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METABOLISM OF THE PERFUSED SWIMBLADDER OF THE EUROPEAN EEL: OXYGEN, CARBON DIOXIDE, GLUCOSE AND LACTATE BALANCE

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Summary

We have measured the metabolic activity in the vascularly isolated, saline-perfused swimbladder of the eel (*Anguilla anguilla*) in order to investigate the pathways for CO_2 formation in the gas gland tissue. Concentrations of O_2, CO_2, glucose and lactate were measured in the arterial inflow and venous outflow of the swimbladder, and metabolic rates were calculated by the direct Fick principle.

1. Total CO_2 production, averaging 55·8 nmol min^{-1}, was about 4·6 times the O_2 consumption (mean 12·0 nmol min^{-1}). This suggests that only about 22% of the CO_2 is formed by aerobic glucose metabolism.

2. CO_2 formation from HCO_3^- or CO_2 washout does not appear to be significant in our experiments with steady perfusion of a saline containing a low level of HCO_3^-.

3. The ratio of lactate production to glucose uptake averaged 1·2, indicating that only 60% of the glucose is converted to lactate. Since only 1–2% of the glucose was found to be oxidized (2 nmol min^{-1}), the extra glucose appears to be anoxidatively metabolized to CO_2.

4. The anoxidative CO_2 formation appears to be of functional importance for producing the high gas partial pressures of both CO_2 and O_2 which are required for secretion of these gases into the swimbladder.

Introduction

Many teleost fishes have developed a gas-filled swimbladder which enables them to keep neutral buoyancy at varying water depths. It is generally accepted that the gas enters the bladder from the blood by diffusion, and it is assumed that the high gas partial pressures in the blood perfusing the swimbladder are generated by acidification of the blood in the gas gland in conjunction with the countercurrent gas exchange in the rete mirabile (Steen, 1970; Fänge, 1983). In fact, the gas gland is known to produce lactic acid even at high O_2 levels. This acid enhances, *via* the Bohr and Root effects, the O_2 partial pressure and, by the conversion of HCO_3^- to CO_2, the CO_2 partial pressure. The formation of lactate by gas gland cells has been demonstrated both in tissue preparations (Ball *et al.* 1955; D'Aoust, 1970; Deck,

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1970) and *in vivo* (Kuhn *et al.* 1962; Steen, 1963*b*; Enns *et al.* 1967). In this model, CO_2 in the swimbladder would derive either from oxidative metabolism or from HCO_3^-.

Oxidative metabolism has been estimated to contribute only 5 % of the total CO_2 formed (Wittenberg *et al.* 1964). Ball *et al.* (1955), Wittenberg *et al.* (1964) and D'Aoust (1970) found that most, if not all the CO_2 was liberated from bicarbonate, presumably by lactic acid. This assumption neglects, however, the presence of nonbicarbonate buffers, which would buffer some of the H^+ released from lactic acid, thus reducing the amount of H^+ available for conversion of HCO_3^- to CO_2. Therefore, a lactate/CO_2 ratio of unity, found by Ball *et al.* (1955) and D'Aoust (1970), can only be explained if CO_2 derives from sources other than bicarbonate.

Such a source could be the decarboxylation reaction of the pentose phosphate shunt, which yields CO_2 from carbohydrates without consuming O_2. Boström *et al.* (1972) measured high activities of enzymes characteristic of the pentose phosphate shunt in the gas gland tissue of the cod, and increases in CO_2 content in blood passing through the gas gland have indeed been measured directly (Steen, 1963*b*; Pelster *et al.* 1988*b*). The contribution of CO_2 *via* this route has not been estimated yet.

In the present study, we have measured the metabolic activity in the saline-perfused eel swimbladder to evaluate the contributions of the various pathways to CO_2 production. The saline-perfused preparation was preferred to the more physiological, blood-perfused swimbladder, as it more easily allows measurement of the perfusion rate and quantitative estimation of metabolic rates.

