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Microcirculatory Pathways in the Spleen of the Rainbow Trout *Oncorhynchus mykiss*

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Abstract Three-dimensional structure of the vascular system of the rainbow trout (*Oncorhynchus mykiss* (=*Salmo gairdneri*)) spleen was studied under a scanning electron microscope using mainly corrosion casts. The specialized vessels related to the periarterial lymphatic sheath and lymphatic nodules which are seen in the mammalian spleen were not found in the rainbow trout. A major trunk artery simply gave off arterioles toward the subcapsular region. Termination of arterial capillaries in reticular meshwork, i.e. open circulation, was clearly observed. Arterioles infrequently showed sphincter-like constrictions which probably regulated blood flow through the organ. Reticular meshwork, which appeared in the cast as clusters of granular resin masses, was considered to be an important site for erythrocyte storage. At the subcapsular region the collecting veins, which showed a plate-like structure and lay on the reticular meshwork, gathered into veins which in turn went straight into the inside of the organ and joined a major trunk vein. These veins running from the reticular meshwork to a larger vein were considered to aid the rapid drainage of erythrocytes from the reticular meshwork at times of urgent need, such as during periods of strenuous exercise.

The spleen of teleosts plays an important role in blood circulation by means of sequestration, storage and release of blood cells. In the yellowtail *Seriola quinqueradiata* during exercise, the spleen was shown to contract and supply erythrocytes into the circulating blood causing elevation of the hematocrit value (Yamamoto et al., 1980). We have also observed in the rainbow trout *Oncorhynchus mykiss* (=*Salmo gairdneri*), that the amount of erythrocytes released from the spleen of the fish during exercise was up to 20% of the total volume of circulating erythrocytes in resting fish (Kita and Itazawa, 1989). The spleen of the Atlantic cod *Gadus morhua* was reported to contract in response to adrenaline, noradrenaline and acetylcholine (Nilsson and Grove, 1974). It was suggested that an important function of the splanchnic nervous control of fish spleen is to release erythrocytes from splenic stores during various states of stress (Fänge and Nilsson, 1985).

In the literature on the mammalian spleen, there is considerable controversy about the intrasplenic arteriovenous intermediate pathways, i.e., whether the blood flows through direct arteriovenous pathways (closed circulation) or through channels lacking endothelium before reaching the venous system (open circulation) (McCuskey, 1985). Recently, with newly developed techniques using a transmission or scanning electron microscope (SEM), many investigators have shown the mammalian intrasplenic circulation to be anatomically "open" (Weiss et al., 1985). Splenic circulation in fish is usually described as "open," although microcirculation studies have not yet been conducted (Fänge and Nilsson, 1985). A clear view of the morphology of the intrasplenic circulatory system is essential to study the regulating mechanisms of blood flow through the organ, for example, where and how blood cells are sequestered and released.

This paper demonstrates the three-dimensional microcirculatory pathway of the teleost spleen, with special emphasis on the arteriovenous intermediate circulation, as observed by scanning electron microscopy of corrosion casts.

Materials and methods

Rainbow trout of both sexes obtained from a fish farm in Oita Prefecture, Japan, and kept in well-aerated water at 16.5–17.5°C were used in this study. The spleens from six fish of 729±53 g (Mean± S.D.) in body mass were used for microcorrosion casting and the organs from six fish of 607±44 g were used for SEM examination.

Microcorrosion casting procedure. Each fish used was instantly sacrificed by a sharp blow on the

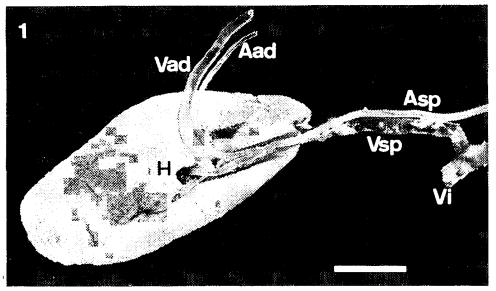

Fig. 1. Whole view of the resin cast of the spleen of the rainbow trout. Splenic artery (Asp) and splenic vein (Vsp) enter and leave the hilum (H) of the spleen. Aad, adipose tissue artery; Vad, adipose tissue vein; Vi, intestinal vein. Bar=0.5 cm.

head and injected with heparinized saline (1,000 i.u. kg^{-1} body mass). The animal was opened laterally on the right side of the body to expose the spleen and vessels. The spleen was in a constricted condition when it was exposed. The coeliac artery was catheterized under microscopic observation, using a polyethylene tube (1 mm in outer diameter) having a tapered tip. The portal vein was also catheterized. Except in those cases in which rupture was feared, all the branches of the coeliac artery were ligated but for the one to the spleen.

