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Scanning Electron Microscope Study of Rainbow Trout Spleen with Special Reference to the Role of the Reticular Meshwork in Erythrocyte Release

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Abstract The fine structure of the perfused spleen of the rainbow trout (*Oncorhynchus mykiss*) was studied by scanning electron microscope. Morphological comparisons were carried out between the spleen in dilated (resting) condition and in contracted condition induced by 5.5 μM adrenaline infusion. Sphincter-like constrictions of the arterioles terminate additional blood supply to the spleen during splenic contraction. It is suggested that reticulum cells contributed to the marked decrease in splenic volume and expulsion of blood cells stored in the reticular meshwork following adrenergic stimulation. The fenestrae found in the walls of the proximal section of the venules function as major passages enabling erythrocytes to be propelled from the reticular meshwork to the vascular lumen.

The spleen of some teleosts plays an important role in blood circulation by means of sequestration, storage and release of erythrocytes (Yamamoto et al., 1980; Yamamoto, 1988; Pearson and Stevens, 1991; Gallaugher et al., 1992). In the rainbow trout, *Oncorhynchus mykiss*, the weight of the spleen showed a 70% decrease from the resting level after forced exercise, the amount of erythrocytes released from the spleen during such exercise being up to 20% of the total volume of circulating erythrocytes in resting fish (Kita and Itazawa, 1989).

A splenic contractile system is needed for rapid expulsion of blood cells from the spleen. The major contractile structures in the mammalian spleen are the capsulo-trabecular system and the arterioles (Saito et al., 1988). However, there are no smooth muscles in the capsule of the teleost spleen, nor does the system of muscular trabeculae (if at all present) attain the prominence of that in mammals (Fänge and Nilsson, 1985).

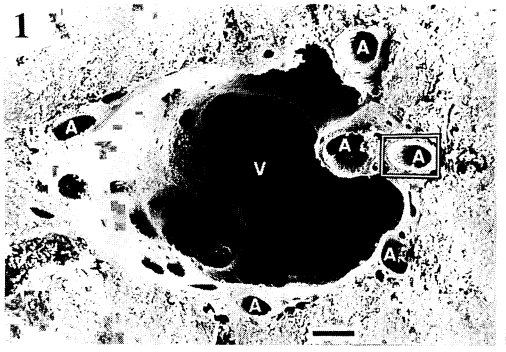
To answer the question therefore, "how are blood cells released from the spleen?," morphological comparisons were made between the spleen in dilated (resting) condition and in contracted condition induced by adrenaline.

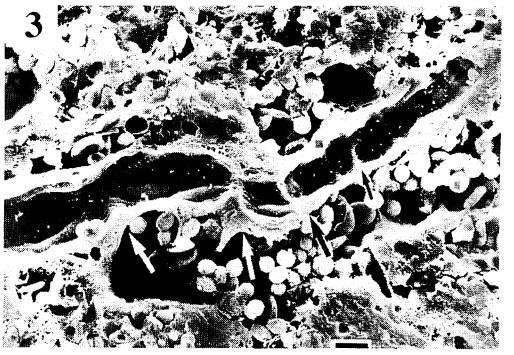
Materials and Methods

Rainbow trout of both sexes were obtained from a fish farm in Oita Prefecture, Japan, and kept in well-aerated water at 16.5–17.5°C. The spleens from seven fish of 737±61 g (Mean±SD) in body mass were used in this study.

Each fish used was sacrificed by a sharp blow on the head and injected with heparinized saline (1000 i.u. kg^{-1} body mass). The spleen was removed and perfused with heparinized saline (5 i.u. ml^{-1}) (Wolf 1963, without glucose); all details of the perfusing technique were identical to those described by Kita and Itazawa (1990).