Materials and methods

Specimens of the freshwater-adapted European eel (*Anguilla anguilla*; body mass 500–1100 g) were purchased from a local supplier and kept in a freshwater aquarium at 12–14 °C until the experiment.

Animal preparation and experimental set-up

The animals were anaesthetized by adding MS 222 ($0{\cdot}1\,g\,l^{-1}$) to the water and were then placed into an 'eel holder', similar to that used by Steen (1963*a*). The gills were irrigated with well-oxygenated tap water (flow rate $4{\cdot}5\,l\,min^{-1}$), containing MS 222 at a concentration of approximately $0{\cdot}05\,g\,l^{-1}$, a level sufficient to keep the animal anaesthetized with stable ventilatory activity.

Preparation of animals was essentially the same as described elsewhere (H. Kobayashi, B. Pelster & P. Scheid, in preparation). Briefly, the body wall was opened ventrally, the swimbladder was exposed and carefully freed from connective tissue. The duct connecting the absorption and secretion part of the bladder was tied off, and small blood vessels and anastomoses bypassing the rete mirabile were carefully ligated without damaging rete vessels or the swimbladder wall. The gas secretion rate was too low to be measured accurately by a catheter inserted

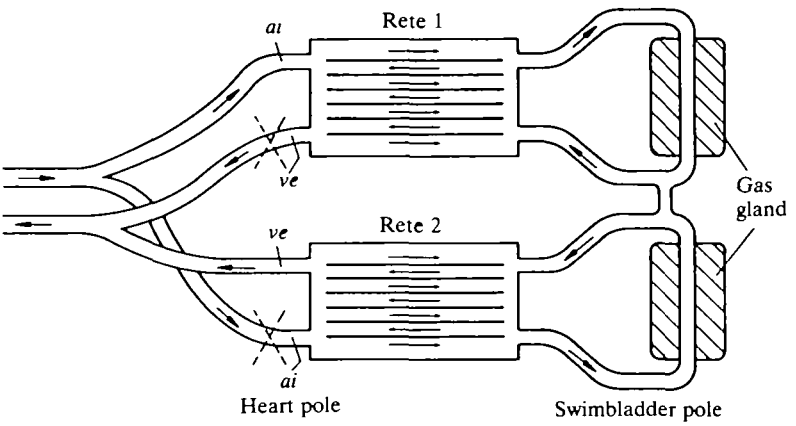

Fig. 1. Diagram of the two retia in the eel swimbladder. The arterial inflow (*ai*) and venous outflow vessels (*ve*) at the heart pole were occlusively cannulated for perfusion. For serial perfusion (type S), *ai* of one and *ve* of the other rete were blocked (see Materials and methods).

into the swimbladder (less than $0{\cdot}1\,ml\,h^{-1}$). All experiments were carried out at 20–22°C.

The artery and vein at the heart pole (*ai* and *ve* in Fig. 1) were occlusively cannulated using PE 50 and PE 100 catheters, respectively, and the rete vessels were perfused with saline solution at $0{\cdot}13$–$0{\cdot}40\,ml\,min^{-1}$ using an infusion pump (Precidor, Type 5003, Infors AG, Basel, Switzerland). This solution was a heparinized ($100\,i.u.\,ml^{-1}$) Ringer's solution which contained (in $mmol\,l^{-1}$) NaCl, 129; KCl, 5; $MgSO_4$, 0·9; $CaCl_2$, 1·1; glucose, 15; insulin, 8 i.u. The colloid osmotic pressure was adjusted to that of eel plasma by adding $5\,g\,l^{-1}$ Dextran FP 70 (Serva, Heidelberg, FRG). The saline contained no bicarbonate and was equilibrated with air so as to remove nearly all CO_2.

