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THE ROLE OF CATECHOLAMINES IN ERYTHROCYTE pH REGULATION AND OXYGEN TRANSPORT IN RAINBOW TROUT (*SALMO GAIRDNERI*) DURING EXERCISE

BY D. R. N. PRIMMETT*, D. J. RANDALL, M. MAZEAUD† AND R. G. BOUTILIER‡

Department of Zoology, University of British Columbia, Vancouver, BC Canada V6T 2A9

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SUMMARY

Rainbow trout were subjected to burst swimming to exhaustion followed by 4 h aerobic swimming at 80 % critical swimming velocity. Severe physiological disturbances, including a marked plasma acidosis caused by the burst swim, were corrected during the 4 h of subsequent aerobic exercise. Erythrocytic pH and arterial oxygen content increased, even though plasma pH was reduced. We suggest that the increase in erythrocytic pH was caused by the action of elevated adrenaline and noradrenaline levels in the blood acting on β-adrenergic receptors on the trout red blood cell, causing the cell to swell and raising intracellular pH, offsetting any effect of a reduction of plasma pH on erythrocyte pH and haemoglobin–oxygen binding. Propranolol blocked the action of catecholamines on trout erythrocytes. We conclude that catecholamines play an important role in maintaining oxygen transport to aerobic muscles, following burst swimming and the associated acidotic conditions.

INTRODUCTION

Burst activity in salmonids is supported by anaerobic glycolysis and results in the production of lactate and H^+ by the muscle which, upon entry into the blood, cause a marked reduction in plasma pH lasting several hours (Heisler, 1982; Holeton, Newmann & Heisler, 1983; Turner, Wood & Clark, 1983). A fall in plasma pH, *in vitro*, will result in a decrease in erythrocytic pH which will reduce haemoglobin–oxygen binding *via* the Bohr and Root effects (Randall, 1970). If a similar drop in red cell pH were observed *in vivo* after exhausting exercise, this could lead to a

* Present address: Department of Human Anatomy, University of Oxford, South Parks Road, Oxford, OX1 3QZ, UK.

† Visiting scientist from Physiology and Pharmacology, INSERM U7-CNRS LA 318, Hopital Necker, 161, Rue de Sevres, 75730 Paris Cedex 16, France.

‡ Present address: Department of Biology, Dalhousie University, Halifax, NS, Canada B3H 4J1.

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decrease in arterial oxygen content and thereby to a decrease in the scope for aerobic exercise following a burst swim.

D. J. Randall, R. G. Boutilier & D. Mense (in preparation) were unable to detect any reduction in aerobic swimming after a burst swim by chinook salmon. One explanation of this result is that the plasma acidosis, as a consequence of burst activity, may not cause a reduction in erythrocytic pH and therefore blood oxygen capacity *in vivo*. Nikinmaa (1982, 1983) and Nikinmaa, Cech & McEnroe (1984) showed that adrenaline could maintain erythrocytic pH, and therefore the blood oxygen transport properties, in the face of a plasma acidosis in both trout and striped bass.

It is the hypothesis of this study that catecholamines are released into trout blood during burst exercise and therein act to maintain red blood cell (RBC) pH, in the face of an extracellular acidosis, such that the aerobic scope for post-burst activity is not curtailed. To test the predictions of this hypothesis, the present series of experiments was designed (1) to evaluate the effect of a burst swim on arterial blood oxygen levels during an ensuing period of aerobic exercise at 80% U_{crit} (critical velocity) in rainbow trout and (2) to examine the effect of propranolol infusion into rainbow trout arterial blood immediately following burst exercise. Propranolol exhibits both competitive and noncompetitive inhibition for adrenergic receptors in fish (e.g. Wood & Shelton, 1980); it is expected that post-exercise propranolol administration will block the postulated maintenance of RBC pH by inhibition of mechanisms responsible for erythrocyte pH regulation.

MATERIALS AND METHODS

Experimental animals

Rainbow trout (*Salmo gairdneri*, 200–300 g) of both sexes were obtained from the Sun Valley Trout Farm (Mission, BC) and were kept in moving, dechlorinated, aerated fresh water (5–15 °C) for at least 4 weeks prior to the measurement of exercise performance (Brett, Hollands & Alderdice, 1958).

