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SPIRAL STRUCTURES IN THE WALL OF THE HEPATIC VENOUS SYSTEM IN THE DOG

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Abstract

Unique spiral structures, located in the wall of the hepatic venous system in the dog, were examined in the central veins and the hepatic venous branches, utilizing microvascular corrosion casting and freeze-fracture technique in scanning electron microscopy and transmission electron microscopy of tissue sections. The whole hepatic venous system was divided into 4 portions: the central, sublobular, collecting and branches of the hepatic veins. The central vein was spindle-shaped with several compressions. Removing the endothelial cells of the central vein, pathways of venous sinusoids were like a labyrinth. In the sublobular veins, spiral structures distinctly appeared as the diameter increased. Beneath the endothelial cells in the constricted portions, smooth muscle bundles were found. The spiral structures gradually became irregular in the collecting veins and discontinuous to form shallow constrictions in cast thicker branches of the intrahepatic veins. A single, fine spindle of the central vein was formed by the arrangement of liver cells. The spiral structures of the sublobular vein were formed by smooth muscle bundles. Irregularity of the spiral structures in the collecting veins was caused by smooth muscle bundles anastomosing with adjacent ones. Disappearance of the spiral structure in cast thicker branches of the intrahepatic veins was due to absence of muscle bundles.

Key Words: Venous sphincter, hepatic venous system, corrosion casting, dog.

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Introduction

Many investigators reported that an anaphylactic shock phenomenon in dog was characterized by intense stasis of the liver, especially by constriction of spiral sphincters of hepatic venous system. Hering, already in 1867, reported the existence of the spiral structures of the hepatic vein in the carnivores; in 1988, Brissaud and Sabourin observed strengthened valves forming peculiar spiral triangular protuberances, composed of smooth muscle fibers on the internal wall of the hepatic venous system in the sea-seal and dog (for a review, see Ohta *et al.*, 1956). Elias and Feller (1931) and other investigators reported on the venous sphincter apparatus in the hepatic venous system, especially on the arrangement and form of smooth muscle cells. Ohta *et al.* (1956) described the spiral structures utilizing a self-developed plastic casting method in an era in which scanning electron microscopy (SEM) had not been applied to observation of microcorrosion casts. The present paper will deal with spiral or ring structures in the intrahepatic venous system of the dog by SEM and transmission electron microscopy (TEM).

Materials and Methods

Ten adult mongrel dogs (8 male and 2 females; aged 2-3 years; 10 to 12 kg body weight) were used for this study. The animals had been raised on a standard diet in compliance with the Guide for Animal Experimentation, with permission from the Committee of Animal Experimentation, Osaka Dental University.

Microvascular corrosion casts for SEM

Six dogs were anesthetized with sodium pentobarbital (50 mg/kg of body weight). An injection needle for perfusion was carefully inserted into the portal vein. The hepatic venous system was washed with 200 ml of heparinized (5000 IU/l) Tyrode solution at 38°C until the efflux of the inferior vena cava became clear. Then, 30 ml oligomer of methyl methacrylate (viscosity: 11 centipoise, Ohta *et al.*, 1990) with 1 g of benzoyl peroxide as a catalyst and 1 ml N, N-dimethylaniline as an