Experimental protocol

Samples of the perfusate were collected at the venous outlet (*ve*), and their composition was compared with that of the perfusate at the inflow (*ai*). Venous collection was about every 30 min for 3–4 h. For calculating metabolic rates from perfusion rate and concentrations in inflow and outflow, steady-state conditions have to prevail. Since countercurrent exchange in the rete during normal flow conditions (perfusion type C) retards attainment of steady state, a serial type of rete flow (type S) was achieved in some experiments by occluding the afferent artery of one and the efferent vein of the other rete at the heart pole (Fig. 1), this flow type enhanced attainment of steady state. Measurements were made in these experiments during both type C and type S flow.

Analytical procedures

Saline samples were analysed for oxygen content (C_{O_2}; using the method of

Tucker, 1967) and total CO_2 content (C_{CO_2}; using the method of Cameron, 1971) immediately after collection. For analysis of glucose and lactate concentrations, part of the sample was deproteinized with perchloric acid and neutralized with K_2CO_3 or KOH. Employing this procedure, no extrusion of proteins from the tissue into the perfusate was detected. The assays were performed enzymatically as outlined by Bergmeyer (1974).

Calculations

The rates of production of CO_2 ($\dot{M}_{CO_2}$) and lactate ($\dot{M}_{La}$) and the rates of consumption of O_2 ($\dot{M}_{O_2}$) and glucose ($\dot{M}_{Glu}$) were calculated by mass balance from the perfusion rate ($\dot{Q}$) and the concentration difference between inflow and outflow. Student's *t*-tests were used to test for statistical significance, and $P < 0{\cdot}01$ was accepted as the limit of significance.

Results

At the onset of perfusion, there were high concentrations of lactate and CO_2 in the venous outflow (*ve*) which gradually decreased to a steady-state level. For both substances this was higher than the arterial level (*ai*; Fig. 2). For calculation of metabolic rates only steady-state values were taken into account.

Table 1 shows concentrations of O_2, CO_2 and lactate in inflow (*ai*) and outflow (*ve*) of the rete during perfusion at two flow rates when there was no countercurrent flow (type S). The observed differences in these concentrations

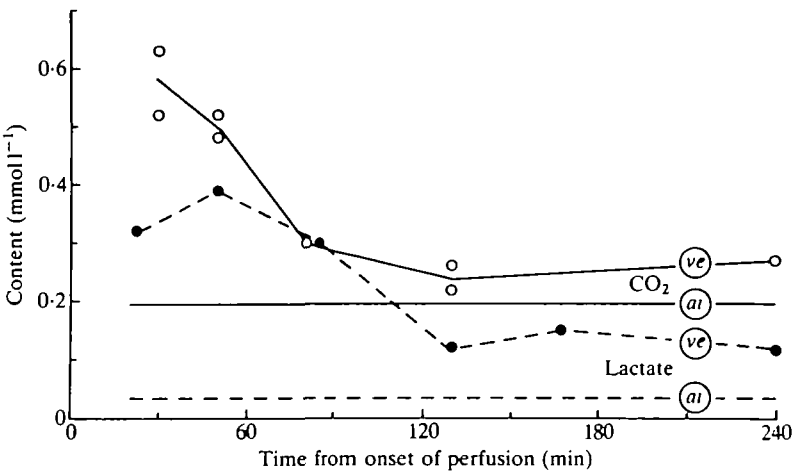

Fig. 2. Concentrations of total CO_2 (open circles, continuous line) and lactate (closed circles, dashed line) in the venous outflow (*ve*) of a saline-perfused swimbladder (perfusion type C) plotted against time from onset of perfusion. Concentrations in the perfusate at the entrance to the rete (*ai*) are presented as horizontal lines. Values of *ve* for $t > 120$ min were averaged in this experiment to yield the steady-state value from which metabolic activity was calculated.