The spleen was perfused via the coeliac artery with heparinized saline (5 i.u. ml^{-1}) (Wolf, 1963) without glucose to wash out the red blood cells from the organ, at the room temperature of 16.5–17.5℃. The pressure of inflowing saline was kept at a constant level corresponding to approximately 65 cm H_2O. The distal end of the outflow catheter was set at the same level as the spleen. The spleen was in a constricted state at the start of perfusion, and then gradually recovered its volume. After the spleen lost its reddish color and the outflowing perfusate appeared colorless, the perfusate was changed to low-viscosity resin (Iwamizu and Itazawa, 1986). The resin injection was continued until the resin appeared in the venous catheter. The arterial and venous catheters were then clamped off and the whole body was left at room temperature for 1 hour. The whole preparation of the organ was then processed to a resin cast in a manner similar to those described by Murakami (1971), Murakami et al. (1973) and Schmidt et al. (1982). The cast was immersed in water and frozen before cutting. The osmium impregnation method was used in order to provide the cast with electrical conductivity.

SEM examination. The spleen was perfused in the same manner as described above. After the outflowing perfusate appeared colorless, the perfusate was changed to 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.9. The splenic artery and the splenic vein were ligated and the organ was excised and kept for 8 hours in the glutaraldehyde-phosphate fixative. The fixed organ was then cut into tissue blocks (ca. 10×5×3 mm), conductive-stained and dehydrated in ascending concentrations of ethanol according to the method of Fujita (1974). The dehydrated specimens were transferred to t-butyl alcohol, frozen in a refrigerator and fractured. These specimens were freeze-dried according to Akahori et al. (1988) and coated with ion-sputtered gold.

Results

Arterial circulation. The spleen of the rainbow trout was supplied by a single splenic artery arising from the coeliac artery. The splenic artery entered the spleen through its hilum (Fig. 1), while branches from the splenic artery entered not the spleen but the adjacent adipose tissue. The splenic artery, on entering the spleen, became a major trunk artery (100–300 μm in diameter) running through the organ (Fig. 2a). This major trunk artery gave off many arterioles (15–65 μm in diameter) running toward the subcapsular region of the organ (Fig. 2c). The arterioles bifurcated repeatedly, some becoming arterial capillaries (3–12 μm in diameter) before reaching the subcapsular region (Fig. 2d, e) and the others becoming arterial capillaries at the subcapsular region (Fig. 2f). There were no anastomoses between arterioles. The major trunk artery and many of the arterioles were accompanied by veins running parallel to the arterial system (Fig. 2a, b). The distal ends of the arteriolar casts were occasionally covered with masses which were commencing portions of the venules (Fig. 4a). The surfaces of the arteriolar casts were usually smooth, and only infrequently shrunk (Fig. 4b). Microcorrosion casts of the trout spleen did not show any evidence of a periarterial macrophage sheath or an ellipsoid.

Intermediate circulation. Arterial capillaries which branched off from arterioles before reaching the subcapsular region as well as those that branched off at the subcapsular region both terminated in the reticular meshwork (Fig. 2d–f). The reticular mesh-