Preparation of the dilated spleen (resting condition): The spleen was perfused first with heparinized saline without adrenaline for at least 30 min, and then with saline containing 1.1 μM adrenaline to contract the organ and expel blood cells from it. After 5 min of splenic contraction, the spleen was perfused again with adrenaline-free saline for at least 30 min. The spleen gradually recovered its volume, returning to the resting condition within 30 min. This procedure was repeated once more to maximize blood cell expulsion from the organ. After the spleen





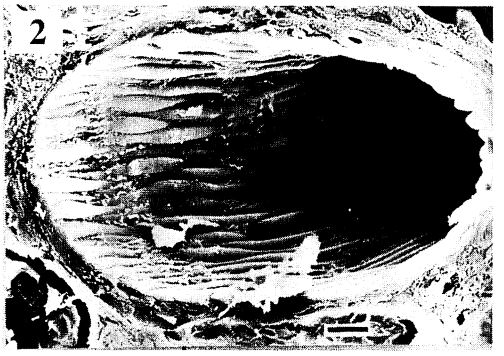


Fig. 1. Overview of the vein (*V*) and arterioles (*A*) in freeze-cracked rainbow trout spleen. 100 μm bar.

Fig. 2. Enlarged view of the artery marked in Figure 1. The luminal aspect of the artery shows a palisade of rod-shaped, endothelial cells with nuclear swellings. 10 μm bar.

Fig. 3. Sphincter constrictions (arrows) of an arteriole in the spleen in 90% contracted condition (spleen infused with 5.5 μm adrenaline). 10 μm bar.

had returned to the resting condition, it was fixed by perfusion with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.8) for 30 min. The splenic artery and vein were ligated and the whole preparation immersed in 2.5% glutaraldehyde in 0.1 M phosphate buffer for 12 h at 4°C.

Preparation of the contracted spleen: Blood cells in the spleen were expelled as described above, and the spleen administered with 5.5 μM adrenaline. Such administration with adrenaline is known to produce 90% splenic contraction (Kita and Itazawa, 1990). Five minutes following administration, the spleen was fixed as described above.

The fixed spleen preparation was cut into blocks (ca. 10×5×3 mm), conductive-stained and dehydrated in ascending concentrations of ethanol following Fujita (1974). The dehydrated specimens were frozen in liquid nitrogen and fractured by mechanical impact (Tokunaga et al., 1974). The fractured specimens were transferred to t-butyl alcohol, and frozen in a refrigerator, before being freeze-dried and coated with ion-sputtered gold (Wheeler et al., 1975; Akahori et al., 1988; Inoué and Osatake, 1988).

The specimens were observed and photographed in a SEM (JSM-T220 A) under accelerating voltages of 10, 15 or 20 kV.

Results

The freeze-fractured surfaces of the tissue blocks were fairly smooth and did not show damage or dislocation of the cellular and fibrous components. In a preliminary experiment in which the spleen was not perfused with saline, the cell components of the spleen could hardly be observed because the freeze-fractured surface was covered with considerable deposits, presumably plasma proteins. On the contrary, the cell surface of the perfused specimens was completely free of such depositions and clearly showed the fine structure of the capsule, lymphoid tissue, and reticular meshwork, as well as the arteries and veins supplying those areas.

Arterial system.—The arterioles were accompanied by much wider veins, running parallel to the arterial system (Fig. 1). The arterial endothelium consisted of a palisade of long, rod-shaped cells running parallel to the longitudinal direction of the arteriole, the central portion being elevated into the lumen of the arteriole and the end portions gradually tapered. The extremities of the rod-shaped cells overlapped

