J. exp. Biol. 131, 427–434 (1987)
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# EFFECT OF BURST SWIMMING AND ADRENALINE INFUSION ON $O_2$ CONSUMPTION AND $CO_2$ EXCRETION IN RAINBOW TROUT, *SALMO GAIRDNERI*

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*Accepted 4 May 1987*

## SUMMARY

1. Immediately following burst swimming, the oxygen consumption of rainbow trout increased by 71%, carbon dioxide excretion by 104% and the respiratory exchange ratio by 17%. 80 min after burst swimming all of these parameters had returned to levels which were not significantly different from control values.

2. Infusion of adrenaline into resting fish had no significant effect on oxygen consumption or carbon dioxide excretion and therefore there was no significant change in the respiratory exchange ratio.

3. This infusion of adrenaline caused a significant elevation in the red blood cell pH which was still present 80 min later.

4. The present results contrast with those of van den Thillart, Randall & Lin (1983), who demonstrated carbon dioxide retention after burst swimming. While it is possible that catecholamines may inhibit bicarbonate flux through the red blood cell, our experiments indicate that this inhibition would not result in detectable changes in carbon dioxide excretion or, therefore, in the respiratory exchange ratio.

## INTRODUCTION

In fish, the rate of carbon dioxide excretion is largely dependent on the catalysed dehydration of plasma bicarbonate by erythrocytic carbonic anhydrase (Randall & Daxboeck, 1984). As in mammals, plasma bicarbonate enters fish erythrocytes *via* a chloride/bicarbonate exchange mechanism on the erythrocyte membrane (Cameron, 1978; Obaid, Critz & Crandall, 1979).

Wood & Perry (1985) have reported that adrenaline inhibits bicarbonate entry into rainbow trout erythrocytes *in vitro*. In the intact animal, this inhibition would result in the retention of plasma bicarbonate during branchial blood transit and, therefore, a reduction of carbon dioxide excretion. Indeed, Perry (1986) proposes that adrenergic control of erythrocytic chloride/bicarbonate exchange may be important

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Key words: $CO_2$ excretion, oxygen consumption, RE, exercise, catecholamines.

for acid–base regulation in fish by increasing internal bicarbonate stores. Circulating catecholamine levels are elevated in fish following acid–base disturbances (Boutilier, Iwama & Randall, 1986; Perry, 1986; Primmett, Randall, Mazeaud & Boutilier, 1986). At present, however, there is little evidence that these hormones modulate carbon dioxide excretion under these conditions in the intact animal. Van den Thillart *et al.* (1983) have reported low respiratory exchange ratios (carbon dioxide retention) in exercising coho salmon (*Oncorhynchus kisutch*) which would indicate a reduction in erythrocytic chloride/bicarbonate exchange. The effect of their experimental protocol on circulating catecholamine levels in these fish, however, is unknown.

The purpose of the present experiment was to determine if catecholamines modulate carbon dioxide excretion and, therefore, the respiratory exchange ratio in the rainbow trout *in vivo*. Burst swimming is known to cause a large increase in circulating catecholamines and an acid–base disturbance in the rainbow trout, *Salmo gairdneri* (Primmett *et al.* 1986). We have, thus, examined the effect of (1) burst swimming and (2) adrenaline infusion on carbon dioxide excretion and the respiratory exchange ratio in these animals.

## MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*), weighing between 285 and 700 g, from the Sun Valley Trout Farm (Mission, BC) were maintained in large outdoor tanks. Water temperature varied from 10 to 15 °C. The fish were regularly fed and in good health. The experimental protocol consisted of two separate series of experiments, in one of which fish were induced to burst swim and in the other adrenaline was infused into resting fish.

The first series of experiments was performed at $15 \pm 0{\cdot}5$ °C in a Brett-type swimming respirometer (Brett, 1964) with a total water volume of 37·5 l. Oxygen consumption was determined over 20-min periods from the decline in oxygen tension of the recirculating water in the closed respirometer, and the amount of pure oxygen injected into the system, as described by van den Thillart *et al.* (1983). Oxygen tension of the water was measured continuously by recirculating a small fraction of the water through an $O_2$ electrode mounted in a cuvette (Radiometer, E-5046 and D-616), as described by Steffensen, Johansen & Bushnell (1984). The $O_2$ electrode was connected to a Radiometer PHM71 acid–base analyser and a chart recorder.

