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SHORT COMMUNICATION

THE SPLEEN IN HYPOXIC AND EXERCISED RAINBOW TROUT

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The teleost spleen is a discrete organ containing, in addition to erythrocytes sequestered from the circulation, erythropoietic tissue involved in the synthesis of new erythrocytes. In this division into supply and synthesis, there appear to be some species differences. The eel spleen is an erythrocyte reservoir but is not thought to be a major erythropoietic organ (Johansson-Sjobeck, 1979). The splenic mass in goldfish does not change with induced anaemia (Houston *et al.* 1988). The trout spleen, in contrast, plays an erythropoietic role during anaemia (Lane, 1979), in addition to serving as a store of erythrocytes (Randall and Daxboeck, 1982). Exercise in trout is accompanied by haemoconcentration (Black *et al.* 1966; Stevens, 1968) and the spleen appears to contribute to elevated circulating haemoglobin levels (Stevens, 1968). Capture stress induced a 25% reduction in spleen haemoglobin concentration, [Hb], in the marine teleost *Girella tricuspidata*, and splenic histology revealed melano-macrophagic centres and erythropoietic tissue indicative of a major role in erythrocyte destruction and synthesis (Ling, 1984). Neither role can be played by the spleen of the icefish, which lacks haemoglobin (Wells *et al.* 1990).

Although storage and synthesis of erythrocytes in the spleen have been examined in few fish, several intraspecific factors affect the size of the spleen. These include reproductive status and body weight (Yamamoto and Itazawa, 1989), exercise (Yamamoto *et al.* 1980; Yamamoto, 1988) and hypoxia (Yamamoto *et al.* 1985; Wells *et al.* 1989). Active fish appear to have highly contractile spleens that assist in the expulsion of erythrocytes (Nilsson and Grove, 1974).

Rainbow trout, more than any other fish, have received the close attention of researchers, and yet we can find little information concerning the possible role of the trout spleen in erythrocyte supply during exercise and hypoxia. Furthermore, no attempt has been made to discern whether erythrocytes stored in the spleen are qualitatively different from those in general circulation. In this study we have addressed these problems.

Female rainbow trout (*Salmo gairdneri* Richardson) were obtained from a

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commercial supplier and kept in large tanks supplied with aerated tapwater ($1.5\,l\,min^{-1}$ flow, $15\pm1\,°C$, $P_{O_2}>18\,kPa$). The fish (approx. body mass 350–450 g) were fed maintenance rations of commercially available pellets for a minimum of 2 weeks before use.

Chronic hypoxia was induced in a group of trout by acclimation for 2 weeks at $7.3\pm0.3\,kPa$ P_{O_2} using a P_{O_2}-stat device, as described previously (Tetens and Lykkeboe, 1985). Acute hypoxia (water $P_{O_2}=4\,kPa$) was induced for 1 h in a second group of fish (see Tetens and Lykkeboe, 1985). A third group was exercised over a period of 5–10 min by repeatedly touching the tail with a rod. The final group was anaesthetized in $0.1\,g\,l^{-1}$ benzocaine and sampled at the first signs of deep anaesthesia.

Trout were rapidly killed by spiking the brain in the manner known to Japanese fishermen as *ike jime*, achieved by thrusting a narrow-bladed knife between the eyes into the cranial cavity and twisting the blade (Boyd *et al.* 1984). This procedure disrupted the medulla oblongata, thus effectively severing the spinal cord from the higher neural centres. Blood was taken from the caudal vein, and the spleen excised from its shallow ventral position near the pelvic mid-line. The spleen was placed on Parafilm, weighed, and erythrocytes collected into glass capillaries from near the sectioned splenic vein after gently massaging the organ. Several aortic cannulations were carried out under anaesthesia, as previously described (Tetens and Lykkeboe, 1985), to sample blood from undisturbed fish after 5 days' recovery from anaesthesia.

Haematocrit values (Hct) were estimated by centrifugation, and haemoglobin concentration ([Hb]) was measured by lysing erythrocytes in distilled water, reading the absorbance at 577 and 542 nm, and applying the respective millimolar extinction coefficients of 15.37 and 14.37 for oxyhaemoglobin. Mean cell haemoglobin concentration (MCHC) was calculated from [Hb]/fractional Hct and used as an index of erythrocyte swelling. Total spleen [Hb] was measured in supernatants obtained from centrifugation of homogenized spleens. Nucleoside triphosphate (NTP) was assayed in deproteinized extracts of erythrocytes from the spleen and circulation using the Sigma enzymatic test kit no. 366-UV for ATP. NTP was assumed to be essentially ATP (Tetens and Lykkeboe, 1985) and its concentration expressed per gram Hb.

Preliminary tests of inequality of treatment variances revealed that sample variances did not differ significantly. Analysis of variance rejected a multisample hypothesis of equal treatment means and established a significant ($P<0.05$) added variance component among spleen masses and total Hb, and among systemic Hct, [Hb], MCHC and ATP. Following ANOVA, Tukey's multiple comparison test was applied *a posteriori* to analyse treatment effects (Zar, 1984).

The spleen from resting, normoxic trout contained densely packed erythrocytes, as judged from Hct values of greater than 90 %. These erythrocytes were released into the circulation after exercise (Table 1). Erythrocytes could not be sampled from the contracted spleens of exercised, anaesthetized or acutely hypoxic fish. The total Hb content of spleens was decreased in exercised trout ($P<0.05$ *vs*

resting normoxic fish). Spleen masses (expressed as a percentage of body mass) were similar for normoxic and chronically hypoxic groups ($P>0.05$). The spleens of acutely hypoxic fish contained less Hb than those from chronically hypoxic or normoxic fish, and the circulating erythrocytes showed evidence of swelling through low MCHC.