The trout were anaesthetized with MS222 (1:10 000, pH 7·5) and implanted with chronic dorsal aortic cannulae (heparinized Clay-Adams PE50 polyethylene tubing) in a manner similar to that of Smith & Bell (1964). After cannulation, the fish were allowed to recover for 18 h in a Brett (1964) respirometer before being experimented on.

After recovery from cannulation, the critical velocity (U_{crit}) of each cannulated fish was determined (Brett, 1964) in the respirometer. The water velocity, and hence swimming velocity, was incrementally increased by 0·2 body lengths per second (0·2 BL s^{-1}) for 60-min intervals until exhaustion was evident (see Hoar & Randall, 1978, for details of U_{crit} determination). These animals were then allowed 18 h of recovery within the respirometer. During all procedures, the respirometer was continually flushed with dechlorinated fresh water.

Experimental series

Series I

This study examined the effect of burst exercise on subsequent aerobic exercise in freshwater rainbow trout at temperatures between 5 and 15°C. Following 18 h of recovery from exercise performance assessment, cannulated fish were swum under three different but successive conditions of activity: (1) resting aerobic level (i.e. 1·0 $BL\,s^{-1}$); (2) burst activity level (i.e. 120% U_{crit} until exhaustion); (3) post-burst aerobic exercise level (i.e. following exhaustion at 120% U_{crit}, the fish were swum at 80% U_{crit} for 4 h). Blood was sampled and variables were measured during condition 1, at the end of condition 2, and at 15 min, 1 h and 4 h into condition 3.

At each sampling time, 500 μl of whole blood was removed from the fish *via* the cannula. Arterial oxygen tension (Pa_{O_2}) and plasma pH (pHe) were measured on whole blood with a Radiometer PHM-71 acid–base analyser and associated micro-pH and oxygen electrodes. The remaining whole blood was then centrifuged in a Brinkman 3200 centrifuge. The plasma fraction was assayed for lactate ($lactate^-$) (Sigma assay), adrenaline (AD) and noradrenaline (NA), using high pressure liquid chromatography (HPLC) with electrochemical detection. The HPLC consisted of a reverse-phase Ultrasphere ODS column and a Spectra-Physics SP 8700 delivery and pump system. The mobile phase was a 0·005 mol l^{-1} citric acid–0·1 mol l^{-1} sodium acetate buffer at pH 4·0 with 0·1 mmol l^{-1} sodium Heptan sulphonic acid. The electrochemical detection system consisted of a thin layer TC-4 flow cell from BAS with a carbon paste electrode set to −700 mV *versus* a Ag/AgCl reference electrode (see Woodward, 1982). Erythrocytic pH was measured from the RBC fraction by the freeze–thaw method of Zeidler & Kim (1977). Ventilatory frequency (f) was determined visually by counting the number of opercular movements per minute.

The experiments were repeated on a second group of fish, but at each sampling time, only 100 μl of whole blood was removed from the fish. 30 μl of whole blood was centrifuged to determine percentage haematocrit (Hct). Whole blood arterial oxygen content (Ca_{O_2}) was determined with a Lex-O_2-Con oxygen content analyser. 20 μl of whole blood was used to determine haemoglobin concentration ([Hb]) using a Perkin-Elmer atomic absorption spectrophotometer (flame analysis for whole blood iron concentration; Zettner & Mensch, 1967).

Series II

This study examined the effect of propranolol infusion into rainbow trout arterial blood immediately following burst exercise. The exercise regime was the same as in the first series of experiments, except that following exhaustion at 120% U_{crit}, the fish were returned to 1·0 $BL\,s^{-1}$. Blood was sampled and the variables measured during the initial resting period, at the end of, and 15 min after, burst exercise. Immediately following burst swimming, the trout were injected *via* the dorsal aortic cannula with either 0·5 ml of 2×10^{-4} mol l^{-1} propranolol (Nikinmaa, 1983) in saline (Wolf, 1963) followed by 0·1 ml of saline to clear the cannula of propranolol, or a control injection of 0·6 ml of saline.

At each sampling time, 700 μl of whole blood was removed from the fish and pHe, RBC pH, AD and NA were measured in the manner described above.

Calculations

Plasma oxygen content was calculated using measured values for Pa_{O_2} and values for plasma oxygen solubility (Boutilier, Iwama & Heming, 1984). Plasma oxygen content was subtracted from measured Ca_{O_2}; the remainder was divided by corresponding values for Hb giving calculated values for the quantity of oxygen bound to haemoglobin (Hb_{O_2}) in terms of mol O_2 mol^{-1} Hb.