Table 1. *Arterial inflow* (ai) *and venous outflow* (ve) *concentrations of* O_2 (C_{O_2}), CO_2 (C_{CO_2}) *and lactate measured in saline-perfused swimbladders at two perfusion rates during type S perfusion (serial flow, no countercurrent)*

	$\dot{Q} = 0{\cdot}2$ ml min^{-1} ($N = 2$)			$\dot{Q} = 0{\cdot}4$ ml min^{-1} ($N = 4$)		
	ai	*ve*	*ve−ai*	*ai*	*ve*	*ve−ai*
C_{O_2} (mmol l^{-1})	0·22 ± 0·01	0·14 ± 0·01	−0·08	0·24 ± 0·02	0·20 ± 0·02	−0·04
C_{CO_2} (mmol l^{-1})	0·15 ± 0·05	0·54 ± 0·41	0·39	0·24 ± 0·11	0·39 ± 0·15	0·15
[Lactate] (mmol l^{-1})	0·07 ± 0·04	0·83 ± 0·22	0·76	0·02 ± 0·01	0·41 ± 0·13	0·39

N, number of preparations.

Table 2. *Rates of oxygen consumption ($\dot{M}_{O_2}$) and CO_2 excretion ($\dot{M}_{CO_2}$) in saline-perfused swimbladder tissue and the corresponding respiratory exchange ratio, R*

Expt no.	Perfusion type	$\dot{M}_{O_2}$ (nmol min^{-1})	$\dot{M}_{CO_2}$ (nmol min^{-1})	R
6	C	13·6	60·0	4·4
6	C†	12·0	82·0	6·8
7	C	3·7	10·4	2·8
8	C	15·5	66·0	4·3
8	S	13·4	26·2	2·0
9	S	17·6	130·8	7·4
11	C	11·2	48·4	4·3
11	S	10·0	38·8	3·9
13	C	8·8	63·6	7·2
13	S	15·6	55·6	3·6
13	C	10·8	32·4	3·0
Mean ± S.D.		12·0 ± 3·8*	55·8 ± 32·1*	4·5 ± 1·8**

Perfusion type C, perfusion with countercurrent flow; type S, serial perfusion, no countercurrent flow.

* Significantly different from zero ($P < 0·01$).

** Significantly different from unity ($P < 0·01$).

† Only one rete mirabile perfused.

between *ai* and *ve* were clearly dependent on the flow rate. Doubling the flow rate resulted in a reduction of the (*ve*–*ai*) differences by a factor of about 2.

The rates of O_2 consumption ($\dot{M}_{O_2}$) and CO_2 production ($\dot{M}_{CO_2}$), calculated from concentration differences in individual experiments, are listed in Table 2. There were no differences between the flow types. In each experiment the CO_2 production rate by far exceeded the O_2 consumption rate. The resulting values of the respiratory exchange ratio, $R = \dot{M}_{CO_2}/\dot{M}_{O_2}$, are significantly above unity. The rates of glucose uptake ($\dot{M}_{Glu}$) and lactate release ($\dot{M}_{La}$) are presented in Table 3. On average, the rate of glucose consumption was higher than the rate of lactate production.

Discussion

For a quantitative evaluation of the metabolic activities of the gas gland its perfusion rate had to be known. Since blood flow rate cannot easily be obtained in the eel swimbladder without altering its function, we had to apply the technique of saline perfusion of the vascularly isolated tissue. The CO_2 and lactate values measured in this study, however, are similar to values that we obtained earlier by non-obstructive blood collection in intact eel swimbladder (Pelster *et al.* 1988*b*). We, therefore, assume the values measured in this study to be representative for eel swimbladder under physiological conditions.

