summary

The contribution of the pentose phosphate shunt to glucose metabolism in the swimbladder tissue of the American eel *Anguilla rostrata* has been evaluated by comparing the rate of $^{14}CO_2$ and [^{14}C]lactate production from [1-^{14}C]glucose and [6-^{14}C]glucose. In blood-perfused swimbladder preparations, 0.18±0.07 nmol min^{-1} of [6-^{14}C]glucose and 3.19±0.57 nmol min^{-1} of [1-^{14}C]glucose were converted to CO_2. The rate of [^{14}C]glucose conversion to [^{14}C]lactate was about the same in preparations perfused with [6-^{14}C]glucose and with [1-^{14}C]glucose. This may indicate that the C_5 skeleton formed in the pentose phosphate shunt is not returned to glycolysis and converted to lactate.

Although gas deposition was usually not measurable in these blood-perfused swimbladder preparations, $^{14}CO_2$ was detected in the eel swimbladder gas of preparations perfused with [1-^{14}C]glucose, but not in preparations perfused with [6-^{14}C]glucose.

The results confirm the hypothesis that, in the eel swimbladder epithelium, some of the glucose taken up from the blood is metabolized in the pentose phosphate shunt. This results in the formation of CO_2, which is released into the swimbladder as well as into the bloodstream.

Introduction

In the swimbladder, high gas partial pressures are generated in two steps, by decreasing the physical gas solubility or the gas-carrying capacity of the blood (the ‘single concentrating effect’), resulting in an initial increase in gas partial pressure in the blood, and by the subsequent multiplication of this single concentrating effect in the countercurrent system of the rete mirabile (Fänge, 1983; Pelster and Scheid, 1992*a*). The single concentrating effect is initiated by the metabolism of the swimbladder tissue. Gas gland cells of the swimbladder tissue produce lactic acid, and the release of lactate and H^+ into the blood results in an increase in the partial pressure of all blood gases through the

*Present address: Institut für Physiologie, Ruhr-Universität Bochum, 44780 Bochum, FRG.

Key words: swimbladder, glucose metabolism, lactate, pentose phosphate shunt, CO_2 formation, eel, *Anguilla rostrata.*

salting-out effect, the Root effect and by conversion of HCO_3^- to CO_2 (Pelster and Scheid, 1993*a*).

Recent studies suggest that, in the metabolism of gas gland cells, some of the glucose is also shifted towards the pentose phosphate shunt, forming CO_2 without concomitant consumption of oxygen (Pelster, 1994). This contention is based on high activities of key enzymes of the pentose phosphate shunt in gas gland tissue of various fishes (Boström *et al.* 1972; Ewart and Driedzic, 1990; Pelster and Scheid, 1991; Walsh and Milligan, 1993) and on CO_2 formation without equivalent consumption of O_2 observed in a Ringer-perfused swimbladder preparation as well as in the active, gas-depositing swimbladder of the European eel (*Anguilla anguilla*) *in situ* (Pelster *et al.* 1989; Pelster and Scheid, 1992*b*). In addition, gas gland tissue of the toadfish *Opsanus beta*, incubated *in vitro* with [1-^{14}C]glucose, produced more $^{14}CO_2$ than when it was incubated with [6-^{14}C]glucose (Walsh and Milligan, 1993).

In spite of this circumstantial evidence, direct proof for the formation of CO_2 in the pentose phosphate shunt *in situ* is still lacking. In the present study, the contribution of the pentose phosphate shunt to the formation of CO_2 in swimbladder tissue of the American eel *Anguilla rostrata* has been analysed by comparing the rate of $^{14}CO_2$ and [^{14}C]lactate production from [1-^{14}C]glucose and from [6-^{14}C]glucose in a blood-perfused swimbladder preparation.

Materials and methods

Specimens of the American eel *Anguilla rostrata* (LeSueur) (body mass 380–646 g) were obtained from a local supplier and kept in a freshwater aquarium with aerated well water at 16–18 °C for a minimum period of 4 days. The fish were not fed, and the photoperiod was 12 h:12 h L:D.