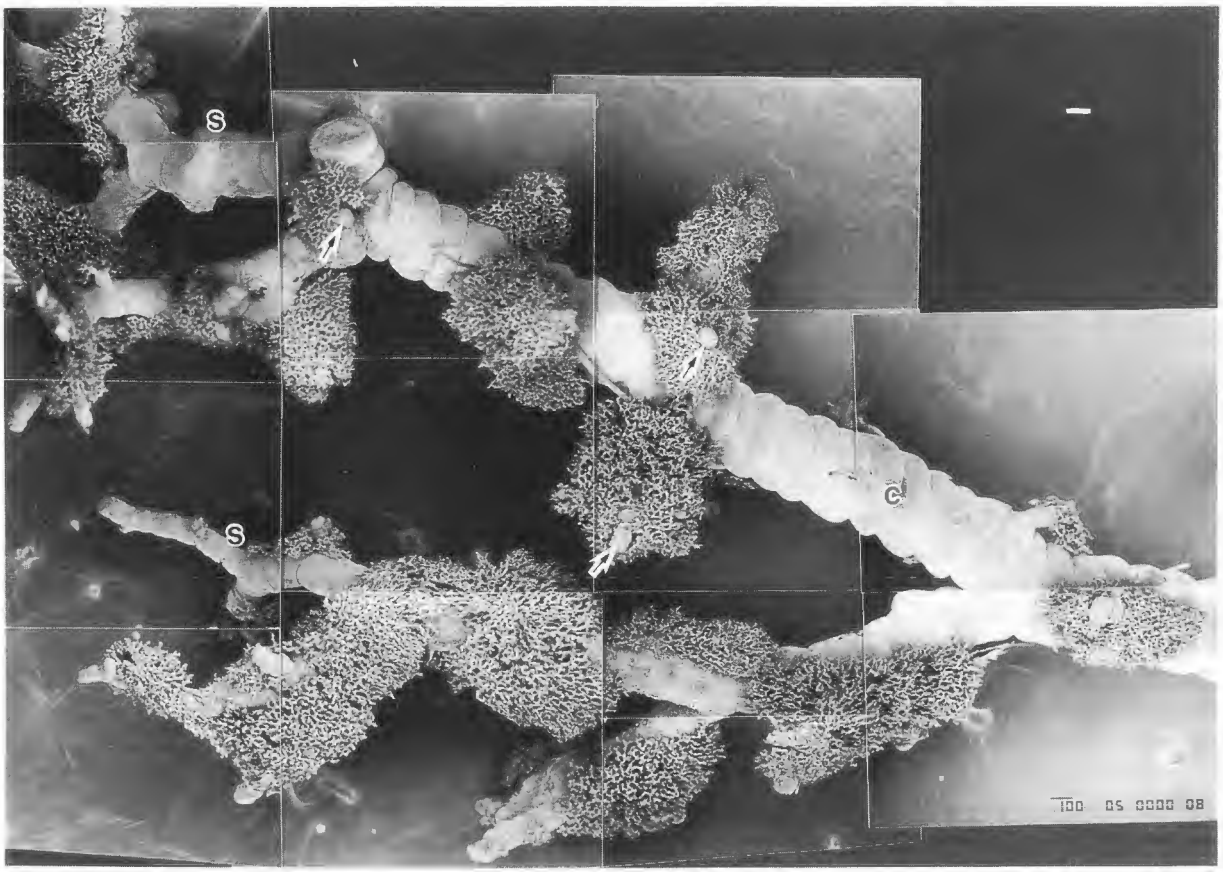

Figure 1. Spiral structures on the internal wall of the hepatic venous system. Central veins (arrows) flow into sublobular veins. Spiral structures are remarkably observed in the sublobular (s) and collecting veins (c). Bar = 100 μm.

accelerator (Aharinejad *et al.*, 1993) was injected. The injected organs were kept at room temperature for 2 hours. The liver was dissected out, soaked in a water bath at 60°C overnight and macerated in 5% KOH solution at 40°C for 1 day with the medium renewed twice. They were rinsed with 5% formic acid for 30 minutes and freeze-dried (Aharinejad and Lametschwandtner, 1990). Corrosion casts were sputtered with gold for SEM (JSM, T-300, JEOL, Tokyo, Japan) at an accelerating voltage of 15 kV and a 45-mm working distance.

Freeze-fracture and NaOH digested specimens

Two dogs were anesthetized as described above. After the blood circulatory system was rinsed by heparinized Tyrode solution, the animals were fixed by perfusion of 2.5% glutaraldehyde. The tissue was washed in phosphate buffer and cut to 1 cm^2 blocks. One specimen was dehydrated and freeze-fractured, the other was treated in 10% NaOH for 1 day at 37°C to remove the endothelial cells of the hepatic venous system from the underlying connective tissue layer. The material was freeze-dried by t-butyl alcohol to observe under a scanning electron microscope in the above condition.