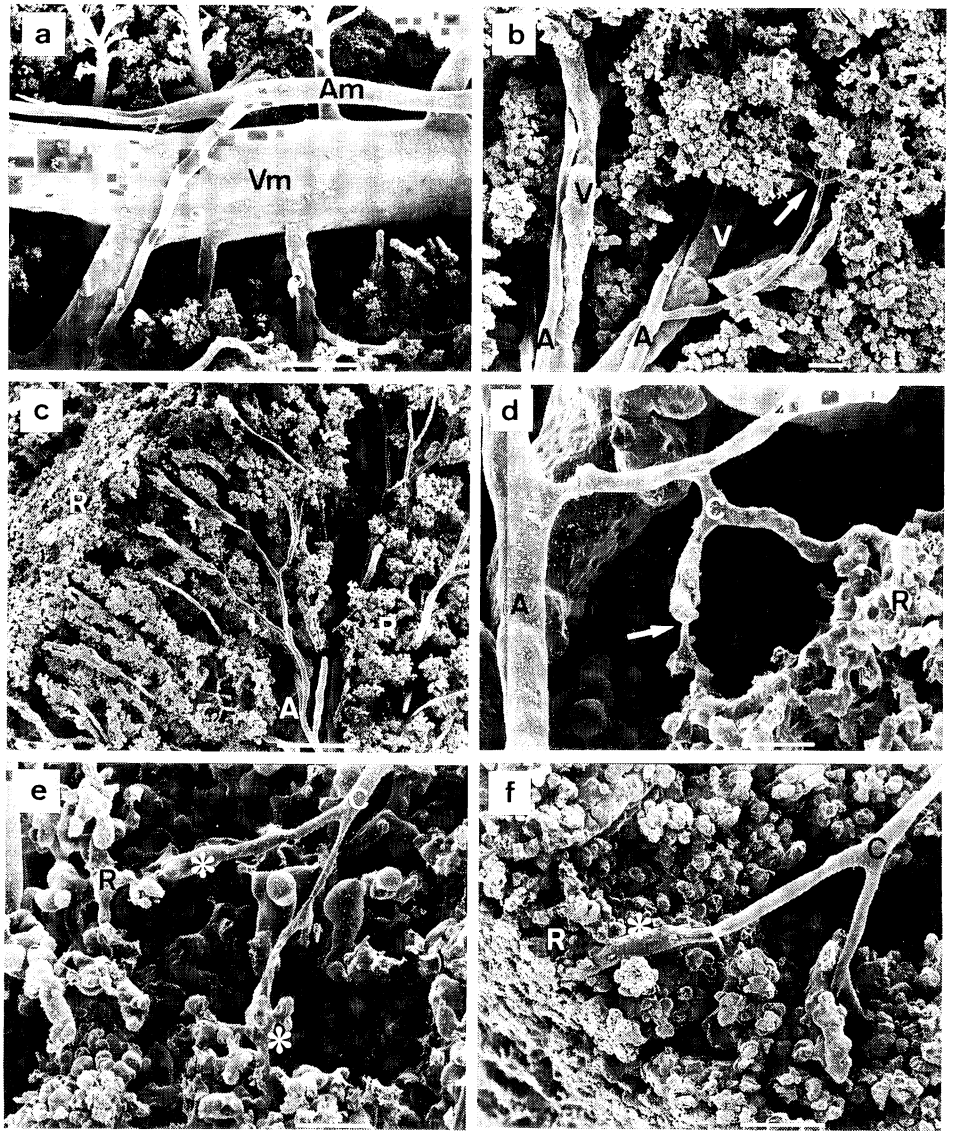

Fig. 2. Resin cast of the arterial pathways. a: Major trunk artery (Am) running parallel with the major trunk vein (Vm). Bar = 1 mm. b: Arterioles (A) accompanied by parallel veins(V). Arterioles travel into capillaries (arrow) and terminate in the reticular meshwork (R). Bar = 100 μm. c: Section of a cast showing the interior of the spleen. The subcapsular region of the organ is seen at the upper left of the picture. Arterioles (A) repeatedly bifurcate and run toward the subcapsular region. R, reticular meshwork. Bar = 500 μm. d: Arterial capillaries (C) arising from arterioles (A) and terminating in reticular meshwork (R). At the interior of the organ, the termination of an arterial capillary in the reticular meshwork shows marked narrowing (arrow), suggesting endothelial contraction. Bar = 50 μm. e: Capillaries terminate in the reticular meshwork via ampullae (*). Bar = 50 μm. f: Capillaries terminate at the subcapsular region (R). Lower left part shows the subcapsular region. *, ampullae. Bar = 100 μm.

work appeared in the cast as clusters of granular resin masses. The SEM of tissues showed the reticular meshwork formed by reticulum cells which had processes connecting with those of adjacent cells (Fig. 3a). The resin clusters of reticular meshwork at the subcapsular region covered the entire organ.