Anaesthesia resulted in contracted spleens and the difference in spleen masses was significant compared with unanaesthetized controls ($P<0.05$).

Exercise and anaesthesia resulted in blood Hct values above resting, normoxic controls ($P<0.05$). It was also apparent from the MCHC measurements that the erythrocytes of exercised and anaesthetized fish were significantly swollen by comparison with other groups ($P<0.05$). These data demonstrate that the Hct increase may be due to both raised erythrocyte supply and erythrocyte swelling.

Splenic erythrocytes from resting normoxic trout contained more ATP than circulating erythrocytes ($P<0.05$). Haematocrits were, as expected (see Railo *et al.* 1985; Korcock *et al.* 1988), slightly lower in cannulated fish than in spiked fish. Nevertheless, data from the latter group were used in Table 1 to secure a valid comparison with splenic cells from spiked fish.

The trout spleen occupied approximately 0.6 % of the body mass of mature fish, a value twice that reported by Lane (1979) for immature trout. This is consistent with an approximate doubling of the spleen size at maturity in the carp (Yamamoto and Itazawa, 1989). The mass of the trout spleen is comparable with that of yellowtail (Yamamoto *et al.* 1980) and lies within the range for cyprinids (Yamamoto, 1987, 1988).

In its fully contracted state, the trout spleen is about half its resting mass. Capture stress in the marine teleost *Girella tricuspidata* resulted in a more modest 25 % decrease in spleen [Hb] (Ling, 1984). The resting spleen in yellowtail stores an estimated 13.6 % of total erythrocytes, and 82 % of these are released within 5 min of exercise (Yamamoto *et al.* 1980). Contractions were completed within a few minutes in cyprinids (Yamamoto, 1988), but graduated contraction has not been clearly documented.

How significant is splenic contraction in the trout in terms of erythrocyte supply? Current research has led to a revision of blood volumes in teleosts because of the recognition of the importance of partitioning between the primary and secondary circulation systems (Vogel and Claviez, 1981). The red cell mass of a trout is approximately 1 % of its body mass (J. Steffensen, personal communication). On the basis of the gravimetric estimations in Table 1, full splenic contraction in a 400 g trout yields about 1 g of erythrocytes. One ml of packed cells added to 4 ml of circulating cells should have a significant impact on total oxygen-carrying capacity. The increase in circulating [Hb] was less than expected and may be explained through the movement of water from blood to muscle, or changes in the distribution of water between primary and secondary circulation systems during exercise and hypoxia. Soivio *et al.* (1980) suggested that removal of plasma water from the primary circulation explained raised blood oxygen-carrying capacity.

Table 1. *Characteristics of the trout spleen and a comparison of splenic and systemic erythrocytes after manipulations*

	Normoxia (N=7)	Hypoxia (N=9)	Exercise (N=5)	Anaesthesia (N=7)	Acute hypoxia (N=4)
Spleen mass (g)	2.02±1.07	2.06±0.32	0.95±0.29	0.89±0.35	1.94±0.30
Spleen (% body mass)	0.56±0.47	0.62±1.11	0.26±0.09	0.31±0.09	0.53±0.18
Total haemoglobin (mmol kg^{-1} spleen)	1.98±0.61	2.22±0.27	1.24±0.43	1.71±0.29	0.83±0.11
Spleen haematocrit (%)	92.6±3.9	95.6±3.68		86.6±0.14	
Systemic haematocrit (%)	25.1±6.1	19.2±3.8	41.8±9.0	38.0±6.4	32.8±1.7
Spleen haemoglobin (mmol l^{-1})	2.96±0.57	3.60±0.06		2.33±0.10	
Systemic haemoglobin (mmol l^{-1})	0.89±0.24	0.70±0.14	1.18±0.32	1.05±0.12	1.03±0.13
Spleen MCHC (mmol l^{-1})	3.21±0.72	3.76±0.08		2.69±0.11	
Systemic MCHC (mmol l^{-1})	3.60±0.88	3.62±0.24	2.81±0.35	2.78±0.26	3.16±0.54
Spleen NTP (μmol g^{-1} Hb)	43.7±12.8				
Systemic NTP (μmol g^{-1} Hb)	32.0±8.3	26.3±6.7	27.9±3.6	18.4±5.3	24.9±6.0

Values are means±S.D.
MCHC, mean cell haemoglobin concentration; NTP, nucleoside triphosphate.

Splenic contraction occurred in response to severe, acute hypoxia, an observation consistent with raised [Hb] in acutely hypoxic trout (Tetens and Lykkeboe, 1985) and in yellowtail (Yamamoto *et al.* 1985).

Chronic hypoxia did not result in splenic contraction. Oxygen consumption, Hct and MCHC are preserved in chronically hypoxic trout, and improved oxygen delivery is secured through an ATP-induced increase of erythrocyte oxygen-affinity (Sovio *et al.* 1980). Red-blooded antarctic nototheniids responded differently to hypoxic acclimation by showing a 39 % reduction in splenic mass as well as increased blood oxygen-affinity (Wells *et al.* 1989).

Tetens and Lykkeboe (1981) found that anoxically incubated trout erythrocytes showed a rapid fall in NTP (mainly ATP), and Weber (1982), assuming low oxygen levels in the spleen, considered this a possible mechanism for an instant splenic supply of ATP-poor cells which have raised affinity for oxygen. Our measurements show that this is not the case. The trout spleen contracts in response to acute emergencies in oxygen supply by ejecting NTP-enhanced erythrocytes into the circulation. The time over which these NTP-enriched cells persist in the circulation is not known. The extent of NTP enrichment is not spectacular, and we cannot be certain that it is the sequestered splenic cells that have this characteristic. It seems more likely that it is the brand new cells released from erythropoietic tissue that have high NTP levels.

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