Animal preparation

The eels were quickly decerebrated and spinally pithed. The immobilized eels were placed into an eel-holder, and the gills were irrigated with aerated water (20±1 °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 part of the swimbladder and the blood vessels from other tissues entering the swimbladder vein were ligated. The artery supplying the retia was carefully separated from the rete effluent vein and occlusively cannulated (PE 20). A second catheter (PE 50) was inserted occlusively into the swimbladder vein for collection of the venous blood.

An additional catheter in the swimbladder allowed the collection of gas samples. After emptying the swimbladder, 500 μl of swimbladder gas was returned to the bladder to prevent the swimbladder walls from sticking together.

At the end of the experiment, the epithelium of the secretory part of the swimbladder was quickly separated from connective tissue, rinsed in Ringer's solution to remove most of the blood, blotted dry and frozen in liquid nitrogen for later analysis of lactate concentration. The mass of the epithelial tissue was usually about 0.3–0.6 g. The tissue was stored in liquid nitrogen until further processing.

Experimental protocol

From immobilized donor animals, a blood pool of about 25 ml was collected for blood perfusion and equilibrated with 0.5 % CO_2 in air. During the experiments, the blood pool was kept under the same gas mixture and placed in a shaker bath. Haematocrit readings in arterial and venous samples remained stable throughout each single experiment, indicating that there was no sedimentation of blood cells, and no haemolysis was detectable.

[1-^{14}C]glucose or [6-^{14}C]glucose (typically about 0.2 μCi ml^{-1} blood; 1 μCi=37 GBq) was added to the blood pool 2–3 min before starting the sampling period at T=0 min.

Using a peristaltic pump, the swimbladder was perfused at a flow rate of about 0.2 ml min^{-1}. Venous blood leaving the swimbladder was collected for 10 min periods, providing six venous samples during the total 1 h perfusion time. The flow rate was determined by measuring the volume of the venous samples. Blood samples (1 ml) from the blood pool (=arterial samples) were taken every 30 min (T=0, 30 and 60 min). Eel red blood cells are metabolically active, and data for them will be presented elsewhere. After the last arterial and venous blood samples had been taken, at T=60 min, all gas was withdrawn from the swimbladder using a gas-tight syringe and the swimbladder tissue was sampled as described earlier.

Sample preparation

Blood samples were centrifuged at 1000 ***g*** for 5 min to separate blood cells, and the plasma was used for further analysis. 500 μl of plasma was transferred to an incubation flask with a CO_2 trap filled with 200 μl of methoxyethanol/ethanolamine (7:1, Eastman Kodak). After the flask had been sealed, the plasma was acidified by injecting 500 μl of perchloric acid (6 %). The flask was incubated for 1 h in a shaker bath, and thereafter the CO_2 trap was transferred to a scintillation vial filled with 10 ml of CytoScint (ICN).

The acidified plasma was neutralized for enzymatic determination of metabolite concentrations. 500 μl of the neutralized plasma extract was applied to a prepacked anion exchange column (BioRad, AG 1-X8) to separate glucose and lactate. Lactate was eluted using 6 mol l^{-1} hydrochloric acid; the enzymatic test revealed a complete separation of glucose and lactate. The fraction with the highest lactate concentration was used for activity determination and calculation of the specific activity of [^{14}C]lactate in this fraction, and thus in plasma, where:

$$\frac{^{14}\text{C activity (disints min}^{-1}\text{ ml}^{-1})}{\text{[lactate] (}\mu\text{mol ml}^{-1}\text{)}} = \text{specific activity of lactate (disints min}^{-1}\,\mu\text{mol}^{-1}\text{)}.$$

The glucose concentration and total activity in the plasma of the first arterial sample taken (at T=0, i.e. 2–3 min after adding the labelled glucose to the blood pool) were determined to calculate the specific activity of [^{14}C]glucose in a similar way.