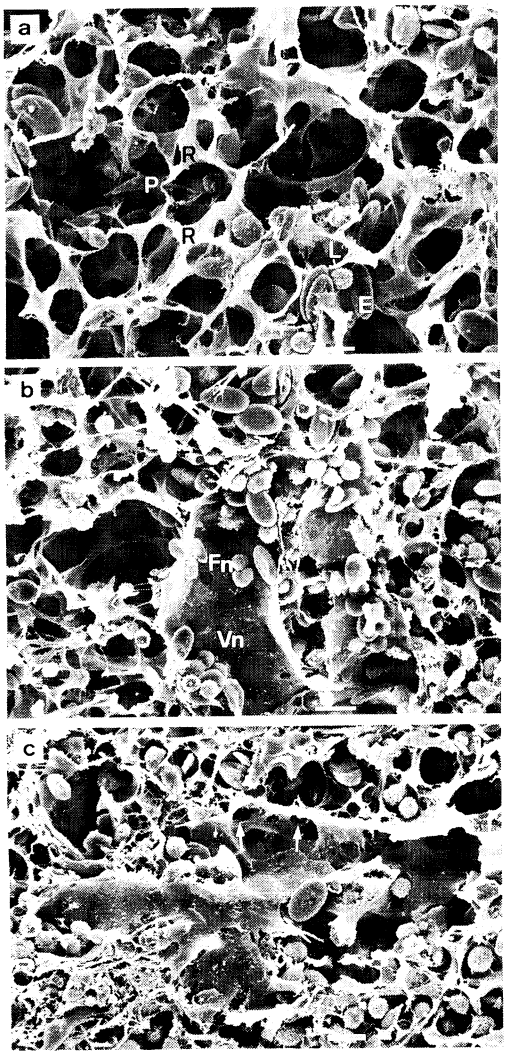

Fig. 3. SEM image of perfusion-fixed and freeze-fractured spleen. a: Reticular meshwork formed by reticulum cells (R) which have processes (P) connecting with those of adjacent cells. E, erythrocyte; L, leukocyte. Bar = 10 μm. b: Inner view of the distal end of the venules (Vn). Fn, fenestrae. Bar = 10 μm. c: The wall of venule has fenestrae (arrow) which are wide enough for blood cells to pass through from the reticular space to the lumen of the venule. Bar = 10 μm.

The capillaries merged into reticular meshwork, usually via ampullae. Some entrances to the reticular meshwork exhibited marked narrowing (Fig. 2d). The arterial capillaries terminated in reticular meshwork, with only one exception in which the capillary connected directly with a venule. This exceptional capillary terminated in reticular meshwork on one side and abutted on the adjacent venule on the other side (Fig. 4c, d).

Venous circulation. The venous drainage from the reticular meshwork at the interior of the organ is shown in Fig. 5a, b. Because the adjacent adipose tissue was not ligated in this cast and the free flow of resin from the adipose tissue filled the splenic vein, resin flowed into the reticular meshwork from the vein (retrograde flow). The primordial veins or venules (35–50 μm in diameter) were much wider than the terminal arteries. Some commencing portions of the venules were covered with granular masses of resin which were much finer than those of the reticular meshwork (Fig. 5b). The SEM of tissues showed that the wall of the commencing portions of the venules had round fenestrae which were wide enough for blood cells to pass through from the reticular meshwork to the lumen of the venules (Fig. 3b, c). Venules joined together and were collected into larger veins and finally into the major trunk vein (0.5–1.5 mm in diameter) of the organ. The major trunk vein became a splenic vein after leaving the spleen through the hilum (Fig. 1). The venous drainage from the reticular meshwork at the subcapsular region had an arrangement prominently different from that from the interior region of the organ. These collecting veins at the subcapsular region were plate-like in form and lay flat on the reticular meshwork. They showed marked interconnections. The casts of their commencing portions were also covered with fine granular masses of resin (Fig. 5c). These collecting veins led into the vein which in turn went straight from the subcapsular to the interior region of the organ and joined either the major trunk vein or the larger veins which were located in close proximity to the major trunk vein (Fig. 5d).

