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SHORT COMMUNICATION

THE ROLE OF THE SPLEEN DURING EXERCISE IN THE ANTARCTIC TELEOST, *PAGOTHENIA BORCHGREVINKI*

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Physiological changes in fish associated with swimming are highly dependent on the intensity of exercise performed (Wood, 1991). Swimming at high speed requires large increases in the delivery of oxygen to the tissues and the circulatory system must be able to meet this demand. Exercise at a speed great enough to cause exhaustion results in an elevation in haematocrit (Hct), which can increase the oxygen content of the blood. The magnitude of the Hct increase varies among different species of fish, although generally the increase is about 50% (Milligan and Wood, 1987; Wells and Weber, 1990; Yamamoto *et al*. 1980; Yamamoto and Itazawa, 1989). The antarctic cryopelagic teleost *Pagothenia borchgrevinki* (Boulenger, 1902, see also *Notothenia hodgsoni*) appears to be an exception as exercise produces an increase in Hct of more than 110% (Davison *et al*. 1988), an increase approximately 2–4 times greater than that in other species of fish studied.

An increase in Hct can result from recruitment of erythrocytes from the spleen of fish (Yamamoto, 1987; Yamamoto and Itazawa, 1989; Wells and Weber, 1990). Erythrocytes are stored in the spleen and are expelled into the systemic circulation by contraction of the spleen (Nilsson and Grove, 1974). An increase in Hct can also result from erythrocyte swelling (Nikinmaa, 1983; Wells and Weber, 1990) and from the movement of water out of the plasma, which results in haemoconcentration.

The present study concentrates on the contribution of the spleen to the massive increase in Hct during strenuous exercise in *Pagothenia borchgrevinki.*

Borchs (*Pagothenia borchgrevinki*) (body mass 75.0±15.6g, mean ± standard deviation, N=40) were caught over deep water, using a baited hook and line, through holes cut in the sea ice off McMurdo Sound, Antarctica. The fish were transported to an aquarium system at Scott Base (New Zealand Antarctic Research Programme) where they were allowed to recover from the stress associated with capture for at least 48 h

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before experimentation. They were kept in sea water at 0°C under a natural photoperiod of 24h of light and were not fed.

The fish were divided into three groups: controls (N=12), sham-operated (N=12) and spleen-ligated (N=16) fish. Half of the fish in each group were sampled after being left undisturbed for 48h (resting fish) and the other half were sampled after being swum at high speed.

Surgery was performed in a room at 0°C to prevent heat damage to the fish. Borchs were anaesthetised in 0.04% benzocaine in sea water and then placed ventral side up on a foam block and their gills irrigated with 0.02% benzocaine to maintain anaesthesia. A 2 cm mid-ventral incision was made into the body cavity to allow access to the spleen. Blood loss from the mesenteric vessels and from the body wall where the incision was made was minimal. A loop of suture thread (Ethicon, 2-0 silk) was placed around the splenic vessels and tied firmly, preventing blood flow to and from the spleen, after which the incision was closed. For the sham-operated fish, the same surgical procedure was followed except that the spleen was not tied off. Fish were allowed to recover from surgery for 48h before being sampled. Control fish were not surgically manipulated.

It is not possible to exercise antarctic fish to exhaustion in the traditional sense by swimming them at very high speeds, as they have a very limited ability to produce lactic acid (Davison *et al.* 1988). The following exercise protocol was designed to ensure that the fish swam at a speed close to their critical swimming speed. Borchs were swum individually in a Blazka-type swim tunnel (Blazka *et al.* 1960). Fish were initially swum at about 0.8bodylengths s^{-1} (15.5cm s^{-1}) for 5min, after which the speed was increased in steps (5min at 20 and 5min at 25cm s^{-1}) to a maximum of 1.4bl s^{-1} (27cm s^{-1}). Each fish was exercised for a maximum of 40min or until it was unable to swim against the current and fell back onto the restraining grid. This was considered to be an exhausted fish and the duration of the exercise period was recorded.