Histological specimens for TEM and LM

Two dogs were anesthetized and fixed as described above. The material was postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.4 for 2 hours and dehydrated in a graded sorts of alcohols and methyl glycidyl ether, and embedded in Spurr's resin (TAAB Laboratories Equipment Ltd., Aldermaston, Berkshire, U.K.). Ultrathin sections were cut and stained with methanolic uranyl acetate and alkaline lead citrate for examination under a transmission electron microscope (JEM-100S, JEOL). The material fixed in a similar way was embedded in paraffin and sectioned at 10 μm thick and stained with Mallory's trichrome.

Results

Unique spiral structures were observed on the casts of the intrahepatic venous system in the dog, the vessel of which measures less than 1 mm in diameter (Fig. 1). The intrahepatic venous system was divided into four portions: central veins (less than 50 μm in diameter) in

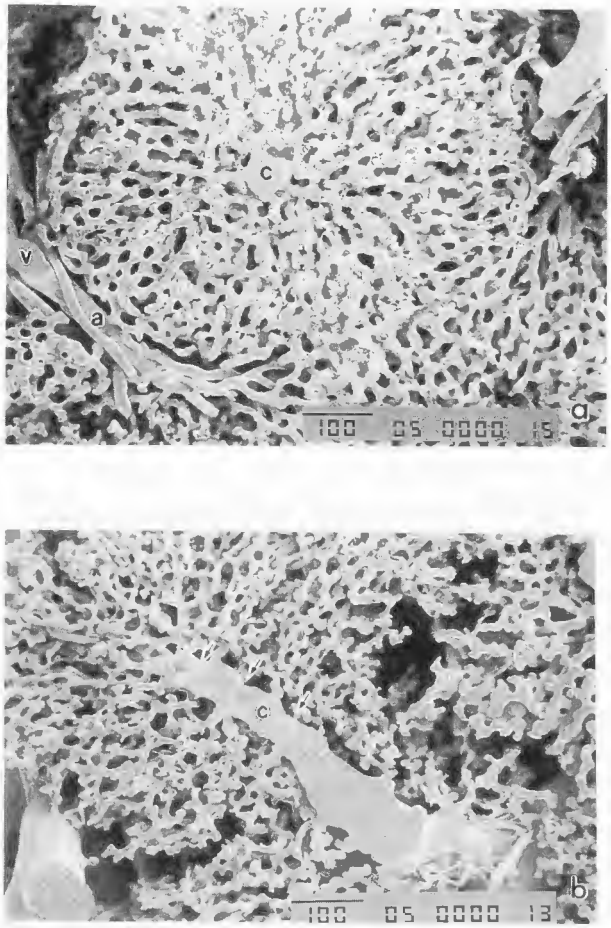

Figure 2. (a) Sinusoidal capillaries in the hepatic lobule. Interlobular artery (a) and portal vein (v) ramify into sinusoidal capillaries which drain centripetally into the central vein (c). (b) Longitudinal section of a central vein (c). The vein is in the shape of a short series of spindles and has three compressions (arrows). Bars = 100 μm.