Table 3. *Rates of lactate production ($\dot{M}_{La}$) and glucose consumption ($\dot{M}_{Glu}$) in saline-perfused swimbladder tissue*

Expt no.	Perfusion type	$\dot{M}_{La}$ (nmol min^{-1})	$\dot{M}_{Glu}$ (nmol min^{-1})	$\dot{M}_{La}/\dot{M}_{Glu}$
1	C	109	36	3·0
2	C	86	44	1·9
3	C	208	83	2·5
4	C	451	204	2·2
5	C	106	112	1·0
6	C	117	432	0·3
7	C†	59	78	0·8
7	C	19	189	0·1
8	C	99	242	0·4
8	S	115	112	1·0
8	C	62	180	0·4
9	S	189	158	1·2
9	C	46	250	0·2
10	C	150	276	0·5
10	S	148	400	0·4
10	C	46	104	0·4
11	C	243	256	1·0
11	S	236	156	1·5
12	C	166	56	3·0
12	S	155	108	1·4
Mean ± S.D.		141 ± 97*	174 ± 110*	1·2 ± 0·9**

Perfusion type C, perfusion with countercurrent flow; type S, perfusion without countercurrent flow.

* Significantly different from zero ($P < 0·01$).

** Significantly different from 2 ($P < 0·01$).

† Only one rete mirabile perfused.

Sources for CO_2 formed in the gas gland

The important results of this study are the demonstration of the high rate of CO_2 production in the swimbladder tissue and the high respiratory exchange ratio, which significantly exceeded the value of unity expected for oxidative glucose breakdown. The values were calculated assuming no gas secretion into the swimbladder, which was indeed below the detection level. In our experiments the CO_2 may derive from three sources: washout from the tissue, oxidative metabolism and anaerobic decarboxylation reactions.

Washout of HCO_3^-/CO_2 from the tissue

As the CO_2 content in the perfusing solution was kept low, to ensure a high sensitivity for arteriovenous CO_2 differences, the swimbladder tissue had probably been cleansed of most of the CO_2 and HCO_3^- during the perfusion period before

measurements were performed. An enhanced conversion of HCO_3^- to CO_2 is to be expected as a result of lactic acid formation.

The exact amount of HCO_3^-/CO_2 washed out from the tissue is difficult to estimate. Let us consider first the conversion of HCO_3^- to CO_2 in the tissue, effected by the protons liberated from lactic acid, and its subsequent release into the perfusate. The amount of lactate produced (Table 1) would, indeed, suggest a large contribution to the CO_2 release into the perfusate, but there are two other possibilities for the H^+ apart from combining with HCO_3^-. One is binding to nonbicarbonate buffers in the tissue, the other binding to any buffer in the perfusate and washout with it. The nominal buffer value of the perfusate was very low, and the pH change in the perfusate from *ai* to *ve* of 1–2 units suggests that the amount of H^+ washed out is probably low. We do not know the buffer value of the swimbladder tissue and, thus, cannot estimate the amount of CO_2 washed out from it by considering the H^+ liberated from lactic acid.

There is another reason to suggest that the contribution of HCO_3^-/CO_2 washout from tissue was probably small. Assuming a bicarbonate concentration of approximately $2\,mmol\,l^{-1}$, typical of muscle tissue, the tissue mass of the swimbladder (about 0·4 g for an 800 g eel; Ball *et al.* 1955; Wittenberg *et al.* 1964) would contain less than $1\,\mu mol$ of HCO_3^-. Under steady-state conditions (see Fig. 2) this cannot contribute very much to the observed rate of CO_2 formation.

CO_2 formation by aerobic glucose metabolism

The rate of CO_2 production by glucose oxidation can best be estimated in our experiments from the O_2 consumption rate, which suggests that $12\,nmol\,min^{-1}$ or about 20% of the CO_2 derives from this pathway. It thus appears that the major fraction of the CO_2 formed must stem from the third source.