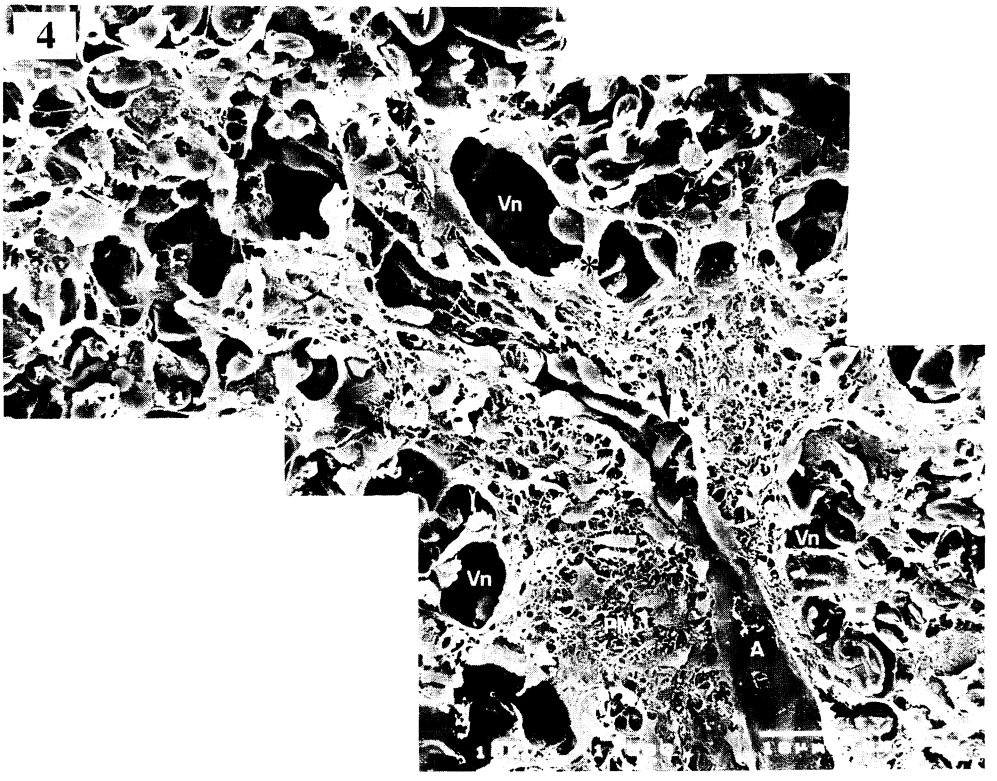


Fig. 4. Relationship between arteriole (*A*) and periarterial macrophage sheath (*PM*). The linkages of the adjoining endothelial cells are loose in the sheathed capillary, the cells forming intervening stomata (arrowhead). Arrow indicates endothelial cells at the branching portion of the sheathed capillary. Venules (*Vn*) surround the periarterial macrophage sheath. Asterisk—luminal trabeculae of the venule. 10 μm bar.

the immediate downstream neighbor, there being no gaps between them (Fig. 2).

Sphincter-like constrictions in the arteriole were observed in the contracted specimen (spleen infused with 5.5 μM adrenaline, Fig. 3), the luminal diameter of the arterioles being reduced from 20 μm to 6 μm. The connective tissue layer of the arteriole became thicker in the sphincter-like constricted area.

As the arterioles proceeded further, they gradually lost the layer of surrounding connective tissue, instead being equipped with a periarterial sheath (sheathed capillary). The linkage of the adjoining endothelial cells of the arterioles became loose and the cells formed intervening stomata coinciding with the disappearance of the surrounding connective tissue layer. The rod-shaped endothelial cells of the sheathed capillary ran parallel with the longitudinal direction of the vessel, except at branching sites, when the cells were bent. The distal end of the sheathed capillary opened directly into the reticular meshwork (Fig. 4). A capillary lacking a periarterial sheath was not observed. The periarterial sheath consisted of an extremely fine meshwork of fibrous reticulum and dendritic cells, presumably of macrophages (Fig. 5).

Reticular meshwork.—The splenic pulp was formed by a three-dimensional meshwork of reticulum cells, i.e., reticular meshwork. The reticulum cells had several fine processes connected with those of adjacent cells. In the reticular meshwork numerous erythrocytes and leukocytes were found.

In the dilated spleen (resting condition), few erythrocytes were found in the meshwork, those present having a normal, oval shape (Fig. 6). In the contracted specimen, however, the meshes were full of deformed, polygonal erythrocytes (Fig. 7).

Venous system.—There was no dramatic morphological change, as occurred in arteries, between the venous systems under dilated and contracted conditions. The venous endothelium consisted of a continuous sheet of closely associated cells, overlapping at