Individual fish were netted from the aquarium system (resting fish) or taken from the exercise machine and killed by transection of the spinal cord. Blood was collected immediately from the caudal vessels using a heparinised syringe and a 20 gauge needle. The body cavity was then opened and the spleen removed and weighed. The fish was weighed and measured. A 5 μl sample of whole blood was taken for determination of haemoglobin concentration and another blood sample collected into a heparinised microtubule and spun at 10000 ***g*** for 3min to determine haematocrit. The rest of the blood was spun at 5000 ***g*** for 3min and the plasma withdrawn. Blood collection and spleen removal were completed within 1min of net capture and the haematocrit microtubules filled within 2min.

Blood haemoglobin concentrations were determined using the cyanmethaemoglobin method (Winton, 1974). Duplicate 20 μl plasma samples were used to determine chloride concentration in a Corning Eel 920 chloride meter. Mean cell haemoglobin concentration (MCHC) was calculated from haemoglobin concentration/fractional haematocrit.

Values are presented as means ± standard deviation. The Student's t-test was used to test for statistical significance.

At the initial swimming speed of 0.8bl s^{-1}, borchs used labriform locomotion to

maintain station in the swimming tunnel. However, at 1.4bl s^{-1}, the fish used both the pectoral muscles and the myotome (intermittently) to maintain position. Four of the control fish swam for the whole of the exercise period of 40min. The mean control swim time was 37.5±4.2min. The haematological and splenic changes associated with this exercise are summarized in Table 1. Exercise caused a massive elevation in haematocrit, increasing from resting levels of 14.8±2.9% to 35.0±5.1%. Haemoglobin concentration also increased significantly. MCHC decreased from 226.4±23.0 to 157.1±17.1 g l^{-1}, indicating red blood cell swelling. Plasma chloride concentration increased from 222 to 235mmol l^{-1} (P<0.05), suggesting haemoconcentration due to movement of water out of the plasma. Exercise also caused the relative spleen mass to decrease by 66%, from 0.50 to 0.17% (Table 1). There was considerable variation in the relative mass of the spleens of the resting fish, whereas there was little variance in spleen mass from exercised fish, the spleens appearing fully contracted and devoid of any excess blood.

As with the control fish, four of the sham-operated fish swam for the whole of the exercise period. The physiological changes associated with exercise were not significantly different from those of control fish, except for the plasma chloride concentration, which was significantly higher in the resting sham-operated fish (P<0.05) and remained unchanged after exercise (Table 1).

Preventing blood flow through the spleen by ligating the splenic vessels significantly reduced the length of time a fish could swim against the water current. The spleen-ligated fish fatigued after 20.1±9.7min. Haematocrit, haemoglobin concentration and MCHC in the resting spleen-ligated fish were similar to those of control and sham-operated resting fish. Following exercise, haematocrit increased significantly, but only to 23.1±2.5%, much lower than the value observed in exercised control fish. The haemoglobin concentration of the blood did not change with exercise. MCHC in spleen-ligated fish decreased significantly after exercise (Table 1).

The relative spleen mass of the resting spleen-ligated fish was not significantly different from that of control resting fish. Exercise had no effect on the mass of these ligated spleens.

The Hct of resting control borchs killed using spinal transection was only 14.8% and compares favourably with haematocrit readings from unstressed borchs in other studies (see Franklin *et al*. 1991; Wells *et al*. 1984, 1989). Similar values were recorded from resting sham-operated animals, indicating that, at least as far as haematological variables are concerned, the fish had recovered from surgery. Hct readings from resting spleen-ligated animals showed only a modest, non-significant increase compared with those of control fish. As the haemoglobin levels were the same, this suggests that the small difference in Hct was a consequence of red cell swelling. As ligating the spleen would prevent any sequestering of blood cells back into the spleen, we must assume that the anaesthesia used in the present study did not produce any splenic contraction, such as is seen in trout (Wells and Weber, 1990).