For determination of tissue lactate concentration, the swimbladder epithelium was extracted with four times its volume of perchloric acid (6 %). A sample of the neutralized tissue extract was applied to the anion exchange column, to separate glucose from labelled anionic intermediates. The fraction with the highest lactate concentration was

used for activity determination. Besides lactate, this fraction may have contained other labelled anionic intermediates. As a result of the high lactate content of the tissue, however, lactate would be the organic compound in greatest concentration in this fraction. Therefore, the specific activity of lactate calculated from these data probably represents a small overestimation. This analysis was carried out because the relative contents of the other intermediates in the fractions analysed were expected to be similar in all of the samples as they had been treated identically, and they therefore provide useful information about the fraction of label incorporated into glycolytic intermediates in the swimbladder tissue at the end of the experiment.

Gas samples taken at the end of the experiment were transferred to a closed vessel containing a CO_2 trap for CO_2 collection.

Analytical procedures

Gas deposition was measured volumetrically by repeated gas sampling using a gas-tight syringe. The pH of the blood pool was measured using a Fisher Accumet model 805MP meter, and the haematocrits (Hct) of arterial and venous samples were determined using an Adams Readacrit microhaematocrit centrifuge. Glucose and lactate concentrations of the neutralized plasma and tissue extract were measured enzymatically using Sigma test kits. ^{14}C activities of CO_2 and lactate were assessed in a LKB Scintillation counter.

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 plasma flow, $\dot{Q}_{pl}$, and the glucose and lactate concentration differences between arterial and venous plasma.

The amount of label present as [^{14}C]lactate in blood (disints min^{-1} ml^{-1}) was calculated from the specific activity of lactate (disints min^{-1} μmol^{-1}) in arterial and venous blood, multiplied by the arterial and venous blood lactate concentrations (μmol ml^{-1}; in some experiments a slowly increasing degree of labelling in arterial blood indicated a low rate of lactate formation in the arterial blood pool, although the blood was equilibrated at high P_{O_2}). The arteriovenous difference in the amount of label present as [^{14}C]lactate in plasma multiplied by plasma flow ($\dot{Q}_{pl}$) yields the amount of label released as [^{14}C]lactate from the swimbladder tissue. The amount of glucose converted into lactate was calculated from the amount of label released as [^{14}C]lactate divided by the specific activity of glucose. Similarly, the amount of glucose converted to CO_2 was calculated from the rate of $^{14}CO_2$ released from the swimbladder tissue divided by the specific activity of glucose. Overall mean values were then calculated by averaging the last 3–4 values of each individual experiment, when the rate of ^{14}C release of the tissue had stabilized.

Statistical analysis

Data are presented as mean ± S.E.M. Statistical differences between groups were tested using Student's *t*-test. Significance of differences was accepted when $P<0.05$.

Results

Variables describing the perfusion conditions and the metabolic activity of swimbladder tissue perfused with either [1-^{14}C]glucose or [6-^{14}C]glucose are listed in Table 1. There was no significant difference between these two series of experiments. Therefore, the net rates of glucose removal from plasma, $\dot{M}_{glu}$, and lactate addition to it, $\dot{M}_{lac}$, presented in Fig. 1 were calculated from pooled values for both types of label. The rates were fairly constant over the 60 min period, indicating stability of the preparation. Overall mean values for lactate addition to, and glucose removal from, the plasma, calculated from mean

Table 1. *Mean values of measured and calculated variables, summarizing the perfusion conditions and the metabolic activity of the swimbladder tissue*

	[1-^{14}C]glucose	[6-^{14}C]glucose
pH	7.74±0.04	7.72±0.05
Haematocrit (%)	22.6±0.7	22.4±0.9
Arterial [glucose] (T=0) (mmol l^{-1})	3.6±0.2	4.0±0.4
Arterial [lactate] (T=0) (mmol l^{-1})	1.4±0.2	1.1±0.1
$\dot{Q}$ (ml min^{-1})	0.19±0.01	0.18±0.01
$\dot{M}_{glu}$ (μmol min^{-1})	0.14±0.02	0.14±0.03
$\dot{M}_{lac}$ (μmol min^{-1})	0.21±0.03	0.18±0.02
Tissue [lactate] (μmol g^{-1} wet mass)*	7.7±0.8	9.1±0.9

Values are mean ± S.E.M., N = 7; *N=5.
$\dot{Q}$, blood flow.