CO_2 formation by anaerobic decarboxylation

An example of anaerobic CO_2 formation is the pentose phosphate shunt. This pathway allows formation of CO_2 by decarboxylation of 6-phosphogluconate formed enzymatically from glucose. The pentose phosphate shunt is known to occur in ocular tissues of fish, where it contributes significantly to the glucose metabolism (Hoffert & Fromm, 1970). Boström *et al.* (1972) found that in the cod glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, key enzymes of the pentose phosphate shunt, displayed higher activities in gas gland cells than in muscle cells. In preliminary measurements we found activities of these two enzymes in the eel gas gland similar in magnitude to those in liver tissue and about 10 times higher than their activities in muscle tissue. It is, thus, to be expected that part of the glucose should be diverted to the pentose phosphate shunt to form CO_2 by decarboxylation catalysed by 6-phosphogluconate dehydrogenase. In this case, an enzymatic reaction reoxidizing the NADPH produced in the pentose phosphate shunt is necessary. This reoxidation might occur by chain elongation of fatty acids.

Another possibility for anaerobic CO_2 formation has been described for goldfish

muscle (Van den Thillart & Verbeek, 1982; Mourik, 1982). This CO_2 formation, which is combined with ethanol formation (Shoubridge & Hochachka, 1980), is predominantly located in the mitochondria of red muscle cells. The observed activities of the pentose phosphate shunt enzymes, and the histological observation that gas gland tissue contains only few mitochondria (Dorn, 1961), lead us to favour the pentose phosphate shunt as a possible pathway for anaerobic CO_2 formation.

Glucose as a source of CO_2 formation

Since the pentose phosphate shunt requires glucose, more glucose should be degraded in our experiments than would be expected from lactate formation and oxidative metabolism. The balance of glucose metabolism is, therefore, of interest in our study.

The glycogen stores in swimbladder tissue are too small to fuel glycolysis for more than a few minutes (D'Aoust, 1970), and blood (or saline) glucose is expected to be the main source for metabolism. Three pathways of glucose degradation have to be considered in our study: aerobic oxidation to CO_2, anaerobic fermentation to lactic acid and anaerobic decarboxylation reactions (for example, the pentose phosphate shunt).

Aerobic metabolism does not seem to be of great importance in gas gland cells. In histological studies Dorn (1961) and Morris & Albright (1975) found only thin and irregularly distributed mitochondria. Boström *et al.* (1972) measured only a very low activity of cytochrome oxidase in gas gland tissue of the cod, *Gadus morhua*. This is in line with the low rate of aerobic glucose metabolism in our experiments, which was estimated from the O_2 consumption rate to be 2 nmol min^{-1} or only 1–2 % of the total glucose metabolized.

Swimbladder tissue is known to produce lactic acid even in the presence of oxygen (Ball *et al.* 1955; D'Aoust, 1970), and its rate of production allows us to estimate the rate of glucose metabolized by this route. Since two lactic acid molecules are formed from each glucose molecule, a $\dot{M}_{La}/\dot{M}_{Glu}$ ratio of 2 would indicate complete degradation of glucose to lactate. The average value in our study of 1·2 indicates that only 60 % of the glucose was used for anaerobic glycolysis. Considering also the oxidatively metabolized glucose, about 38 % of the glucose metabolized could have entered the pentose phosphate shunt. It is not possible to predict the ratio of CO_2 to glucose in this pathway, but our data seem to indicate that most of the CO_2 was formed by the pentose phosphate shunt.

Physiological significance of anaerobic CO_2 formation

Anaerobic formation of CO_2 appears to have several advantages for gas secretion into the swimbladder, which requires generation of high gas partial pressures, particularly of O_2 and CO_2, in blood (Fänge, 1983). Let us first consider a situation in which high O_2 and CO_2 gas partial pressures are formed in a swimbladder by aerobic CO_2 and anaerobic lactic acid formation, but with no extra CO_2 production (Fig. 3). P_{CO_2} would mainly be increased by aerobic CO_2

