Release of Erythrocytes from the Spleen during Exercise and Splenic Constriction by Adrenaline Infusion in the Rainbow Trout

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Abstract Erythrocyte-supplying function of the spleen was examined in the rainbow trout *Salmo gairdneri* under exercise. The spleen showed remarkable reduction, about 70% in weight and about 85% in hemoglobin content, after forced exercise of 15 min. The amount of erythrocytes released from the spleen was 2.33 ml/kg body, and this amount corresponds to about 20% of the total volume of circulating erythrocytes in resting condition. No damage was observed at the spleen, splenic artery and splenic vein after the exercise. Examination of the vascular system by a corrosion casting method showed that no place other than the venous circulation exists for the erythrocytes released from the contracted spleen. The spleen was strongly constricted by infusion of adrenaline into the organ. These facts imply that the fish spleen supplies stored hemoglobin into the circulating blood in response to an increased demand of oxygen during exercise, under the control of the sympathetic nervous system.

The spleen of mammals, e.g. horse and cat, containing a high amount of trabeculae rich in smooth muscle cells is known to contract and release a considerable amount of erythrocytes under forced exercise (Hartwig and Hartwig, 1985, for review). The spleen of fish, on the other hand, usually have no trabeculae and no smooth muscle cells (Tischendorf, 1985; Fänge and Nilsson, 1985, for review). In the dogfish, *Squalus acanthias*, faradic stimulation of the splenic pedicel or the spleen itself showed no visible evidence of change in the size of the organ (Opdyke and Opdyke, 1971). In the rainbow trout, *Salmo gairdneri*, it was reported that there was no significant change in splenic weight associated with forced exercise of 15 min (Stevens, 1968).

In the yellowtail, *Seriola quinqueradiata*, the spleen was shown to contract and supply erythrocytes into the circulating blood both under forced exercise and under severe hypoxia (Yamamoto et al., 1980, 1983). But the effect of sympathomimetic agents on the spleen and the mechanism of contraction of the organ are left unknown. The only case in which positive effects of catecholamines upon fish spleen was experimentally shown is the case of the Atlantic cod, *Gadus morhua* (Nilsson and Grove, 1974).

The present study investigates the effects of forced exercise on the weight and hemoglobin content of the spleen of the rainbow trout and attempts to confirm the blood storage function of the organ in this fish. The study also presents an illustration of the vascular system of the fish on the basis of the corrosion cast of the system and the results of a preliminary experiment on the effect of adrenaline upon the spleen.

Materials and methods

Rainbow trout of 519±64 g (Mean±S.D., the same will apply hereinafter) in body weight of both sexes were obtained from a fish farm in Oita Prefecture and kept in well-aerated water at 16.5–17.5°C for at least one week prior to the experiment. They were fed once a day with trout pellets at 0.5% of the body weight until two days before experiment. The studies were performed from September, 1986 to January, 1987.

Responses of the spleen to exercise. A fish was anesthetized in 1: 10,000 quinaldine solution for 2 min and allowed to recover from the anesthesia in a respiration chamber for at least 38 h for resting. The respiration chamber was darkened and irrigated with well-aerated water of 6.5 ml/l or about 156 mmHg in oxygen level. The temperature of the water was maintained at 16.5–17.5°C. The flow rate of water through the chamber was adjusted to 600 ml/min or 1.5 mm/s in average speed (Fig. 1). The fish was forced

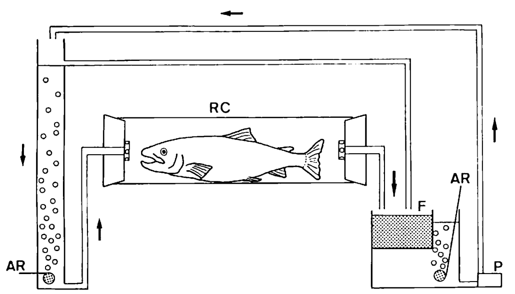

Fig. 1. The respiration chamber system. P, lift pump; AR, aeration; F, filter; RC, respiration chamber.