Discussion

The present study has demonstrated that the vascular organization of the rainbow trout spleen is rather simple; arteries repeatedly bifurcate toward the subcapsular region and do not show any specialized structure concerned with splenic lymphoid tissue. In studies on mammals (dog, cat, rat and mouse), Schmidt et al. (1983a, b, 1985a, b) showed by the microcorrosion casting method the specialized vascular architecture in the marginal zone and the

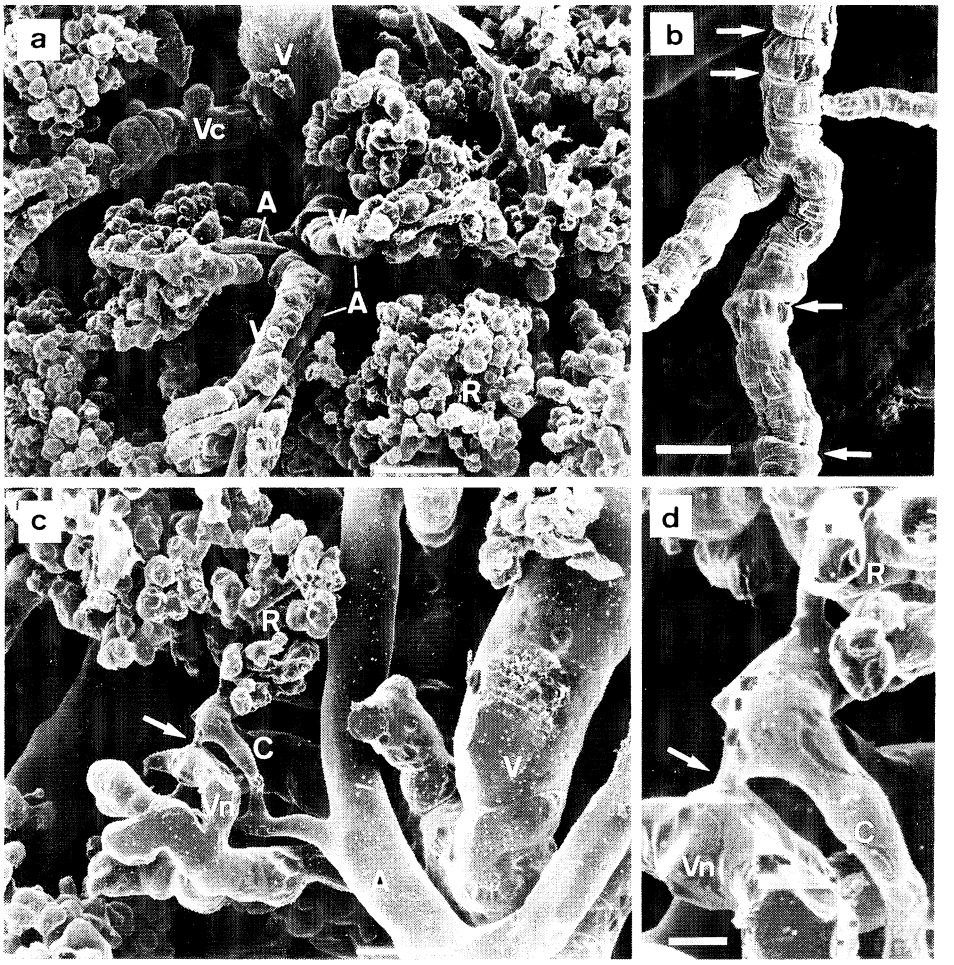

Fig. 4. Resin cast showing the relationship between arteries and veins and arterial constriction. a: Distal end of the arterioles (A) covered with resin masses which is considered to be commencing portions (Vc) of a vein(V). R, reticular meshwork. Bar = 100 μm. b: Arteriole shows shrunk surfaces which have sphincter-like constrictions (arrows). Bar = 50 μm. c: Direct connection of capillary (C) to venule (Vn) (arrow). A, arteriole; V, vein; R, reticular meshwork. Bar = 50 μm. d: Higher magnification of the direct connection shown in c. Bar = 10 μm.

marginal sinus. This difference between teleosts and mammals probably stems from the fact that the mammalian spleen is involved in the immune system, while the fish spleen does not play a significant role in the immune response (Seifert and Marks, 1985). Splenic lymphoid tissue ("white pulp" or "periarterial lymphatic sheath") is poorly developed in the rainbow trout.