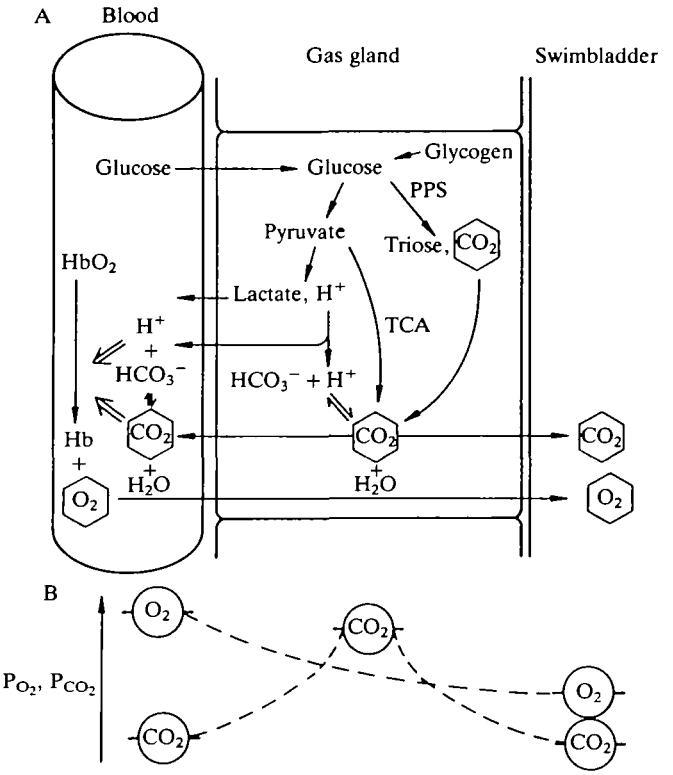

Fig. 3. (A) Diagram of metabolic processes in gas gland cells contributing to the formation of CO_2 and to the delivery of oxygen from the haemoglobin (Hb) in the capillaries. The anaerobic decarboxylation reaction was assumed to occur in the pentose phosphate shunt (PPS). Open arrows (⇐) indicate an influence on haemoglobin oxygen-affinity. TCA, tricarboxylic acid cycle. (B) The diffusion gradients for the gases CO_2 and O_2 shown schematically.

formation, and countercurrent enhancement in the rete (Kuhn *et al.* 1963) would further increase P_{CO_2}. The ensuing combined respiratory and metabolic acidosis (lactic acid formation) would release O_2 from the haemoglobin bond *via* the Bohr and Root effects (see Steen, 1970), and the increased P_{O_2} would be further enhanced in the rete countercurrent system.

It should, however, be noted that both CO_2 and O_2 secretion into the swimbladder would counteract the increases in their partial pressure. Moreover, any CO_2 molecule formed would consume one O_2 molecule, thus also reducing P_{O_2}. And, even if some CO_2 were formed from HCO_3^-, this would reduce the total salt concentration which is required for raising the partial pressures of inert gases (e.g. N_2 and Ar) by the salting-out effect (Gerth & Hemmingsen, 1982; Enns *et al.* 1967; Pelster *et al.* 1988*a*).

In the presence of an anaerobic route for CO_2 formation (Fig. 3), the efficiency of raising all partial pressures would be significantly increased. P_{CO_2} itself can

thereby attain higher values, and this would not reduce P_{O_2} levels. The highest P_{CO_2} values will be found in the gas gland cells, where CO_2 is formed, and CO_2 will diffuse from the tissue to the swimbladder and to the blood (Fig. 3). The concomitantly more pronounced reduction in pH would yield higher P_{O_2} levels in the blood by the Root and Bohr effect. Furthermore, HCO_3^- could be formed from CO_2 despite the lactacidosis, and this would increase the salt concentration for the salting-out effect.

It is thus evident that anoxidative CO_2 formation, for example *via* the pentose phosphate shunt, may be of great significance for the formation of high gas partial pressures in the swimbladder.

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References

Ball, E. G., Strittmatter, C. F. & Cooper, O. (1955). Metabolic studies on the gas gland of the swim bladder. *Biol. Bull. mar. biol. Lab., Woods Hole* **108**, 1–17.