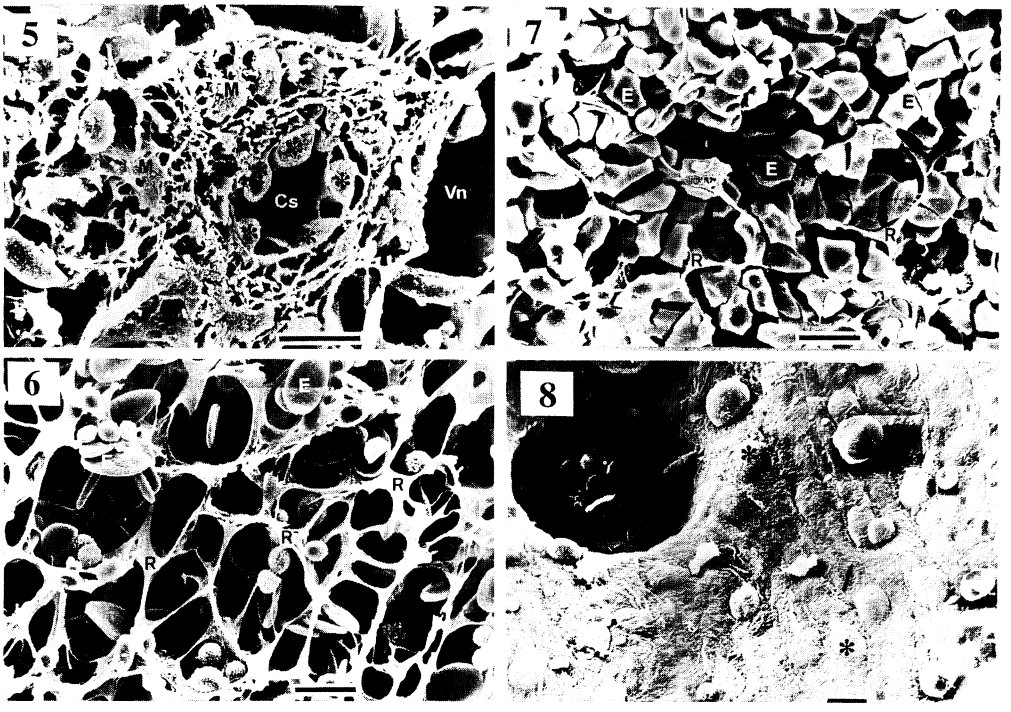


Fig. 5. High magnification view of a cross-fractured, sheathed capillary (*Cs*). Cells, presumably of macrophages (*M*), are held in fixed positions by reticular fibers. Asterisks—endothelial cells. *Vn*—venule. 10 μm bar.

Fig. 6. Reticular meshwork in the dilated spleen. The erythrocytes (*E*) found in the mesh show a normal, oval shape. *R*—reticulum cells. 10 μm bar.

Fig. 7. Reticular meshwork in the contracted (induced by 5.5 μM adrenaline infusion) spleen. The meshes of the reticulum cells (*R*) are full of deformed erythrocytes (*E*). 10 μm bar.

Fig. 8. Enlarged view of the luminal endothelial surface of the vein shown in Figure 1. The edges of the endothelial cells are overlapped (arrow). Asterisks indicate endothelial cell nuclei. 10 μm bar.

their edges and with a raised central area (Fig. 8). Thin reticulum cell processes supported the outside wall of the venule. The wall of the proximal section of the venule had irregularly localized fenestrae, round or oval in section. A medially constricted erythrocyte, arrested in the act of passing through such a fenestrae was frequently observed (Fig. 9).

The venules surrounding the periarterial sheath ramified markedly, the wall coming into contact with the sheath on one side (no fenestrations observed) and with the reticular meshwork on the other. The lumen of the venule was bridged by luminal trabeculae (Fig. 4).

Venules were also found in the subcapsular region, i.e. subcapsular venules, the walls being in contact with the epithelial cells of the capsule on one side and with the reticular meshwork on the other. In the contracted specimen, although the reticular space was reduced and the blood cells in the space deformed, the blood cells in the lumen of the venule retained their normal shape (Fig. 10). Erythrocytes passing through the fenestrae of the lumen of the subcapsular venule were frequently observed. Although the portions of such erythrocytes remaining in the reticular space were deformed, those parts protruding into the subcapsular venule were not (Fig. 11).

Capsule.—The capsule consisted of a single layer of squamous epithelium, the capsular tissue being continuous with the reticular meshwork. The reticulum cells were fixed to the inner surface of the capsule by extended cell processes (Fig. 12).