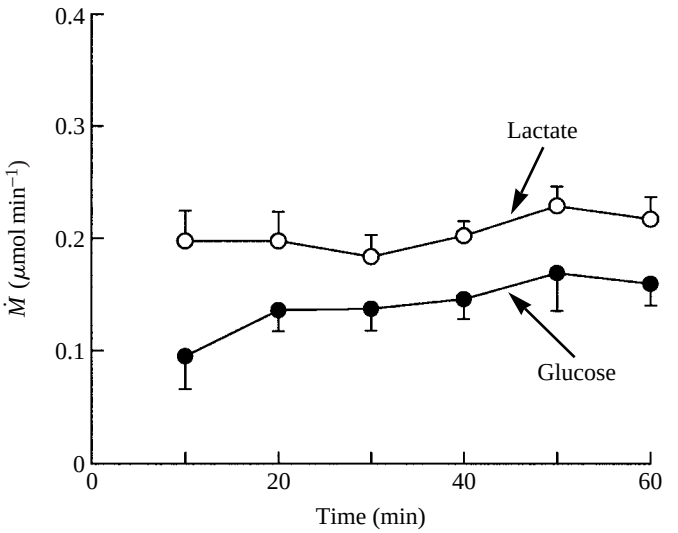

Fig. 1. Rates of glucose removal from ($\dot{M}_{glu}$), and lactate addition to the plasma ($\dot{M}_{lac}$), calculated from the arteriovenous concentration difference in plasma and the plasma flow through the swimbladder over the course of the experiments (N=14). Values are mean ± S.E.M.

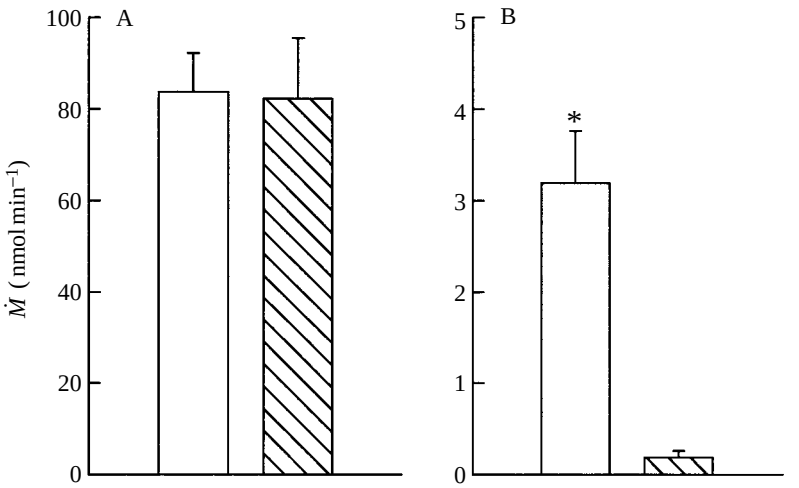

Fig. 2. Rates of [^{14}C]lactate (A) and $^{14}CO_2$ (B) production from [1-^{14}C]glucose (open bars) and [6-^{14}C]glucose (hatched bars). Values are mean + S.E.M., N=7. * indicates significant differences between values.

values obtained at the six sampling times, were 0.20±0.01 μmol min^{-1} and 0.14±0.01 μmol min^{-1}, respectively, giving a ratio of $\dot{M}_{lac}/\dot{M}_{glu}$ of 1.43.

The release of [^{14}C]lactate from swimbladder tissue was usually detectable in the first venous sample, collected over the first 10 min after starting the perfusion with [^{14}C]glucose. The later venous samples always showed the release of [^{14}C]lactate in both [1-^{14}C]glucose and [6-^{14}C]glucose perfusions. The release of $^{14}CO_2$ was also detectable in the first venous sample, but only in perfusions containing [1-^{14}C]glucose. In experiments with [6-^{14}C]glucose, traces of $^{14}CO_2$ produced in the swimbladder tissue could be detected in only some of the venous samples, collected towards the end of the experiment. Fig. 2 shows the amount of glucose converted to either [^{14}C]lactate or $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose. While only traces of [6-^{14}C]glucose were converted to $^{14}CO_2$, a significantly greater amount of $^{14}CO_2$ was released from the swimbladder tissue from [1-^{14}C]glucose. In contrast, the amounts of [^{14}C]lactate released from the tissue did not differ significantly in these experiments. The rates of [^{14}C]lactate production were about 20 and 400 times higher than the rates of $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose, respectively.