The present study has also shown that the cast of arterial capillaries are continuous with the clusters of granular resin masses which correspond to the reticular space revealed by the SEM examination of a perfusion-fixed and freeze-fractured spleen. The irregularly shaped resin masses in the rat spleen were reported to represent the interstitial space among reticulum cells, and the slender pores in the masses to have been formed by the processes of the reticulum cells (Yamamoto et al., 1982). The center of the reticulum cells in the rainbow trout was broader than that in mammals (Fig. 3a, cf. with Hataba et al., 1981 and Hataba and Suzuki, 1989). This was reflected in the cast of the trout spleen, in which some parts of the resin masses exhibited rather tubiform shapes (Fig. 2d, e). Although the resin masses

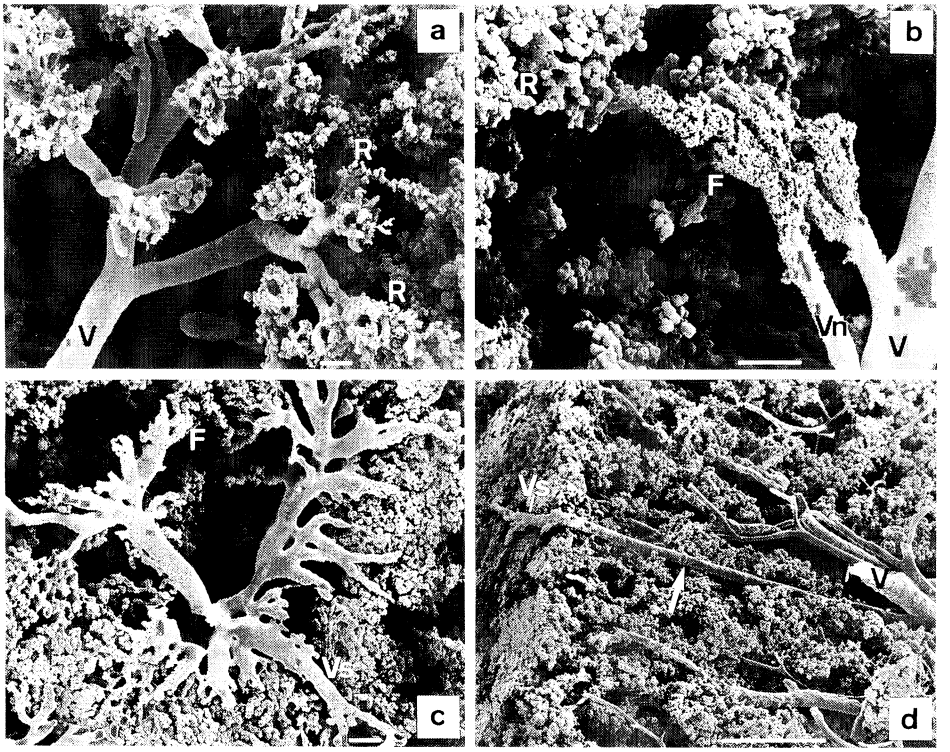

Fig. 5. Resin cast showing the venous drainage from the reticular meshwork (R). a: Cast in which the retrograde flow of resin occurred. V, vein. Bar=100 μm. b: Venules (Vn) covered with fine granular masses of resin (F). Bar=100 μm. c: Collecting veins (Vs) at the subcapsular region. Note their marked interconnection. F, fine granular masses of resin. Bar=100 μm. d: Collecting vein (Vs) going straight into the organ (arrow). Dark left part shows the surface of cast or the subcapsular region, and right part shows section of the cast or interior of the organ. V, vein. Bar=500 μm.

in Fig. 2d, e were somewhat similar to those of the venous sinus in the human spleen (Kashimura, 1985), no true venous sinus with its characteristic annular fibers was found by SEM examination of the trout spleen. The cast of the trout spleen showed sinus-like venules (Fig. 5a) which were apparently different from the resin masses replicating the reticular meshwork. These observations, therefore, indicate that the arterial capillaries terminated in the reticular meshwork (i.e., open circulation).