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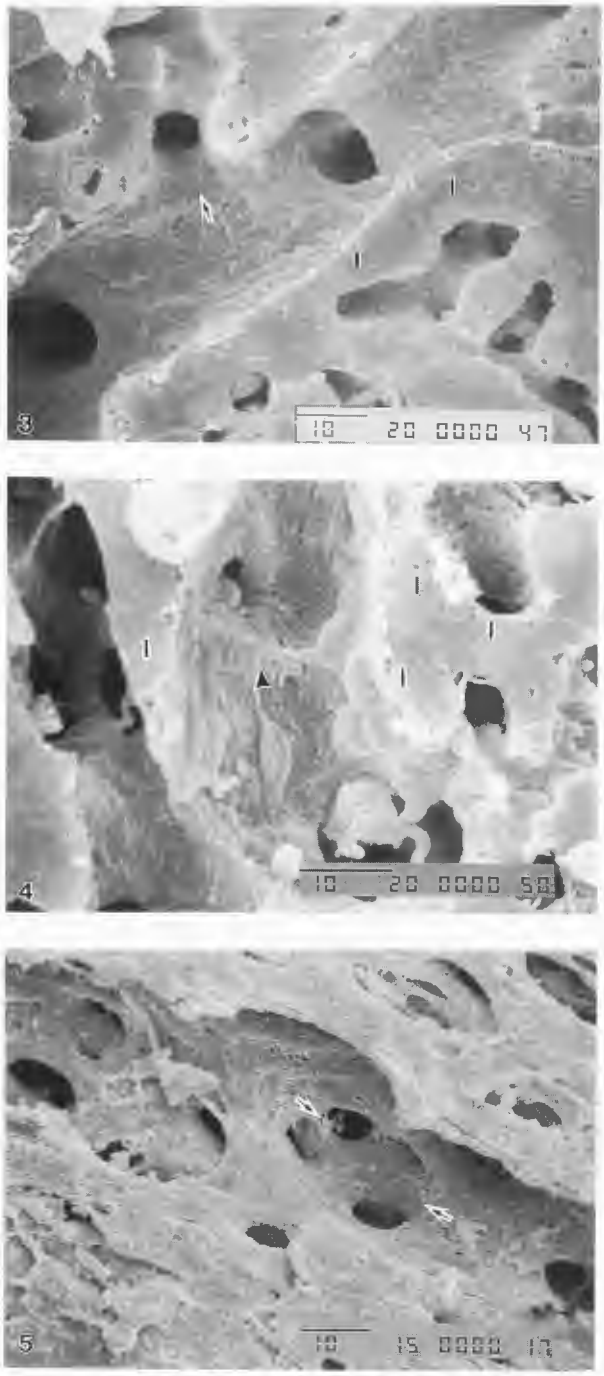
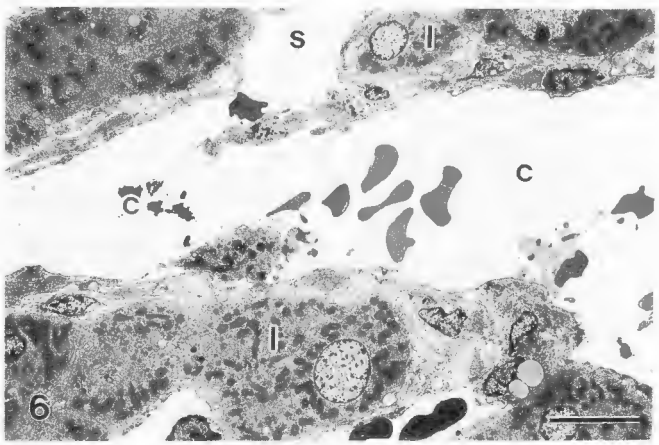
the hepatic lobule, sublobular veins (50 to approximately 300 μm in diameter), collecting veins (300 to approximately 500 μm in diameter) and branches of the hepatic veins (less than 1 mm in diameter).

Central veins

The microvasculature of the hepatic lobule was observed characterized by interlobular arteries and portal veins branching into sinusoidal capillaries which drained centripetally into the central vein (Fig. 2a). The central vein was approximately 500 μm in full length and had the configuration of a short series of spindles. Generally, three to five compressions were found in one vein (Fig. 2b). Sinusoidal capillaries entered primarily expanded areas between the compressions of the spindles (Fig. 2b). Liver cells were regularly arranged in these



Figures 3-5. Central vein: freeze-fractured (**Figs. 3 and 4**) and NaOH digested (**Fig. 5**) specimens. **Figure 3.** Expanded area. Sinusoidal capillary drains into the expanded area (arrow), and liver cells (l) are regularly arranged on this area. **Figure 4.** Compressed area. Liver cells (l) protrude into the lumen and small longitudinal plicae (arrowhead) are present on the wall of the central vein. **Figure 5.** The sinusoidal lumina opening into the central vein are observed in the shape of a labyrinth (arrows). Bars = 10 μm.