$CO_2$ excretion from the fish was calculated from the difference in total $CO_2$ content of the water, determined every 20 min by analysing water samples using a Carle Series 111 analytical gas chromatograph with a Poropak Q column. Total $CO_2$ was determined as described by Boutilier, Iwama, Heming & Randall (1985) with the following modifications. A water sample of 2·0 ml was injected into a glass syringe (10 ml) containing pure nitrogen, and acidified with 50 $\mu$l of 1·0 mol $l^{-1}$ HCl. After 2 min of shaking, at least 6 ml of the gas in the syringe was injected into the gas chromatograph loop (volume = 1·0 ml) to ensure complete wash-out of the loop. The

$CO_2$ concentration was calculated by integrating the signal from the gas chromatograph with an HP 3497 data acquisition/control unit and an HP 9135A computer.

The water pH in the swimming respirometer was kept constant at 6·80 by means of a pH electrode (Canlab, GK2401C) permanently mounted in the system, connected to a Radiometer PHM71 acid–base analyser and a comparator. The recorder output was connected to a circuit controlling a Harvard Linear displacement pump injecting 0·25 mol $l^{-1}$ NaOH, as described by van den Thillart *et al.* (1983). When the pH of the water fell below 6·80, the pump was activated and NaOH injected until the pH was re-established.

The fish were acclimated in the respirometer for 24 h before the experiment. During this period, the respirometer was continuously flushed with thermostatically controlled, aerated water of pH 6·80. The experiment was started by closing the respirometer. Control $O_2$ consumption and $CO_2$ excretion were measured for 20-min periods for 80 min at the acclimation swimming speed (40 cm $s^{-1}$). The swimming speed was then increased to 80–85 cm $s^{-1}$ for 10 min, causing the fish to burst swim to 'exhaustion'. Burst swimming causes an increase in catecholamines in trout (Primmett *et al.* 1986). After 10 min, the speed was returned to 40 cm $s^{-1}$ and the same parameters were measured during the following 80 min, when the experiment was terminated.

In the second series of experiments, the fish were anaesthetized with MS-222 and cannulated in the dorsal aorta with PE 50 polyethylene tubing, as described by Soivio & Oikari (1976). The cannula was used to sample blood for determination of red blood cell (RBC) pH ($pH_i$), according to the freeze–thaw method described by Zeidler & Kim (1977) with a Radiometer PHM71 acid–base analyser and a micro-pH unit. In addition, the cannula was used to infuse 0·25 ml of $10^{-4}$ mol $l^{-1}$ adrenaline in the dorsal aorta. This large dose ensured that the $\beta$-adrenoceptors were stimulated and saturated. The increased RBC $pH_i$ indicated that the bolus acted on the $\beta$-receptors of the RBC. The adrenaline solution was prepared no more than 10 min before the infusion. The fish was acclimated for at least 24 h in the respirometer. Control measurements were performed for 10-min periods for 70 min. Adrenaline was then injected and the measurements were continued for the following 60 min. $O_2$ consumption, $CO_2$ excretion and erythrocyte pH ($pH_i$) were measured in resting fish before and after infusion of adrenaline. The fish were housed in a flow-through respirometer with a volume of 3·0 l. The respirometer was constructed with a recirculating circuit to ensure adequate mixing. Water temperature was 10·0 ± 0·5 °C.

Oxygen tension, $P_{O_2}$ and total $CO_2$ were measured in the water entering and leaving the respirometer, as described above, every 10 min. Oxygen consumption was calculated from the measured incurrent and excurrent $P_{O_2}$ and water flow ($\dot{V}$) according to the following equation:

$$\dot{V}_{O_2} = \beta[(P_{I_{O_2}} - P_{E_{O_2 t=1/2}})\dot{V} + (\Delta P_{E_{O_2}}/\Delta t)V]\ bm^{-1}$$

(Ultsch, Ott & Heisler, 1980), where $\beta$ is the solubility of oxygen in water; $P_{I_{O_2}}$ is the partial pressure of oxygen in incurrent water; $P_{E_{O_2}}$ is the partial pressure in excurrent

water; $P_{E_{O_2}t=1/2}$ is the partial pressure of oxygen in excurrent water after the elapse of one-half of the time interval $\Delta t$; V is the volume of the respirometer; and bm is body mass. $CO_2$ excretion was calculated in a similar manner.

In both series of experiments the ambient oxygen tension was always kept above 14·66 kPa.