The mean cellular haemoglobin concentration (MCHC) was calculated using the formula:

$$MCHC = \frac{Hb \times 100}{Hct}.$$

RESULTS

Series I

Burst exercise resulted in a reduction in blood pH and an increase in plasma lactate levels (Fig. 1). The reduction in plasma pH peaked soon after the exhaustive exercise terminated, whereas lactate levels peaked about 1 h into the aerobic swimming period. Although there was a sharp reduction in plasma pH, this disturbance did not result in a fall in RBC pH, which was elevated above pre-exercise resting levels during the entire 4-h aerobic swim at 80 % U_{crit} (Fig. 1).

Severe activity caused about a 35-fold increase in plasma adrenaline and 25-fold increase in plasma noradrenaline concentrations (Fig. 2); the levels of both of these catecholamines decreased during post-burst aerobic exercise but still remained elevated over resting levels. Dopamine levels remained below the detection limit (1×10^{-9} mol l^{-1}) of our system at all times.

The effects of burst exercise and ensuing aerobic exercise at 80 % U_{crit} on Hct, [Hb] and MCHC are shown in Fig. 3. During the burst swim, Hct increased by about 40 %, [Hb] increased by about 20 % and MCHC decreased by about 15–20 %. These data indicate that exhaustive activity resulted in about a 40 % increase in the red cell fraction of trout whole blood; about one-half of this rise was due to red cell swelling and the other half was the result of haemoconcentration.

Fig. 4 shows the effect of burst exercise followed by aerobic activity on Ca_{O_2}, Hb_{O_2}, Pa_{O_2} and f. Arterial blood oxygen content was maintained during the burst swim, became significantly elevated above resting levels within 1 h of post-burst exercise, and was then restored to resting levels after 4 h of aerobic swimming. Hb_{O_2}, Pa_{O_2} and f showed a sharp decrease following burst exercise. Hb_{O_2} and Pa_{O_2} were restored to resting levels within 15 min of post-burst exercise, and Pa_{O_2} was elevated over resting levels after 1 h of recovery. Following burst activity, f overshot within 15 min of aerobic recovery and then was restored to resting levels by 1 h of post-burst exercise.

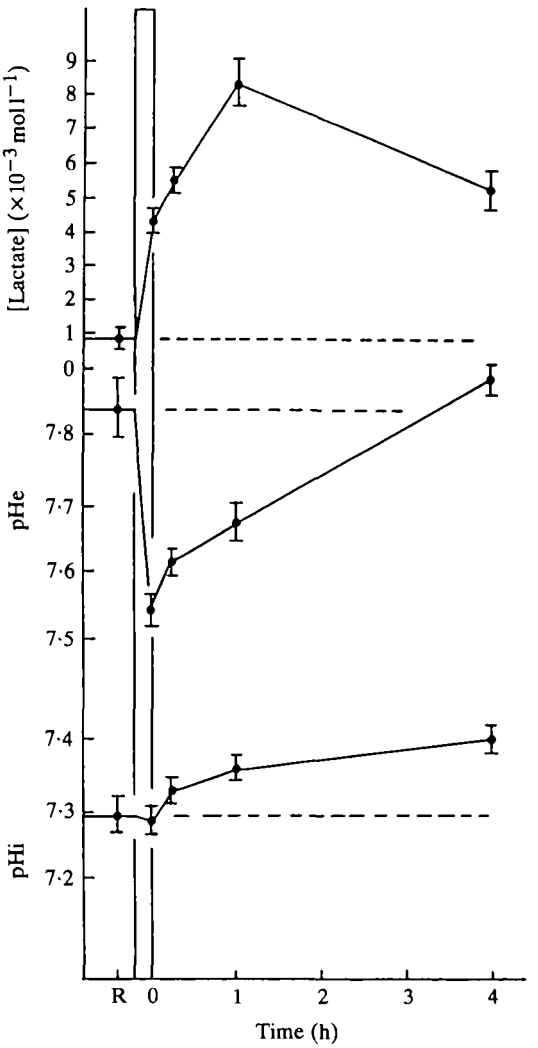

Fig. 1. Plasma lactate levels ($N = 10$), blood pH (pHe) and red blood cell pH (pHi) ($N = 13$) in trout during a burst swim and an ensuing aerobic swim at 80% U_{crit}; ($\bar{x} \pm$ S.E.). R, rest; bar, burst activity to exhaustion; 0 h, start of post-burst aerobic activity at 80% U_{crit}; * significantly different from rest using Student's t-test ($P < 0{\cdot}05$).