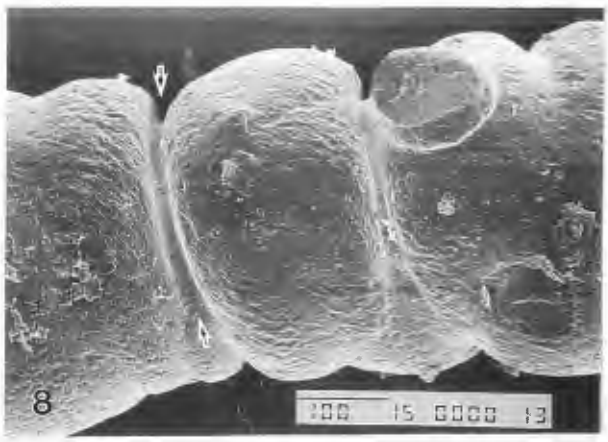


Figure 6. Transmission electron micrograph of the central vein. No valve-like structures and smooth muscle cells are found in the wall of the central vain (c); s: sinusoidal capillary; l: liver cell. Bar = 10 μm.

Figures 7 and 8. Sublobular vein: shows series of circles and become spiral structures as the diameter increased (**Fig. 7**); constricted portion, constrictions (arrows) account about 1/3 of the diameter of the vessel (**Fig. 8**). Bars = 100 μm.

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expanded areas to form sinusoidal walls, into which sinusoidal capillaries drained (Fig. 3). In the compressed areas, liver cells protruded into the lumen and small longitudinal plicae appeared on the wall of these central vein (Fig. 4). In NaOH digested specimens, in which endothelial cells were removed from the underlying connective tissue, sinusoidal lumina opening into the central veins were clearly observed as having the

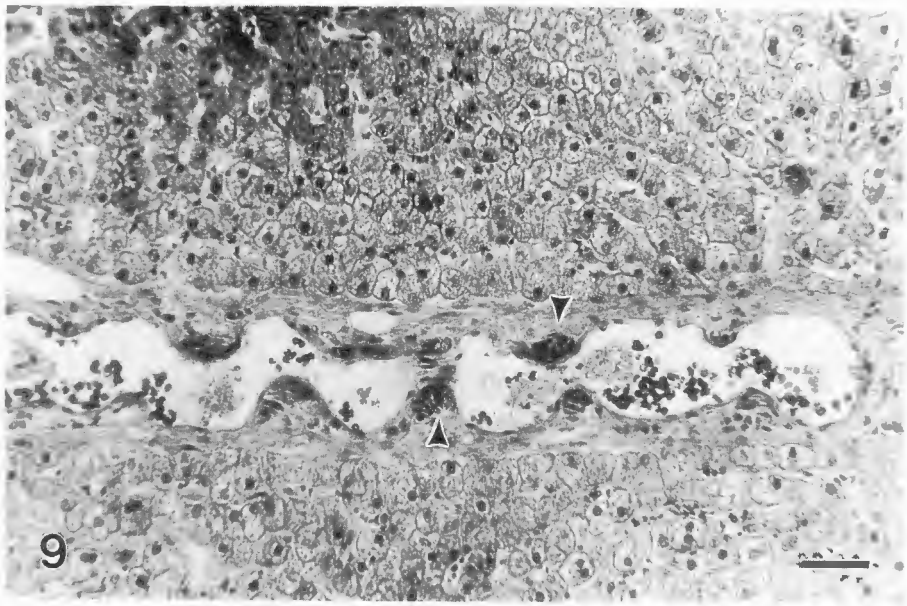

Figure 9. Sublobular veins stained with Mallory's trichrome. Smooth muscle bundles (arrowheads) are found in the constricted portions. Bar = 50 μm.

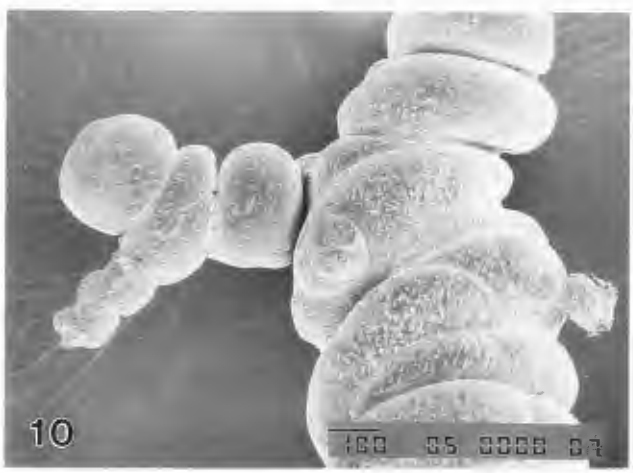

Figure 10. Spiral structures of the collecting vein. Constrictions account for about half of the vessel diameter. Bar = 100 μm.