At the beginning of an experiment 500 μl of gas was left in the swimbladder, and withdrawal of gas at the end of the perfusion revealed no measurable increase in the gas volume in these preparations. Nevertheless, the gas samples were analysed for the presence of activity, and $^{14}CO_2$ was never detected in preparations perfused with [6-^{14}C]glucose. In preparations perfused with [1-^{14}C]glucose, however, $^{14}CO_2$ was always detected in the gas samples, indicating the decarboxylation of 1.1±0.4 nmol h^{-1} (0.018 nmol min^{-1}) of glucose during the experiment.

The lactate content of the tissue samples taken at the end of the experiment amounted to 8.43±0.64 μmol g^{-1} wet mass (N=10). The ratio of the specific activity of tissue

'lactate' to glucose was 0.23±0.09 in preparations perfused with [6-^{14}C]glucose and 0.18±0.08 in preparations perfused with [1-^{14}C]glucose (N=5). Thus, ^{14}C activity in the anion fraction of swimbladder tissue perfused with [6-^{14}C]glucose was 22 % higher than in tissue perfused with [1-^{14}C]glucose.

Discussion

Metabolic activity of the swimbladder tissue

A comparison of the rates of lactate release and glucose uptake of the blood-perfused swimbladder preparation indicated that about 72 % of the glucose removed from the blood was converted into lactate. Thus, the ratio of $\dot{M}_{lac}/\dot{M}_{glu}$ as well as the metabolic activity of the swimbladder are very similar to the values obtained with a saline-perfused swimbladder preparation of the European eel ($\dot{M}_{lac}$=0.14 μmol min^{-1}; Pelster *et al.* 1989). *In situ* a much higher metabolic activity has been observed in the swimbladder of the European eel ($\dot{M}_{lac}$=1.16 μmol min^{-1}), but the ratio of $\dot{M}_{lac}/\dot{M}_{glu}$ was also about 1.6 (Pelster and Scheid, 1993*b*). These studies demonstrate that in the eel swimbladder 70–80 % of the glucose removed from the blood is converted to lactate. The results indicate that the metabolic activity of the tissue is correlated to the rate of gas deposition. In artificially perfused preparations, the rate of gas deposition was not measurable, while gas was deposited at a rate of 0.56 ml h^{-1} *in situ*, and the metabolic activity was significantly higher (Pelster and Scheid, 1993*b*). Thus, the results obtained with the blood-perfused swimbladder preparation of the American eel are similar to those reported for the swimbladder of the European eel.

Pathways for CO_2 formation

A generalized diagram of possible metabolic pathways for the fate of labelled carbon in the swimbladder tissue is presented in Fig. 3. In preparations perfused with [6-^{14}C]glucose, only traces of $^{14}CO_2$ were detected in the venous blood leaving the swimbladder. Therefore, aerobic metabolism of the swimbladder tissue must be very low, confirming previous studies where, based on the measurement of oxygen consumption, a low aerobic activity of swimbladder tissue was observed *in vitro* (Ewart and Driedzic, 1990) and *in situ* (Pelster and Scheid, 1992*b*). In contrast, significant amounts of $^{14}CO_2$ were detected in the venous blood in experiments with [1-^{14}C]glucose, and a comparison of the rate of $^{14}CO_2$ production from [1-^{14}C]glucose and [6-^{14}C]glucose clearly demonstrates that most of the CO_2 is produced in the swimbladder tissue *via* the decarboxylation reaction of the enzyme 6-phosphogluconate dehydrogenase in the pentose phosphate shunt (PPS, Fig. 3).