Exercise caused a 136% increase in Hct in control fish. This is the largest increase in Hct reported for a fish that has been exercised and is higher than the 110% increase recorded in an earlier study (Davison *et al.* 1988). In exercise studies on temperate-water fishes much smaller increases have been observed, usually in the range 40–60% (Wells

Table 1. *Summary of blood and splenic variables from control, sham-operated and spleen-ligated* Pagothenia borchgrevinki *that are resting or have been exercised to exhaustion*

	Controls		Sham-operated		Ligated spleen	
	Resting (N=6)	Exercised (N=6)	Resting (N=6)	Exercised (N=6)	Resting (N=8)	Exercised (N=8)
Body mass (g)	83.9±18.2	71.7±3.5	81.5±29.8	73.4±7.6	74.0±12.2	68.4±8.3
Exercise duration (min)	–	37.5±4.2	–	37.5±4.8	–	20.1±9.7†
Haematocrit (%)	14.8±2.9	35.0±5.1*	15.4±5.7	33.6±4.9*	16.3±4.5	23.1±2.5*†
Systemic haemoglobin ($g\,l^{-1}$)	33.3±5.1	52.6±7.4*	32.0±8.2	51.6±7.7*	33.8±10.1	38.4±3.3†
MCHC ($g\,l^{-1}$)	226.4±23.0	157.1±17.1*	216.6±39.6	153.4±9.0*	208.4±31.5	167.5±22.6*
Plasma [chloride] ($mmol\,l^{-1}$)	222±6	235±9	235±8	239±12	238±12	231±3
Spleen mass (g)	0.42±0.19	0.12±0.03*	0.42±0.20	0.12±0.04*	0.22±0.11	0.19±0.03†
Relative spleen mass (% body mass)	0.50±0.21	0.17±0.03*	0.51±0.18	0.17±0.04*	0.31±0.18	0.28±0.05†

Values are means ± standard deviation.
MCHC, mean cell haemoglobin concentration.
* indicates a significant difference between resting and exercised values in the control, sham-operated or spleen-ligated fish (t-test, P<0.01).
† indicates a significant difference between the control fish and sham-operated or spleen-ligated fish (t-test, P<0.01).

and Weber, 1990; Yamamoto *et al.* 1980; Yamamato and Itazawa, 1989). In some investigations Hct hardly changed with exhaustive exercise. For example, Turner *et al*. (1983) reported only a 14.6% Hct increase in flathead sole (*Hippoglossoides elassodon*) and Egginton *et al*. (1991) measured a decrease in an antarctic teleost (*Notothenia neglecta*). The increase in Hct shown by the borchs is the product of several changes in blood variables. There appears to be a small amount of haemoconcentration, though this was only shown by the control fish. There was a significant swelling of erythrocytes, which was particularly noticeable in the spleen-ligated animals where the number of cells did not increase (as indicated by the lack of change in [Hb]) yet the Hct did. The most important component of the increase in Hct was the increase in red cell number.

Compared to other species of fish, why is the increase in Hct in *P. borchgrevinki* so much greater? Wells *et al*. (1980) found that the MCHCs of antarctic fish species were low compared with those of temperate and tropical species. As it is [Hb] that determines oxygen-carrying capacity of the blood, a greater increase in Hct might be needed to achieve a certain [Hb]. Recently Axelsson *et al*. (1992) in a study of the cardiovascular system of *P. borchgrevinki* found that this fish could only double its myocardial power output during exercise. This scope for cardiac work is low, especially when compared with the trout or sea raven where cardiac power can be increased by 4–5 times (Farrell, 1991). The borch's strategy to increase Hct greatly during exercise may be to off-set the limitations set by the cardiovascular system and low MCHC. Also of importance is the viscosity of blood at low temperatures. Antarctic fish reduce their Hcts to lower blood viscosity. In borchs, these excess red cells are stored in the spleen. During exercise, the Hct is allowed to rise but at the expense of increased viscosity and increased work load for the heart (Macdonald and Wells, 1991).

The significance of the increase in Hct and thus oxygen-carrying capacity of the blood was very apparent in fish with ligated spleens. No fish completed the exercise regime, the animals quickly becoming exhausted once the highest speed had been achieved. At this highest speed, intermittent use of the myotome suggested that the pectoral red muscle fibres were working at maximum capacity, requiring a high, constant supply of oxygen. The anaerobic capacity of this fish has been reported to be limited (Davison *et al.* 1988), indicating that adequate supply of oxygen to the working muscles is crucial at high speed. Greater than 60% of the increase in control fish Hct can be attributed to the release of erythrocytes from the spleen and this contribution of erythrocytes greatly enhances swimming performance. Wood (1991) has suggested that the increase in Hct during exercise in fish assists post-exercise recovery. This is unlikely to be the case for borchs as it has been shown that recovery is rapid in these fish because they do not build up an oxygen debt (Davison *et al.* 1988).

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