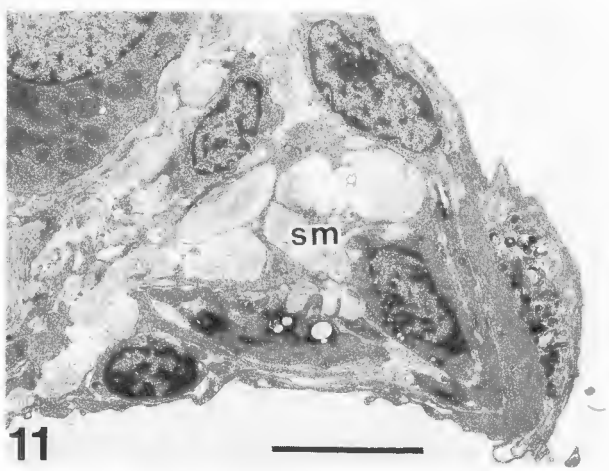

Figure 11. Bottom of the constriction. Smooth muscle bundles (sm) protrude into the lumen, forming a sharp edge. Bar = 5 μm.

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configuration of a labyrinth (Fig. 5). However, no valve-like structures were found in either this draining site or the smooth muscle cells beneath the endothelial cells (Fig. 6).

Sublobular veins

Sublobular veins showed no spiral structures on their walls at the beginning area but series of circles were seen as the diameter increased and spiral structures were present in vessels of large diameter (Figs. 1 and 7). Constrictions accounted for a loss of approximately 1/3 of the diameter of the vessel (Fig. 8). Beneath the endothelial cells in the constricted portion, smooth muscle bundles were identified (Fig. 9). Muscle bundles showed the morphology of serial circles or spirals which formed the corresponding structures on the walls of the sublobular veins (Fig. 9).

Collecting veins

The spiral structures gradually became irregular and occupied approximately half of the lumen of the vessel at the confluence of the collecting vein and the sublobular veins. Intervals between the constrictions broadened incrementally (Fig. 10). In the constricted portion, larger numbers of smooth muscle bundles protruded into the lumen forming a sharp edge (Fig. 11).