Series II

Burst exercise followed by a saline injection resulted in increased plasma lactate concentration, decreased pHe, increased plasma AD levels and maintenance of RBC pH (Table 1). These results closely paralleled those seen in series I.

Burst exercise followed by a propranolol injection resulted in the same response as seen with a saline injection except that there was a significant decrease in RBC pH (Fig. 5) associated with a fall in plasma pH.

Post-burst injections of saline and of propranolol had no effect on plasma NA levels.

DISCUSSION

Severe physiological disturbances, including a marked plasma acidosis, were observed in freshwater rainbow trout during aerobic exercise following a period of burst swimming; for the most part, these were corrected within the first 4 h of post-burst aerobic exercise at 80 % U_{crit}. The plasma acidosis was not transferred into the erythrocyte; in fact, erythrocytic pH increased. This increase in red blood cell pH was associated with a rise in blood adrenaline and noradrenaline levels. A reduction in plasma pH, *in vitro*, results in a fall in RBC pH and a decrease in haemoglobin–oxygen binding (Root & Irving, 1943). The addition of catecholamines to blood, *in vitro*, causes a rise in erythrocyte pH (Nikinmaa, 1982; T. Heming & D. J. Randall, in preparation). We conclude that the absence of an erythrocyte pH drop associated with the plasma acidosis in our experiments was due to the release and subsequent action of catecholamines on trout red blood cells. The action of the catecholamines appears to be *via* β-adrenergic receptors (Nikinmaa, 1983). This conclusion is supported further by the results of the experiments in series II, in which a plasma acidosis was associated with a fall in erythrocyte pH in the presence of the β-adrenergic antagonist, propranolol, in spite of a significant rise in blood catecholamine levels.

Catecholamines also cause trout red blood cells to swell (Baroin, Garcia-Romeu, Lamare & Motais, 1984) and catecholamines probably caused the increased RBC volume in our experiments, as indicated by the increased haematocrit and decreased

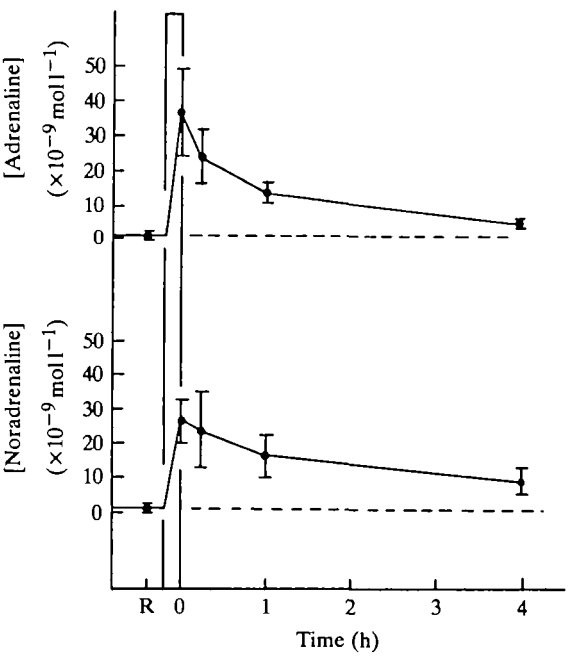

Fig. 2. Relationship between plasma adrenaline and noradrenaline levels in trout during a burst swim and an ensuing aerobic swim at 80 % U_{crit}; $N = 8$ ($\bar{x} \pm$ S.E.). See Fig. 1 for further explanation.