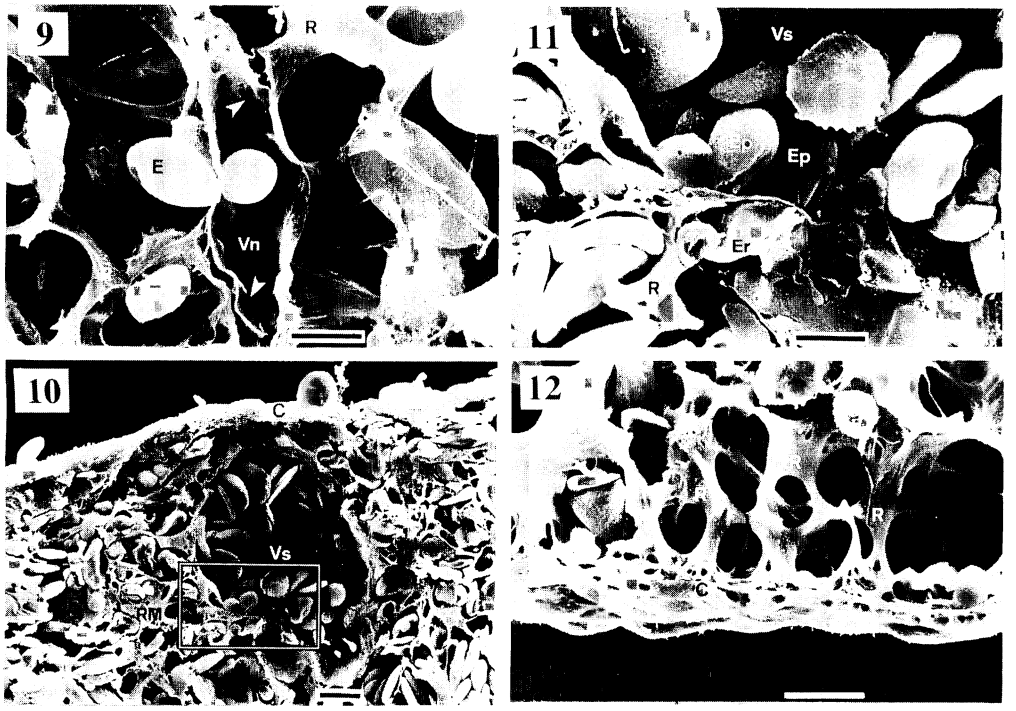


Fig. 9. Erythrocyte (*E*) passing through one of the fenestrae (arrowheads) on the wall of the venule (*Vn*). Reticulum cell (*R*) processes support the outside wall of the venule. 5 μm bar.

Fig. 10. Subcapsular venule (*Vs*) in the contracted spleen. Note that the blood cells in the reticular space are deformed, whereas the blood cells in the lumen of the venule are normal. *C*—capsule; *RM*—reticular meshwork. 10 μm bar.

Fig. 11. Enlarged view of the area marked in Figure 10. Note that the residual body of the erythrocyte (*Er*) in the reticular space is deformed, whereas the body protruding into the venule (*Ep*) is normal. *R*—reticulum cell. 5 μm bar.

Fig. 12. Capsule (*C*) of the dilated state spleen. The capsule consists of a single layer of squamous epithelium. Reticulum cells (*R*) are fixed to the inner surface of the capsule. 10 μm bar.

Discussion

Sphincter-like constrictions of the arteriole following adrenaline administration, that were observed in this study, are consistent with the resin casts of arterioles examined previously (Kita and Itazawa, 1990). A dramatic narrowing of the luminal diameter of arterioles in the contracted spleen was found, which had prevented the casting material from penetrating further in the earlier study. Such constricted areas in the arterioles in the trout spleen may be consistent with the so-called precapillary sphincter areas of mammalian arterioles. Precapillary sphincter areas vary in length and may extend for 10 to 15 μm along the vessel, or, instead, be represented by one or two smooth muscle cells, forming a true sphincter around the entrance of a capillary (Rhodin, 1984).