Branches of the intrahepatic veins

Spiral structures eventually disappeared, becoming

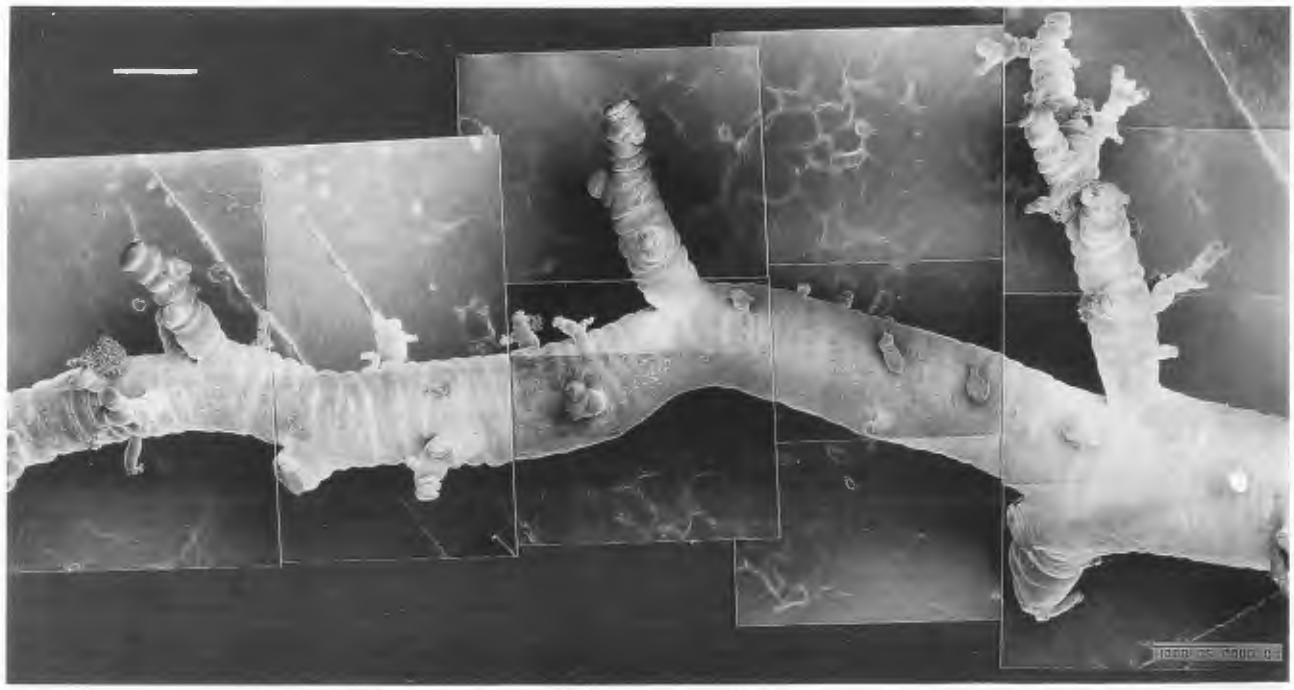

Figure 12. Branches of the intrahepatic vein. Spiral structures have eventually disappeared. Bar = 1 mm.

first discontinuous and showing very shallow constrictions, making the length of a constriction decrease to less than half that of the vessel diameter (Fig. 12). Smooth muscle bundles also disappeared but gradually changed to a layered structure. The spiral structure in the intrahepatic vein wall finally disappeared completely in all vessels more than 1 mm in diameter (Fig. 12).

Discussion

When large amounts of blood circulate in the portal system of the carnivore liver, the outflow must be kept at a continuous average by mechanisms such as constriction and dilation of the sinusoidal and hepatic venous vessels. The spiral structures of the hepatic venous system in the dog were reported on already in 1922 by Zimmermann. Ohtani (1980) reported short circular constrictions of the pulmonary vein in the rat, revealed by microcorrosion castings, but did not give a detailed description of the structures and functional significance in the venous sphincter. Konerding *et al.* (1988) described a venous sphincter of the sciatic nerve of the chicken and considered it to possibly control blood flow in the vasa nervorum. Schraufnagel (1987), Schraufnagel and Schmidt (1988a,b), and Schraufnagel and Patel (1990) demonstrated that the circular constrictions in rat pulmonary veins were innervated. They proposed the name "venous sphincter" for these constrictions. Aharinejad *et al.* (1990, 1992) reported the venous sphincter found in the excretory portion of the rat pancreas and a similar apparatus in the pulmonary vein of the rat in detail. Although the spiral structures in the intrahepatic venous systems of carnivores were described in Miller's text book on the anatomy of the dog (Evans and Christensen, 1979), the detailed structure and possible functions have not been investigated. Fujita (1964) recorded that mast cells are numerous around the sublobular branches being specifically gathered beneath the endothelium. Takahashi-Iwanaga *et al.* (1990) reported that mast cell granules are known to contain various mediators which cause the constriction of the smooth muscle cells. They concluded the hepatic vein branches, their mast cell sheath and the accompanying lymphatic plexus provide a structural unit which plays an important role in the immunological reaction of the canine organism. Ohta *et al.* (1956) examined these structures three-dimensionally utilizing plastic corrosion casts. Thus, this study is a significant contribution in that further detailed re-examination of the aforementioned structures is described (Table 1).

Fine spindle structures found in the central vein differ morphologically from the venous sphincters in sublobular and thicker venous branches. The former is not composed of smooth muscle bundles but rather of a thin arrangement or thickening of individual liver cells existing on the central venous wall at the site of confluence of the sinusoidal capillaries. Accordingly, they do not have an active function as with the venous sphincter.

Table 1. Patterns of spiral structures on the internal wall of the hepatic venous system; () indicates diameter of the intrahepatic vein.