to swim for 15 min by continuous chasing in a tank of 60×40×35 (depth) cm. The fish was then instantly killed by a sharp blow on the head. The spleen was quickly excised from the killed fish after ligation of the splenic artery and splenic vein, and weighed on a direct reading balance to a precision of 1 mg. The hemoglobin content of the spleen was determined by applying the cyanmethemoglobin method to the tissue homogenate after Yamamoto et al. (1980). The hemoglobin content per unit body weight was then calculated. Control fish kept in the resting condition were treated in the same procedures except chasing.

The vascular system. The entire vascular system of the fish was observed by the corrosion casting method after Iwamizu and Itazawa (1986) to examine, from the point of view of erythrocyte supply from the spleen into the circulating blood, the position of the organ in the general blood circulation.

Effects of adrenaline upon the spleen. A sympathomimetic agent, 0.3 m*l* of *l*-Adrenaline hydrogen tartrate solution of 10 mg/*l* in concentration, was injected into the spleen in situ of resting condition at the point opposite to the entrance and the exit of the splenic vessels to examine the effects of adrenergic stimulation upon the organ.

Results

Responses of the spleen to exercise. Weight of the spleen showed 70% decrease from the resting level of 3.98±1.32 g/kg body to 1.20±0.51 g/kg body after the forced exercise. Hemoglobin content of the spleen per unit body weight showed 85% decrease from the resting level of 0.79±0.32 g/kg body to 0.12±0.04 g/kg body after the forced exercise (Fig. 2). The relationship between the spleen weight (SW, g/kg body) and the spleen hemoglobin per unit body weight (SHb, g/kg body) is expressed as SHb=0.214SW−0.083 (r=0.966) (Fig. 3).

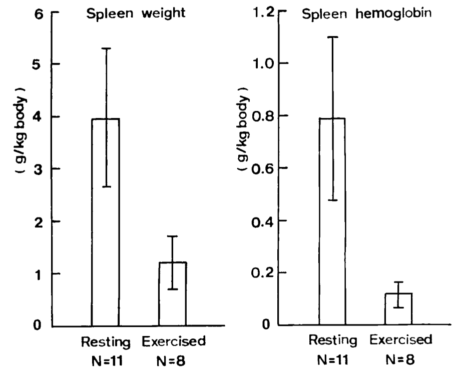

Fig. 2. Weight and hemoglobin content of the spleen in the resting and exercised rainbow trout. Bars indicate the mean values and vertical lines the standard deviations.

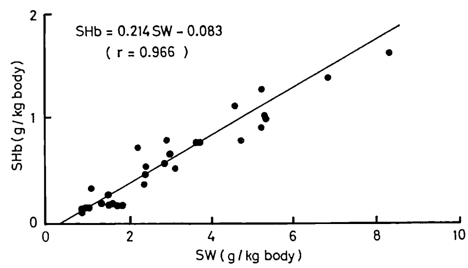

Fig. 3. The relationship between weight of the spleen (SW in g/kg body) and hemoglobin content of the organ (SHb in g/kg body).

The vascular system. A large portion of arterial blood flows into the coeliac artery via the dorsal aorta after leaving the gills. The coeliac artery sends off branches to the stomach, spleen and intestine. The spleen receives the arterial blood from one of these branches, the splenic artery. Venous blood from the spleen drains into the hepatic portal vein leading to the liver. The blood is then collected into the hepatic veins which enter into the sinus venosus. If the spleen is constricted, it is able to release sequestered erythrocytes into the venous circulation (Fig. 4).