Bergmeyer, H. U. (ed.) (1974). *Methoden der Enzymatischen Analyse*. Weinheim: Verlag Chemie, Bd. I u. II.

Boström, S. L., Fänge, R. & Johansson, R. G. (1972). Enzyme activity patterns in gas gland tissue of the swimbladder of the cod (*Gadus morhua*). *Comp. Biochem. Physiol.* **43B**, 473–478.

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

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

Deck, J. E. (1970). Lactic acid production by the swimbladder gas gland *in vitro* as influenced by glucagon and epinephrine. *Comp. Biochem. Physiol.* **34**, 317–324.

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

Enns, T., Douglas, E. & Scholander, P. F. (1967). Role of the swimbladder rete of fish in secretion in inert gas and oxygen. *Adv. Biol. Med. Phys.* **11**, 231–244.

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

Gerth, W. A. & Hemmingsen, E. A. (1982). Limits of gas secretion by the salting-out effect in fish swimbladder rete. *J. comp. Physiol.* **146**, 129–136.

Hoffert, J. R. & Fromm, P. O. (1970). Quantitative aspects of glucose catabolism by rainbow and lake trout ocular tissues including alterations resulting from various pathological conditions. *Expl Eye Res.* **10**, 263–272.

Kuhn, H. J., Moser, P. & Kuhn, W. (1962). Haarnadelgegenstrom als Grundlage zur Erzeugung hoher Gasdrücke in der Schwimmblase von Tiefseefischen. *Pflügers Arch. ges. Physiol.* **275**, 231–237.

Kuhn, W., Ramel, A., Kuhn, H. J. & Marti, E. (1963). The filling mechanism of the swimbladder. Generation of high gas pressures through hairpin countercurrent multiplication. *Experientia* **19**, 497–511.

Morris, S. M. & Albright, J. T. (1975). The ultrastructure of the swimbladder of the toadfish, *Opsanus tau* L. *Cell. Tissue Res.* **164**, 85–104.

Mourik, J. (1982). Anaerobic metabolism in red skeletal muscle of goldfish (*Carassius auratus L.*). PhD thesis, University of Leiden, 163 pp.

PELSTER, B., KOBAYASHI, H. & SCHEID, P. (1988*a*). Solubility of nitrogen and argon in eel whole blood and its relationship to pH. *J. exp. Biol.* **135**, 243–252.

PELSTER, B., KOBAYASHI, H. & SCHEID, P. (1988*b*). Production of acid by the gas gland of the swimbladder. *Pflügers Arch. ges. Physiol.* **412**, R40.

SHOUBRIDGE, E. A. & HOCHACHKA, P. W. (1980). Ethanol: novel endproduct of vertebrate anaerobic metabolism. *Science* **209**, 308–309.

STEEN, J. B. (1963*a*). The physiology of the swimbladder in the eel *Anguilla vulgaris*. II. The reabsorption of gases. *Acta physiol. Scand.* **58**, 138–149.

STEEN, J. B. (1963*b*). The physiology of the swimbladder in the eel *Anguilla vulgaris*. III. The mechanism of gas secretion. *Acta physiol. Scand.* **59**, 221–241.

STEEN, J. B. (1970). The swim bladder as a hydrostatic organ. In *Fish Physiology*, vol. IV (ed. W. S. Hoar & D. J. Randall), pp. 413–443. New York: Academic Press.

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

VAN DEN THILLART, G. & VERBEEK, R. (1982). Substrates for anaerobic CO_2-production by the goldfish, *Carassius auratus* (*L.*): Decarboxylation of ^{14}C-labeled metabolites. *J. comp. Physiol.* **149**, 75–81.

WITTENBERG, J. B., SCHWEND, M. J. & WITTENBERG, B. A. (1964). The secretion of oxygen into the swim-bladder of fish. III. The role of carbon dioxide. *J. gen. Physiol.* **48**, 337–355.