Statistical analysis was based on Student's *t*-test. All values in the text and tables are given as mean ± standard deviation.

## RESULTS

Results of oxygen consumption, $CO_2$ excretion and respiratory exchange ratio (RE) for six fish swimming at 40 cm $s^{-1}$ before and after burst swimming are summarized in Table 1. Control oxygen consumption at a swimming speed of 40 cm $s^{-1}$ ranged from 118·3 to 135·6 $\mu$mol $kg^{-1}$ $min^{-1}$ (mean ± S.D. = 126·8 ± 29·2 $\mu$mol $kg^{-1}$ $min^{-1}$). $CO_2$ excretion varied from 86·7 to 102·1 $\mu$mol $kg^{-1}$ $min^{-1}$ (94·2 ± 21·3 $\mu$mol $kg^{-1}$ $min^{-1}$). The calculated respiratory exchange ratio $\dot{V}_{CO_2}/\dot{V}_{O_2}$ varied from 0·68 to 0·80 with a mean of 0·74 ± 0·08.

After burst swimming for 10 min, oxygen consumption increased significantly, by 71 %, to 217·3 ± 32·1 $\mu$mol $kg^{-1}$ $min^{-1}$ during the first 20 min of recovery. $CO_2$

Table 1. *$O_2$ consumption, $CO_2$ excretion and respiratory exchange ratio (RE) of rainbow trout swimming at 40 cm $s^{-1}$ before and after burst swimming*

| Time (min) | $O_2$ consumption ($\mu$mol $kg^{-1}$ $min^{-1}$) | $CO_2$ excretion ($\mu$mol $kg^{-1}$ $min^{-1}$) | RE ($\dot{V}_{CO_2}/\dot{V}_{O_2}$) |
|---|---|---|---|
| Before burst swimming: | | | |
| 0–20 | 135·6 (35·8) | 98·5 (21·3) | 0·74 (0·10) |
| 20–40 | 129·1 (36·5) | 89·4 (36·5) | 0·68 (0·13) |
| 40–60 | 124·1 (24·3) | 102·1 (37·2) | 0·80 (0·13) |
| 60–80 | 118·3 (28·2) | 86·7 (24·3) | 0·74 (0·13) |
| Mean of control period: | 126·8 (29·2) | 94·2 (21·3) | 0·74 (0·08) |
| Burst swimming for 10 min (80–85 cm $s^{-1}$): | | | |
| 0–20 | 217·3 (32·1) +71 %* | 192·1 (49·9) +104 %* | 0·87 (0·11) +17 %* |
| 20–40 | 167·1 (26·9) +32 %* | 141·6 (23·8) +50 %* | 0·86 (0·18) NS |
| 40–60 | 152·8 (25·7) +21 %* | 118·4 (34·5) +26 %* | 0·86 (0·12) NS |
| 60–80 | 138·4 (18·6) NS | 97·1 (9·8) NS | 0·71 (0·10) NS |

Values are mean (±standard deviation), $N = 6$. Temperature = 15 °C.

Paired *t*-tests were used to compare to mean of control: *$P \leq 0{\cdot}025$; NS, no significant difference.

Water pH was kept constant at 6·8 and oxygen tension ≥14·66 kPa.

Fish mass ranged from 520 to 700 g (mean ± S.D. = 607 ± 64 g).

excretion increased by 104% to 192·1 ± 49·9 $\mu$mol kg$^{-1}$ min$^{-1}$; thus RE increased significantly, by 17%, to 0·87 ± 0·11.

20–40 min after burst exercise, $O_2$ consumption and $CO_2$ excretion were still significantly elevated as compared to the control values, 32% (167·1 ± 26·9 $\mu$mol kg$^{-1}$ min$^{-1}$) and 50% (141·6 ± 23·8 $\mu$mol kg$^{-1}$ min$^{-1}$), respectively. RE was not significantly different from the control value.

During the following 20 min, oxygen consumption was 152·8 ± 25·7 $\mu$mol kg$^{-1}$ min$^{-1}$ and $CO_2$ excretion was 118·4 ± 34·5 $\mu$mol kg$^{-1}$ min$^{-1}$, or 21% and 26% higher than control, respectively. RE was not significantly different from the control value.

During the last period (60–80 min) none of the measured parameters was significantly different from control values.