Additional support for this conclusion is provided from the presence or absence of $^{14}CO_2$ in the swimbladder lumen. In these preparations, gas deposition was not measurable (i.e. below 0.1 ml h^{-1}), and we can assume that there was not *net* diffusion of CO_2 into the swimbladder lumen. The presence of $^{14}CO_2$ in the swimbladder gas phase indicates diffusional exchange between gas gland cells and the swimbladder gas and thus the presence of $^{14}CO_2$ in the gas gland cells. That $^{14}CO_2$ was always detectable in the gas phase of the swimbladder in experiments with [1-^{14}C]glucose, but never in experiments

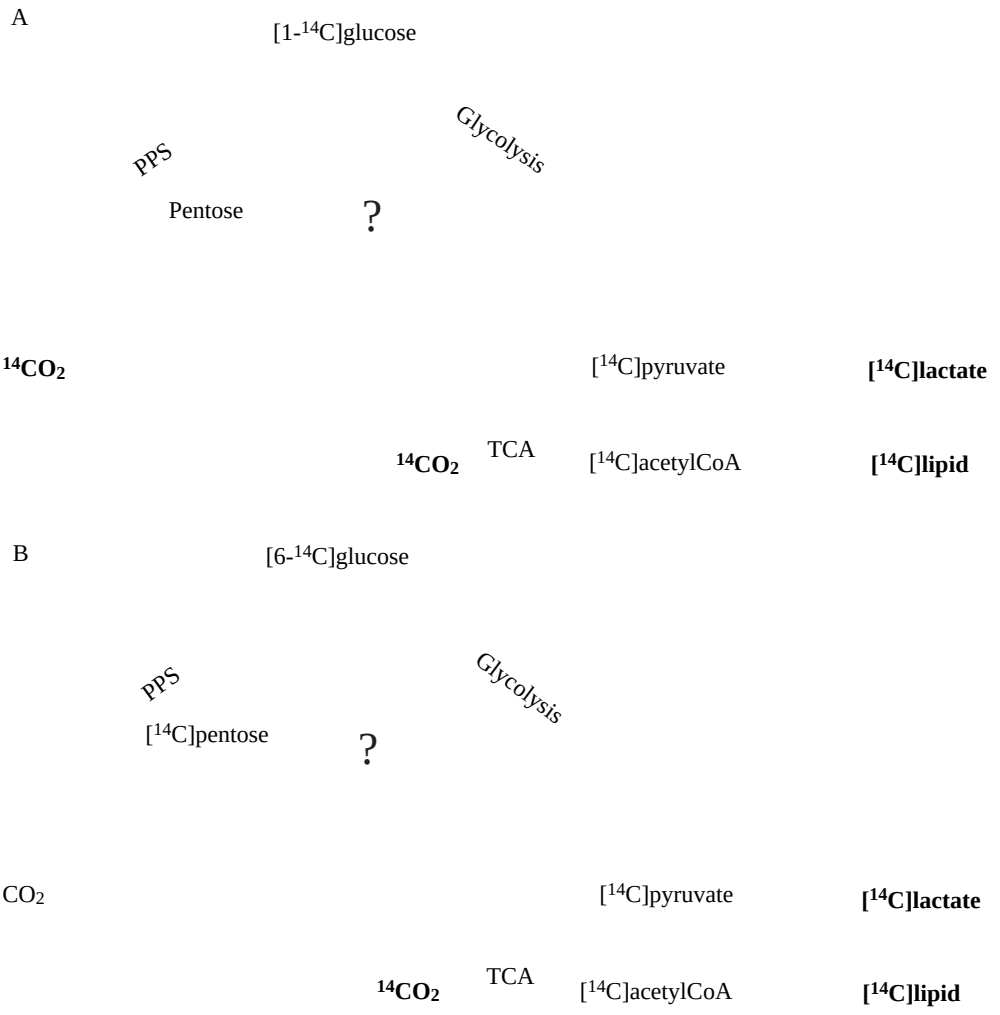

Fig. 3. Schematic diagram of possible metabolic pathways for the catabolism of [1-^{14}C]glucose (A) and [6-^{14}C]glucose (B) in swimbladder tissue. The labelled metabolic end products measured in the present study are presented in bold type. PPS, pentose phosphate shunt; TCA, tricarboxylic acid cycle.