	Constrictions	Venous sphincters	Microvascular corrosion casts
Central vein (below 50 μm)	+	not visible	short series of fine spindles
Sublobular vein (50-300 μm)	++	circle and spiral	spiral shape with short pitches (100-150 μm)
Collecting vein (300-500 μm)	+++	circle and spiral	spiral shape with short pitches (100-150 μm)
Branches of hepatic vein (below 1 mm)	+	incomplete	spiral shape with long pitches (200 μm)

Smooth muscle elements, however, are clearly observed on the walls of sublobular veins. Proportional to the participation of this element, the depressions are first observed as a partially circular pattern which gradually develops into a continuous spiral pattern. Many investigators have been interested in this evolving pattern. Circular structures may not have a continuous constrictive function except for a partial reaction. On the contrary, spiral structures may be able to maintain a continuous constriction in some portions. The smooth muscle bundles contribute to an increase in the intensity and duration of constriction. Plical patterns found in the compression of the central vein are not composed of muscular elements but rather of only a protrusion of liver cells. Comparable plicae found in the sublobular veins are, on the other hand, formed on the endothelial cytoplasm by a constriction of smooth muscle bundles, and this thickened cytoplasm may be a resistant form of constriction.

Strong depressions which accounted for approximately half diameter of the vessel, especially as observed at the confluence of the sublobular veins with the collecting veins, intensity the constriction by increasing the smooth muscle element and adhesion to adjacent structures. Such an enforced pattern offers the advantage of constriction. Adhesion of grooves in the constriction may widen the pitch of the spiral groove, thereby augmenting constriction in a longer portion of the vein. It can be said that the spiral pattern should offer advantageous control of blood flow as compared to the series of circles pattern. In branches of intrahepatic veins, disappearance of the spiral or ring constriction is attributed to global thickening of the muscle element in the hypertrophied wall, leading to a layered structure. Such a pattern may not have a role in blood flow control.

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Discussion with Reviewers

O. Ohtani: The sphincters of the intrahepatic venous system have been described only in dogs and seals. Why do you think the sphincters exist only these animals?
Authors: The possibility of the liver being the shock organ in dogs and seals remains to be investigated. Hepatic vein branches, their mast cell sheath and the accompanying lymphatic plexus are believed to provide a structural unit which plays an important role in the immunological reaction of the canine organism.

S. Aharinejad: Have you ever observed nerve terminals closely related to smooth muscle sphincters in the central or sublobular vein?
Authors: We could observe no nerve innervation to the smooth muscle sphincter in the sublobular vein.

S. Aharinejad: You describe smooth muscle tufts in the wall of sublobular veins which have a width of approximately 25 μm (Fig. 9). The width of constrictions on the casts is 10-12 μm (Fig. 8). How do you explain this discrepancy?
Authors: Sublobular vein in Figure 8 is a transforming area from circle constriction to spiral one and shows the constrictions which is gradually thickening proceeding to the collecting vein.

A. Lametschwandtner: You have shown very elegantly that in the sublobular veins the circular structures change into spiral structures. You discuss that the circular structures may serve for short constrictions while the spiral ones might maintain even longer constrictions. Do you have any hypothesis what the stimuli (nervous, hormonal, local) might be which cause these circular and/or spiral structures to constrict?
Authors: Since vasomotor innervation has not been demonstrated in the lateral wall, the flow regulation must depend on a local control. Mast cells are numerous around the sublobular veins. These granules are known to contain various mediators which trigger inflammatory reaction, i.e., histamine which causes the constriction of the smooth muscle cells.

D.E. Schraufnagel: What are the similarities and differences between the structures in dog hepatic veins you describe and the sphincters of rat lung veins that we described?
Authors: Circle constrictions in dog hepatic vein are similar as the sphincter of rat lung, but spiral structures are characteristic and differ from ones in the rat lung.

D.E. Schraufnagel: Aharinejad and colleagues could find no nerve connections in rat lung veins. Did you see anatomic evidence for innervation in the dog?
Authors: We could not observe nerve innervation to the venous sphincter.

D.E. Schraufnagel: Are the contractions uniform in all of the veins of a given size? If they are, perhaps there purpose is to retard blood flow to allow more time for exchange of material within the liver.
Authors: Yes, we agree with your opinion.