The effects of infusing adrenaline into nine resting rainbow trout are summarized in Table 2. Oxygen consumption during the 70-min control period was 47·9 ± 8·0 $\mu$mol kg$^{-1}$ min$^{-1}$, $CO_2$ excretion was 35·2 ± 6·7 $\mu$mol kg$^{-1}$ min$^{-1}$, and RE, consequently, was 0·74 ± 0·09. Intracellular pH measured prior to adrenaline infusion was 7·391 ± 0·017. Adrenaline had no significant effect on either $O_2$ consumption, $CO_2$ excretion or RE during the following 60 min. 10 min after

Table 2. *$O_2$ consumption, $CO_2$ excretion, respiratory exchange ratio (RE) and $pH_i$ of resting rainbow trout before and after infusion of adrenaline*

| Time (min) | $O_2$ consumption ($\mu$mol kg$^{-1}$ min$^{-1}$) | $CO_2$ excretion ($\mu$mol kg$^{-1}$ min$^{-1}$) | RE $\dot{V}_{CO_2}/\dot{V}_{O_2}$ | $pH_i$ |
|---|---|---|---|---|
| Before infusion: | | | | |
| 0–10 | 47·3 (8·2) | 34·3 (6·0) | 0·73 (0·09) | |
| 10–20 | 47·7 (7·5) | 34·3 (6·3) | 0·72 (0·08) | |
| 20–30 | 47·6 (7·5) | 35·6 (7·0) | 0·75 (0·10) | |
| 30–40 | 47·2 (8·1) | 33·9 (7·7) | 0·74 (0·10) | |
| 40–50 | 47·6 (9·2) | 33·9 (9·2) | 0·71 (0·13) | |
| 50–60 | 47·9 (8·4) | 35·2 (6·8) | 0·74 (0·11) | |
| 60–70 | 49·7 (8·1) | 38·2 (6·4) | 0·78 (0·09) | 7·391 (0·017) |
| Mean of control period: | 47·9 (8·0) | 35·2 (6·7) | 0·74 (0·09) | |
| Infusion of 0·25 ml of $10^{-4}$ mol l$^{-1}$ adrenaline: | | | | |
| 0–10 | 55·2 (15·0)NS | 40·9 (11·3)NS | 0·75 (0·10)NS | 7·447 (0·034)* |
| 10–20 | 58·9 (23·9)NS | 42·5 (16·2)NS | 0·74 (0·10)NS | |
| 20–30 | 59·9 (24·5)NS | 41·7 (14·5)NS | 0·71 (0·08)NS | |
| 30–40 | 52·3 (14·0)NS | 39·6 (14·3)NS | 0·73 (0·09)NS | |
| 40–50 | 52·4 (16·0)NS | 39·7 (12·3)NS | 0·76 (0·09)NS | |
| 50–60 | 50·3 (18·5)NS ($N$ = 9) | 37·3 (14·5)NS ($N$ = 9) | 0·72 (0·19)NS ($N$ = 9) | 7·432 (0·051)† ($N$ = 8) |

Values are mean (±standard deviation).

Paired $t$-tests were used to compare to mean of control: *$P$ = 0·001; †$P$ = 0·025; NS, no significant difference.

Water temperature was 10°C and oxygen tension ≥14·66 kPa.

Fish mass ranged from 285 to 380 g (mean ± S.D. = 327 ± 34 g).

injection of adrenaline, $pH_i$ had increased to 7·447 and remained elevated (7·432) for 60 min after adrenaline infusion.

## DISCUSSION

Several types of stress have been shown to cause an increase in the levels of circulating catecholamines in fish (Nakano & Tomlinson, 1968; Mazeaud & Mazeaud, 1981; Boutilier *et al.* 1986; Perry, 1986). Recently, Primmett *et al.* (1986) have documented 25- to 35-fold increases in circulating adrenaline and noradrenaline levels in the rainbow trout following a burst swim. It was therefore assumed that the burst swim in the present experiment would cause a similar increase in the levels of circulating catecholamines.