with [6-^{14}C]glucose, indicates that gas gland cells produce more $^{14}CO_2$ from [1-^{14}C]glucose than from [6-^{14}C]glucose. Without overall gas deposition, the amount of glucose that must have been decarboxylated to account for this $^{14}CO_2$ was negligible (1.1 nmol h^{-1}) compared with the amount that was decarboxylated to account for the release of $^{14}CO_2$ into the bloodstream (Fig. 2). During periods of gas deposition, the metabolic activity of the swimbladder tissue is greatly increased (as discussed above) and the proportion of CO_2 in newly secreted gas amounts to about 25 % (Kobayashi *et al.* 1990). In this situation, the CO_2 produced in the pentose phosphate shunt will contribute significantly to the filling of the swimbladder.

In preparations perfused with [1-^{14}C]glucose, part of the label is removed as $^{14}CO_2$ as a result of the decarboxylation reaction in the pentose phosphate shunt. Nevertheless, in these preparations the release of [^{14}C]lactate was not significantly different from that measured in preparations perfused with [6-^{14}C]glucose. This may indicate that the C_5 skeleton (pentose) formed in the pentose phosphate shunt is not returned to glycolysis for conversion to lactate. On the basis of measurements of enzyme activities, Walsh and Milligan (1993) also suggested that the pentose was not returned to glycolysis but was used for anabolic metabolism, such as RNA production and protein synthesis. In preliminary experiments, we have performed a lipid extraction on some of the tissue samples taken after 1 h of perfusion with [^{14}C]glucose and, in all of these samples, there was ^{14}C activity in the lipid fraction. Part of the carbon skeleton, therefore, may have been used for the synthesis of fatty acids. It appears quite possible that the pentose produced by decarboxylation of glucose is shifted towards other metabolic pathways.

Measurement of $^{14}CO_2$ production relative to that of [^{14}C]lactate from [1-^{14}C]glucose reveals that approximately 4–5 % of the carbon flow is *via* the shunt. Owing to diffusional loss of CO_2 during the collection of venous blood samples, this value could be an underestimate. The relative amounts of labelled anionic intermediates in the swimbladder tissue were about 22 % lower following perfusion with [1-^{14}C]glucose than in preparations perfused with [6-^{14}C]glucose. On the basis of indirect evidence, Pelster and Scheid (1993*b*) suggested that, in the European eel swimbladder, about 20 % of glucose removed from the blood was shifted towards the pentose phosphate shunt.

$\dot{M}_{lac}$ *and [^{14}C]lactate formation*

On the basis of the specific activities it was calculated that about 80 nmol min^{-1} of glucose was converted into lactate, resulting in a lactate production of 160 nmol min^{-1}. From the arteriovenous concentration difference for lactate and the perfusion rate, a value of 200 nmol min^{-1} was calculated for $\dot{M}_{lac}$. This discrepancy may be due to incomplete mixing of the labelled glucose and intermediates with endogenous substrate pools within the short period of our perfusion experiment (Sephton *et al.* 1991). For instance, it is probable that gas gland cells synthesize lipids, because a high lipid concentration is usually found in the swimbladder tissue (Phleger, 1991) and we observed ^{14}C activity in the lipid fraction of the tissue (see above).

Similar comparisons of $\dot{M}_{glu}$, calculated from $\dot{M}_{O_2}$ and the disappearance of labelled glucose, or of $\dot{M}_{CO_2}$, assessed either by measuring C_{CO_2} (total concentration of CO_2) or by measuring $^{14}CO_2$, have been reported for fish red blood cells (Walsh *et al.* 1990; Sephton *et al.* 1991) and for fish heart muscle (Sephton *et al.* 1990; Bailey and Driedzic, 1993). In all of these studies, the metabolic activity calculated from the production or disappearance of ^{14}C-labelled metabolites represented an underestimate, in one study by a factor of 1000 (Sephton *et al.* 1991). In the swimbladder experiments, the mismatch was much smaller than in most other studies, probably because of the relatively high glycolytic flux, which causes a more rapid equilibration of the label with the endogenous substrate pool. [^{14}C]lactate was detected even in the venous samples taken 10 min after starting the perfusion, indicating that the whole glycolytic pathway, starting with glucose uptake from the blood and ending with the release of lactate into the blood, can be

completed within 10 min in eel swimbladder tissue. When perfusing with [1-^{14}C]glucose, the same was observed for $^{14}CO_2$.