Effects of adrenaline upon the spleen. After the adrenaline injection, the spleen gradually contracted and reached a full contraction state about

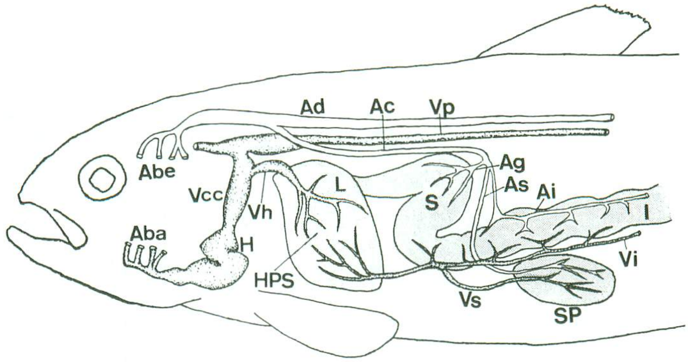

Fig. 4. Arrangement of the splenic artery and the splenic vein in the general circulation of the rainbow trout reconstructed by a corrosion casting method. Aba, afferent branchial artery; Abe, efferent branchial artery; Ac, coeliac artery; Ad, dorsal aorta; Ag, gastric artery; Ai, intestinal artery; As, splenic artery; H, heart; HPS, hepatic portal system; I, intestine; L, liver; S, stomach; Sp, spleen; Vcc, common cardinal vein; Vh, hepatic vein; Vi, intestinal vein; Vp, post-cardinal vein; Vs, splenic vein.

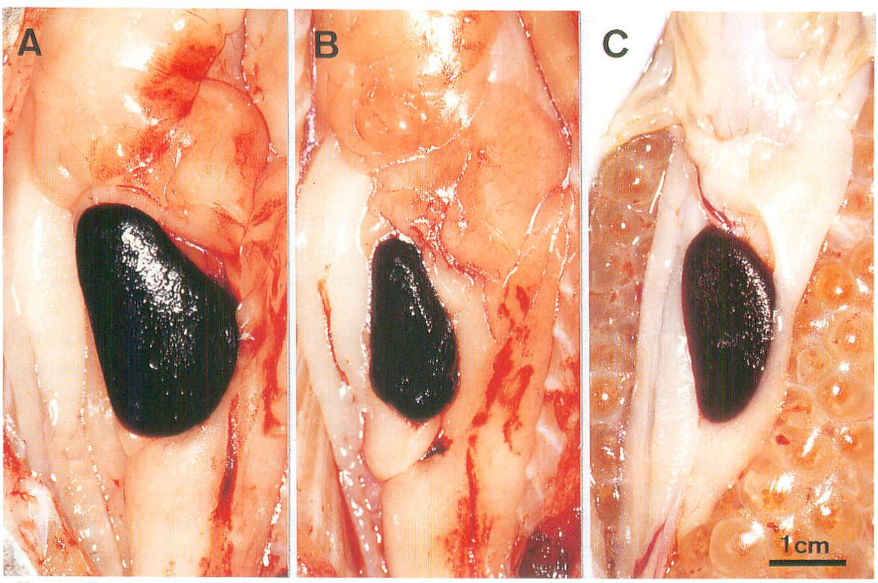

Fig. 5. External appearance of the spleen of rainbow trout A) at rest, B) after adrenaline infusion to the organ, and C) just after severe exercise for 15 min.

5 min after the injection. Splenic hemorrhage was not observed during the contraction of the organ. The spleen contracted by adrenaline seemed very similar to that contracted by forced exercise. The spleen in the resting condition was large and soft, and that in the constricted condition caused both by exercise and by adrenaline infusion was small and hard. The external appearances of the spleen in the resting condition (A), after forced exercise of 15 min (B), and 5 min after adrenaline infusion (C) are shown in Fig. 5.

Discussion

Responses of the spleen to exercise. The spleen contraction ratio or the ratio of reduction to the initial level was, in the present study, 70% in splenic weight and 85% in hemoglobin content. These ratios are not very different from those documented in yellowtail (71% in weight and 81% in hemoglobin content, Yamamoto et al., 1980) and freshwater teleosts (47–85% in weight and 56–93% in hemoglobin content, Yamamoto, 1988).