The terminal branches of the arterioles appeared to govern blood flow to various regions of the capillary network. In some vascular beds, there is a specialized smooth muscle cell (precapillary sphincter) which regulates blood flow through individual capillaries (Johnson, 1984). Peripheral arterioles, metarterioles, precapillary sphincters, and muscular venules in mammals are known to contain specific receptors for many neurohumoral substances and reagents, which elicit contraction, including catecholamines (Altura, 1984). Besides the nerve trunks, which accompany the arteries in the rainbow trout spleen, numerous unmyelinated nerves have been found by TEM along the capillaries (Zwillenberg, 1964).

It can thus be concluded that arterial blood flow in

the spleen of rainbow trout is regulated by adrenergic-mediating, sphincter-like constriction of the arteriole and/or endothelial contraction of the capillaries. The decreased splenic outflow rate following adrenaline administration (Kita and Itazawa, 1990) can be explained by these mechanisms. Arterial constriction terminates additional blood supply to the spleen during splenic contraction.

Because expulsion of blood cells from the spleen before fixation were maximized by the same procedure in the contracted and dilated spleen, residual erythrocytes of both were the same in number. In the contracted spleen, however, the reticular meshwork held more blood cells than the dilated spleen, indicating that the space for holding blood cells in the spleen had decreased considerably. In fact, the volume of the rainbow trout spleen is decreased by 70% during splenic contraction (Kita and Itazawa, 1989).

Nikinmaa and Huestis (1984) reported that adrenergic stimulation induces swelling of erythrocytes in rainbow trout. Adrenaline administration to an excised rainbow trout spleen, however, induced deformation, rather than swelling, of erythrocytes remaining in the reticular meshwork, indicating that such deformation was caused by mechanical force resulting from the decrease in the space containing the blood cells. It is believed that contractile reticulum cells (reticular meshwork) are one of the main effector units causing a prominent decrease in the volume of the rainbow trout spleen, since there are neither smooth muscles in the capsule of the teleostean spleen, nor an effective system of muscular trabeculae (Zwillenberg, 1964; Fänge and Nilsson, 1985). The reticulum cells of some mammals are thought to be contractile because they are innervated by adrenergic nerves and contain abundant microfilaments. Such reticulum cells probably participate, along with the smooth muscle of the capsule and trabeculae, in causing a prominent decrease in splenic volume following adrenergic stimulation (Weiss, 1985). The relative importance of neuronal (splanchnic nervous supply) and humoral (circulating catecholamines) systems in controlling the reticulum cells in rainbow trout is, however, still unknown.

The lack of dramatic morphological change, as occurred in arteries, between the venous systems of rainbow trout spleen under dilated and contracted conditions can be explained as follows. Venules are less sensitive to adrenergic stimulation than arterioles and precapillary sphincters (Altura, 1984). As the reticulum cells, which adhere to the basal surface of the venule and extend into the reticulum of the pulp, contract by adrenergic stimulation, they would prevent contraction of the venule by pulling the venular wall outwards. In addition, the luminal trabeculae observed in the lumen of the venules may participate in preventing venular contraction.

The fenestrae found in the wall of the proximal section of the venules function as major passages for blood cells shifting from the reticular meshwork to the vascular lumen. In the contracted spleen, the erythrocytes remaining in the reticular space were deformed, whereas those in the venule were not, indicating a higher pressure in the reticular space than in the vascular lumen. If the reticulum cells supporting the outside wall of the venule were contractile, they could propel blood cells through the fenestrae into the lumen as the cells contract following adrenergic stimulation. Rapid expulsion of stored blood cells after severe exercise (Kita and Itazawa, 1989), and in consequence of adrenaline administration to the spleen, may be caused by contraction of the reticular meshwork.

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ニジマス脾臓の走査電顕による観察、特に赤血球の放出における細網細胞の役割について

喜田　潤・板沢靖男

灌流したニジマス脾臓の微細構造を走査型電子顕微鏡を用いて観察した．安静状態にある膨らんだ脾臓と，5.5μM のアドレナリン投与によって収縮した脾臓の形態学的比較を行った．収縮した脾臓では，細動脈の括約筋様の収縮により脾臓への新たな血液の供給が止められる．アドレナリン性刺激による脾臓体積の著しい減少，および細網細胞の網目構造に貯えられた血球の放出に細網細胞が寄与していることが示唆された．細静脈起部の壁にある窓は，赤血球が細網構造から血管内に押し出されることを可能にする主要な通路となっている．

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