It has been reported that catecholamines affect $CO_2$ transport by inhibition of bicarbonate flux through the erythrocyte (Wood & Perry, 1985). This inhibition of erythrocytic chloride/bicarbonate exchange could explain the low RE values reported by van den Thillart *et al.* (1983) in the coho salmon (*Oncorhynchus kisutch*). In coho salmo (exercised in sea water at pH 7·0) the RE was found to be 0·21. Van den Thillart *et al.* (1983) also measured $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ after burst swimming in normal sea water, but only as mean rates for a 6-h period 'since most rates did not change very much during each run'. They determined RE to be 0·64 ($N = 4$). It appears, however, that in their representative fish (their fig. 3), the bicarbonate excretion during the first hour after burst swimming was only 1/15 that of the following 3 h. Accordingly, RE must have been approximately 0·1 during the first hour. The results of the present experiments, in contrast, provide no evidence that increased circulating catecholamines lead to a reduction in carbon dioxide excretion in the whole animal.

Infusion of adrenaline into resting rainbow trout *in vivo* caused no significant changes in either $CO_2$ excretion or $O_2$ consumption and, consequently, the RE value did not change significantly from its control value of 0·74. In addition, burst swimming, which is associated with an increase in blood catecholamines, caused an increase in both $O_2$ consumption (71%) and carbon dioxide excretion (104%) during the recovery period. Thus, in contrast to the '$CO_2$ retention' proposal of Wood & Perry (1985), there was, in fact, a significant increase in the respiratory exchange ratio to 0·87. The increased RE following the burst swim is likely to result, as in other animals, from the titration of the blood bicarbonate pool by protons entering the blood from the exercising tissues.

It is possible that the absence of an effect of catecholamines on $CO_2$ excretion in our study could have been due to seasonal variation in inhibition of erythrocytic chloride/bicarbonate exchange by catecholamines caused by seasonal variation in $\beta$-receptor activity. It has been documented that the gills and heart (Peyraud-Waitzenegger, Barthelemy & Peyraud, 1980) of eels may lose $\beta$-adrenergic sensitivity during the winter. Similarly, Nikinmaa & Jensen (1986) have suggested that this may also be the case for the erythrocytes of the rainbow trout. However, the fact that an elevation in catecholamine levels caused a significant increase in the erythrocyte pH,

as shown in other studies (Nikinmaa, 1983; Nikinmaa & Huestis, 1984; Heming *et al.* 1986), indicates that the $\beta$-adrenergic receptors were still functional in the trout used in this study. Thus, the absence of an effect of catecholamines on $CO_2$ excretion in the present study cannot be attributed to reduced activity of erythrocytic $\beta$-adrenergic receptors.

There is also a discrepancy between the present experiment and those of van den Thillart *et al.* (1983) concerning oxygen uptake following burst swimming. These authors reported no change in oxygen consumption during the approximately 4-h recovery period, whereas we found $O_2$ consumption had initially increased, but decreased to control values within 80 min. Brett (1964) reported an oxygen debt replacement up to 5 h after fatigue in yearling sockeye salmon (*Oncorhynchus nerka*). Similarly, Stevens & Randall (1967) and Steffensen *et al.* (1984) found that the $O_2$ consumption of rainbow trout decreased to control values within 0·5–4 h after strenuous exercise. Why van den Thillart *et al.* (1983) found no such increase in $O_2$ consumption (or oxygen debt) after burst swimming is not clear: an oxygen debt after strenuous exercise can be expected, since lactate is removed metabolically. Holeton, Neumann & Heisler (1983) found that strenuous exercise resulted in a severe lactacidosis, which was corrected within 4 h by a transient net transfer of $H^+$ to the environmental water. The lactate was removed metabolically within 6–8 h. This curious lack of an oxygen debt after burst swimming in the study of van den Thillart *et al.* (1983) indicates that their surprisingly low RE values may be due to technical limitations.

In summary, the present experiments provide no evidence to support the view that increased catecholamine levels in fish cause a reduction in carbon dioxide excretion (Wood & Perry, 1985; Perry, 1986). In addition, carbon dioxide excretion is proportional to haematocrit and inhibition by the anion exchange blocker 4-acetamido-4′-isothiocyanatosilbene-2,2′ disulphonic acid (SITS) in the blood-perfused trout preparation (Perry, Davie, Daxboeck & Randall, 1982). The present experiment also provides no evidence that erythrocytic chloride/bicarbonate exchange is inhibited in the rainbow trout, *in vivo* by elevated catecholamines and contrasts with the results obtained with *in vitro* preparations (Wood & Perry, 1985).

JFS was supported by a NATO Science Fellowship, the Danish National Science Research Council and the Carlsberg Foundation, Denmark. BLT and DJR were supported by the National Science and Engineering Research Council of Canada. Assistance from D. Mense and L. E. Fidler is gratefully acknowledged.

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