The volume of erythrocytes supplied from the spleen into the circulating blood, $RBC_{supplied}$ (m*l*/kg body), is calculated as follows:

$$RBC_{supplied}=100(SHb_r-SHb_e)/MCHC$$

Where SHb_r: hemoglobin content of the spleen in the resting condition (g/kg body), SHb_e: hemoglobin content of the spleen after exercise (g/kg body), and MCHC: mean corpuscular hemoglobin concentration (g/100 m*l*). The value of MCHC is calculated as follows:

$$MCHC=100Hb/Hct$$

where Hb: hemoglobin concentration of the whole blood (g/100 m*l*), and Hct: hematocrit value (%). Blood samples for MCHC determination were taken from 7 fish in the above-mentioned resting condition through the cannula inserted into the dorsal aorta. As the results, $RBC_{supplied}$ is calculated to have been 2.33 m*l*/kg body. This value is close to that (2.90 m*l*/kg body) observed in exercised yellowtail (Yamamoto et al., 1980). The total volume of erythrocytes in the resting condition was estimated, by the dilution method with Evans blue, to have been 12.6 m*l*/kg body with 5 rainbow trout (unpublished). Therefore, $RBC_{supplied}$ corresponds to about 20% of the total volume of circulating erythrocytes in the resting condition.

The vascular system. The route of intrasplenic circulation and the controlling mechanisms of splenic blood flow are still in question and ought to be subjects of further investigation.

As the conclusion from the results of the exercise experiment and the observation of the vascular system, the spleen of rainbow trout is considered to sequester a considerable amont of erythrocytes under the resting condition, and release the sequestered erythrocytes into the venous circulation under the exercised condition in response to an increased demand in oxygen.

Effects of adrenaline upon the spleen. Nilsson and Grove (1974) reported that in the Atlantic cod the splanchnic nerve to the spleen contained a mixed supply of adrenergic and cholinergic neurons. Fänge and Nilsson (1985) stated: "It is reasonable to believe that one important function of the splanchnic nervous control of the fish spleen (and/or control via circulating catecholamines and possibly other hormones) is to release erythrocytes from splenic stores during various states of 'stess' such as hypoxia." The levels of plasma adrenaline and noradrenaline are known to increase in exercised rainbow trout (Butler et al., 1986; Primett et al., 1986; Milligan and Wood, 1987).

In our experiments, the spleen contracted by an adrenergic stimulation and was considered to release erythrocytes into the circulating blood. It is, therefore, suggested that circulating catecholamines probably control the erythrocyte-supplying function of the spleen in the rainbow trout under exercise.

Acknowledgments

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ニジマスの運動時における脾臓からの赤血球の供給とアドレナリン注入による脾臓の収縮

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ニジマスの運動時における脾臓の赤血球供給機能についてしらべた．15 分間の強制運動の直後には，安静時の値に比べ，脾臓重量は約 70% 減少し，脾臓中ヘモグロビン含量は約 85% 減少した．脾臓から放出された赤血球の量は，2.33 ml/kg body であった．これは，安静時における赤血球量の約 20% に相当する．これらの事実は，運動後に脾臓，脾動脈ならびに脾静脈の破損がみられないこと，および腐食鋳型法による血管系の観察結果より考えて，脾臓から放出された赤血球は脾静脈を経て静脈血循環系に供給される以外にはあり得ないことと併せて，脾臓が循環血液中に赤血球を供給したことを示すものである．アドレナリンの脾内注入によって，脾臓は著しく収縮した．この事実は従来実証例の少なかった魚の脾臓に対するカテコールアミンの収縮効果を示しており，運動時の酸素需要増大に対し交感神経の支配によって脾臓が貯蔵ヘモグロビンを循環血液中に供給する能力を持つことを示唆するものである．

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