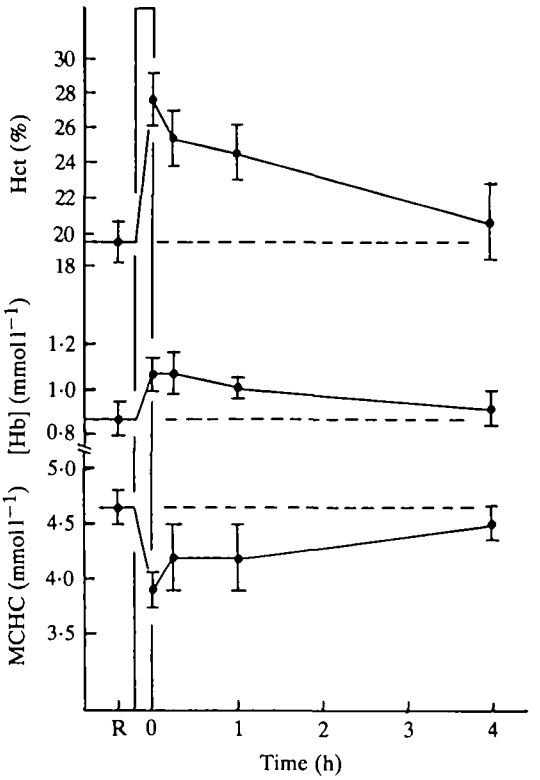

Fig. 3. Effect of a burst swim and ensuing aerobic swim at 80 % U_{crit} on haematocrit (Hct), blood haemoglobin concentration (Hb) and mean cellular haemoglobin concentration (MCHC); $N = 6$ ($\bar{x} \pm$ S.E.). See Fig. 1 for further explanation.

mean corpuscular haemoglobin concentration. The rise in haematocrit was also associated with an increase in total blood haemoglobin content, indicating the addition of erythrocytes to the circulation, probably from the spleen which is also under adrenergic control (Nilsson & Grove, 1974). The rise in haemoglobin concentration probably also reflects a decrease in plasma volume (Yamamoto, Itazawa & Kobayashi, 1980).

The maintained erythrocytic pH and the increased levels of circulating erythrocytes following burst activity resulted in a rise in blood oxygen content even though Pa_{O_2} was reduced immediately after the burst swim. During the switch from burst to aerobic swimming, there was a reduction in gill ventilation frequency associated with exhaustion, which undoubtedly contributed to the fall in Pa_{O_2} seen during this period. Oxygen saturation of haemoglobin was reduced to around 80 % immediately following burst exercise. It is not clear if this is simply a reflection of the fall in Pa_{O_2} or if some other factors had caused an additional right shift of the haemoglobin–oxygen dissociation curve. Arterial P_{O_2} rose quickly and, within 1 h of the transition from burst activity to aerobic swimming, it was elevated above that observed in the resting fish and haemoglobin–oxygen saturation rose to close to 100 % (Fig. 4).

A similar acidification of plasma *in vitro* to that observed *in vivo* in these experiments would have reduced haemoglobin–oxygen saturation by up to 30 or 40 % (Cameron, 1971; Nikinmaa, 1983). Jones (1971) found that a reduction in haematocrit or hypoxic conditions resulted in a marked reduction in maximum critical velocity. He estimated that, in his experiments when fish were exposed to hypoxia at 22 °C, the blood oxygen content was reduced by 30–40 % and this led to a 30 % reduction in swimming speed. Clearly, the maintenance of oxygen transport to the tissues is crucial for aerobic swimming at high speeds, hence the release of catecholamines and their action in elevating RBC pH is of selective advantage to the fish. Catecholamines also cause erythrocytes to swell, which in turn dilutes the haemoglobin and organic phosphates within the red blood cell. Whether these changes are also of selective advantage or are simply secondary to pH regulation is difficult to assess at this time.

Catecholamine release into the circulation is associated with stress (Mazeaud & Mazeaud, 1981) and a number of other factors, including burst swimming as shown in this study. The stimulus could have been either the burst swim itself or some