The results presented here demonstrate that the metabolism of the eel swimbladder tissue is highly specialized to produce acidic metabolites at a very low rate of citric acid cycle activity, despite high levels of available oxygen. The results provide proof that, in the swimbladder tissue of the eel, CO_2 is formed in the pentose phosphate shunt and is released from the cells into the bloodstream as well as into the swimbladder. The respiratory exchange ratio is far above unity and, in terms of gas deposition, gas gland cells thus not only produce acid to reduce the gas transport capacity of the blood but actually produce gas molecules that enter the swimbladder lumen.

Financial support by the Bundesministerium für Forschung und Technologie, Germany, through the International Exchange Office GKSS (KAN AQ-38) to B.P., and of the Natural Sciences and Engineering Research Council of Canada to W.R.D. is gratefully acknowledged.

References

BAILEY, J. R. AND DRIEDZIC, W. R. (1993). Influence of low temperature acclimation on fate of metabolic fuels in rainbow trout (*Oncorhynchus mykiss*) hearts. *Can. J. Zool.* **71**, 2167–2173.

BOSTRÖM, S. L., FÄNGE, R. AND JOHANSSON, R. G (1972). Enzyme activity patterns in gas gland tissue of the swimbladder of the cod (*Gadus morhua*). *Comp. Biochem. Physiol.* **43**B, 473–478.

EWART, H. S. AND DRIEDZIC, W. R. (1990). Enzyme activity levels underestimate lactate production rates in cod (*Gadus morhua*) gas gland. *Can. J. Zool.* **68**, 193–197.

FÄNGE, R. (1983). Gas exchange in fish swim bladder. *Rev. Physiol. Biochem. Pharmac.* **97**, 111–158.

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.

PELSTER, B. (1994). Metabolism of the swimbladder tissue. *Biochem. molec. Biol. Fishes* (in press).

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

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*a*). Countercurrent concentration and gas secretion in the fish swimbladder. *Physiol. Zool.* **65**, 1–16.

PELSTER, B. AND SCHEID, P. (1992*b*). The influence of gas gland metabolism and blood flow on gas deposition into the swimbladder of the eel *Anguilla anguilla*. *J. exp. Biol.* **173**, 205–216.

PELSTER, B. AND SCHEID, P. (1993*a*). Metabolism of the swimbladder epithelium and the single concentrating effect. *Comp. Biochem. Physiol.* **105**A, 383–388.

PELSTER, B. AND SCHEID, P. (1993*b*). Glucose metabolism of the swimbladder tissue of the European eel *Anguilla anguilla*. *J. exp. Biol.* **185**, 169–178.

PHLEGER, C. F. (1991). Biochemical aspects of buoyancy in fishes. *Biochem. molec. Biol. Fishes* **1**, 209–247.

SEPHTON, D. H., BAILEY, J. AND DRIEDZIC, W. R. (1990). Impact of acute temperature transition on enzyme activity levels, oxygen consumption and exogenous fuel utilization in sea raven (*Hemipterus americanus*). *J. comp. Physiol.* B **160**, 511–518.

SEPHTON, D. H., MACPHEE, W. L. AND DRIEDZIC, W. R. (1991). Metabolic enzyme activities, oxygen consumption and glucose utilization in sea raven (*Hemipterus americanus*) erythrocytes. *J. exp. Biol.* **159**, 407–418.

WALSH, P. J. AND MILLIGAN, C. L. (1993). Roles of buffering capacity and pentose phosphate pathway activity in the gas gland of the gulf toadfish, *Opsanus beta*. *J. exp. Biol.* **176**, 311–316.

WALSH, P. J., WOOD, C. M., THOMAS, S. AND PERRY, S. F. (1990). Characterization of red blood cell metabolism in rainbow trout. *J. exp. Biol.* **154**, 475–489.