However, the possibility of 'closed circulation' in the trout spleen cannot be completely ruled out, since we found one case of direct connection of a capillary to a venule (Fig. 4c, d). This connection resembles the direct pathways reported in the rat spleen in which a capillary directly leads into a venous sinus while others open to the reticular meshwork. The frequency of direct capillary-to-sinus connections was reported to be substantially lower in the rat than in the dog (Schmidt et al., 1985a). However, we cannot exclude the possibility that the direct communication presented here might be an artifact. Further examination is needed to confirm whether there are such direct pathways in the spleen of the rainbow trout or not.

The commencing portions of the venules appear to cover the distal end of the arterioles. This structure seems to be related to the periarterial macrophage sheath or ellipsoid, because the distal ends of the arterioles have periarterial macrophage sheaths in the rainbow trout. It was reported in *Lophius piscatorius*, that the reticular meshwork in the ellipsoidal wall stood out very clearly, and the surroundings of the ellipsoids were large, sinus-like spaces (Yoffey, 1929).

In the interior of the organ, the blood from the reticular meshwork is collected by venules via open ends and fenestrae in venular walls as demonstrated

in Figs. 3b, 3c and 5a. The structure represented by the fine granular resin masses covering some commencing portions of the venules is presently unknown.

The structure of the venous system in the subcapsular region of the spleen is completely different from that in the interior of the organ. Since most of the subcapsular veins collecting smaller veins from the reticular meshwork directly join the major trunk vein, these veins are considered to aid in rapid drainage of erythrocytes from the reticular meshwork at times of urgent need, such as during strenuous exercise. The reticular meshwork is considered to play an important role in blood storage.

The veins which drain the blood from the reticular meshwork at the subcapsular region (Fig. 5c) are very similar to those of the nonsinusal spleen of mammals. In mammals the sinusal spleen (e.g. rat, dog) is known to possess the dense arrays of subcapsular venous sinuses forming an almost continuous layer, while in nonsinusal spleens (e.g. cat, mouse), a much smaller area of the subcapsular region is occupied by venous channels (Schmidt et al., 1985b). The fish spleen has been reported to be nonsinusal (Osogoe, 1954). This similarity between the fish spleen and the mammalian nonsinusal spleen is interesting, although its significance is presently unknown.

The constrictions of the arterial casts (Fig. 4b) suggest the contraction of the arterial smooth muscle which seems to be involved in the regulation of arterial blood flow. Marked narrowing of the capillary casts also suggests that blood flow seems to be controlled by endothelial contraction as described in capillaries of the spleen of the rat and mouse (Ragan et al., 1988). Constriction of vessels in the spleen of a relaxed rainbow trout is not as remarkable as that in mammals reported by Schmidt et al. (1983c) who observed vessels in a contracted spleen injected with norepinephrine. This difference in constriction probably reflects the difference in the physiological state of the spleen.

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ニジマス脾臓の微小循環系

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ニジマス *Oncorhynchus mykiss* (=*Salmo gairdneri*) 脾臓の血管系の三次元構築を，腐食鋳型法を主に用いて走査型電子顕微鏡で観察した．ニジマスの脾臓には，哺乳類において見られる周動脈リンパ様組織鞘やリンパ小節に関連して特殊化した血管系は見られない．主要動脈は，被膜下域に向けて細動脈を分出する．動脈性毛細血管が細網組織の網目構造に終わるいわゆる開放型循環が明確に示された．鋳型では樹脂の顆粒状の塊として現れる細網組織の網目構造は、赤血球貯蔵の重要な場所であると考えられる．被膜下域にある静脈は，細網組織の網目構造の上に偏平に存在する．これらの静脈は一点に集まり，そこから脾臓の深部へ向かい，その大部分が直接中心静脈に連なる．細網組織の網目構造から中心静脈へ走るこれらの静脈は，激しい運動のような緊急の必要時に，赤血球を細網組織の網目構造からすばやく排出して循環血液中へ供給するのに適していると考えられる．

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