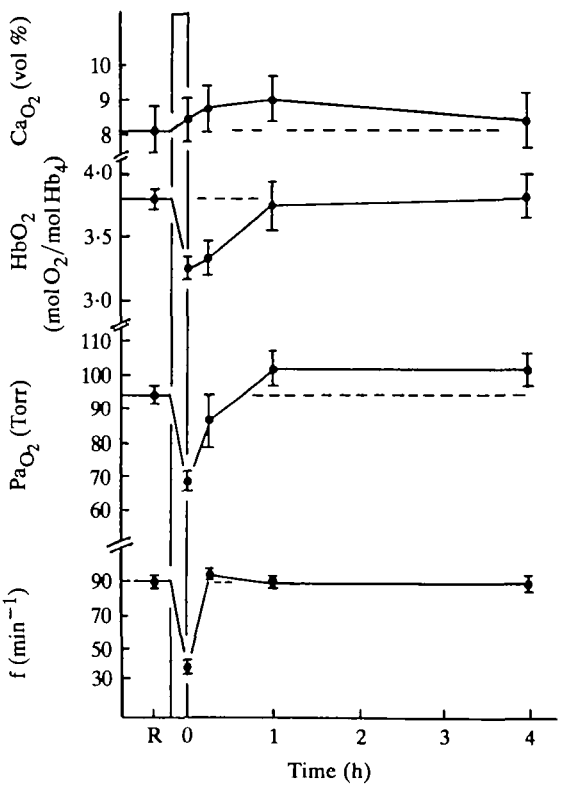

Fig. 4. Effect of a burst swim and ensuing aerobic swim at 80 % U_{crit} on arterial oxygen content (Ca_{O_2}), the amount of oxygen bound to haemoglobin in arterial blood (Hb_{O_2}), arterial oxygen tension (Pa_{O_2}) and ventilatory frequency (f); $N = 6$ for all variables except f where $N = 3$ ($\bar{x} \pm$ S.E.). See Fig. 1 for further explanation.

Table 1. *Effects of burst exercise followed by sham injection (Ex.) and burst exercise followed by propranolol injection (Exp.) in rainbow trout (5·0–7·5 °C)*

Variable	Rest ($N = 12 \pm$ S.E.)	Sig.*	Ex. ($N = 6 \pm$ S.E.)	Sig.†	Exp. ($N = 6 \pm$ S.E.)	Sig.‡
Lactate ($mmol\,l^{-1}$)	0·61 (±0·09)	0·05	5·32 (±0·40)	NS	4·83 (±0·73)	0·05
Adrenaline ($\times 10^{-9}\,mol\,l^{-1}$)	0·91 (±0·13)	0·05	5·82 (±1·44)	NS	4·90 (±1·15)	0·05
Noradrenaline ($\times 10^{-9}\,mol\,l^{-1}$)	0·74 (±0·10)	NS	0·50 (±0·06)	NS	0·59 (±0·06)	NS
Blood pH (pHe)	8·115 (±0·024)	0·05	7·920 (±0·045)	NS	7·833 (±0·025)	0·05
RBC pH (pHi)	7·491 (±0·015)	NS	7·476 (±0·028)	0·05	7·418 (±0·011)	0·05

Significance test between variables: Student's t-test ($t_{0.95}$), where Sig.* is Rest compared to Ex., Sig.† is Ex. compared to Exp., Sig.‡ is Rest compared to Exp.

Note: Ex. and Exp. resting values are not significantly different ($t_{0.95}$); these values are pooled above ($N = 12$).

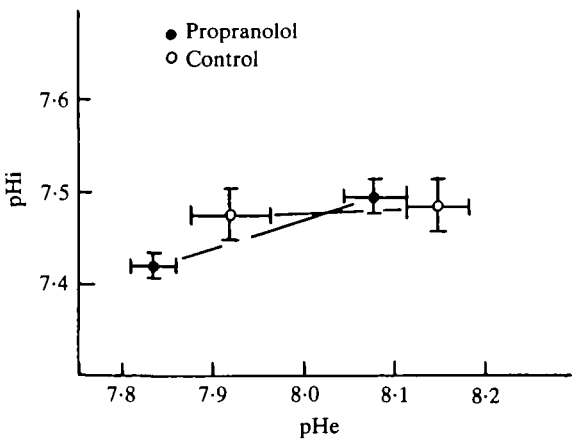

Fig. 5. The effect of propranolol treatment following burst exercise on extracellular (pHe) and erythrocytic pH (pHi); $N = 6$ ($\bar{x} \pm$ S.E.). *, significantly different from resting.

change associated with the burst, for example the resultant acid conditions in the body. Aerobic swimming was not a requirement for catecholamine release because circulating adrenaline levels increased in the series II experiments when there was no aerobic swim following the burst activity.

We conclude that catecholamines are released into the general circulation of fish during strenuous exercise and that these hormones play an important role in regulating erythrocyte pH *in vivo*, and hence in the maintenance of blood oxygen transport, during the associated plasma acid–base disturbances.

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