Collagen Fiber Arrangement in Canine Hepatic Venules

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ABSTRACT. Cell-maceration/scanning electron microscopy, serial sections and scanning electron microscopy of vascular resin casts were employed to demonstrate the arrangement of collagen fibers in the terminal hepatic venules, involving the central, intercalated and collecting veins in dog liver. In cell-maceration specimens, each collagen fiber was observed to run in various directions, forming a sheath with a compact meshwork of collagen fibers. The collagenous meshwork in the hepatic venules was looser than those of the terminal portal venules and hepatic arterioles. Some collagen fibers formed bundles with an elongated spiral arrangement encircling the wall of the terminal hepatic venules. In resin casts, these venules were observed as a twisted configuration caused by spiral collagen bundles. A helical modification of such connective tissue bundles might provide a mechanically stable vascular structure and permit reversible changes in linear and circumferential vascular dimensions at the terminal tributaries of veins. Round or oval pores with diameters of approximately 9 μm were also observed in the sheath of collagen fibers. These pores, together with the relatively loose collagenous meshwork in the hepatic venules, might play a role in lymphocyte migration from these venules into the surrounding tissue and provide high permeability to the venule walls. No such helical configuration and pores were observed in either the portal venules or the hepatic arterioles.—KEY WORDS: cell-maceration/SEM, collagen fiber, hepatic venule, resin cast, spiral bundle.

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The liver plays an important role as a blood reservoir. Approximately 60% of the hepatic blood is contained in peripheral capacitance vessels such as the intrahepatic portal veins, hepatic veins and sinusoids [1]. Hepatic blood flow is also believed to vary during respiratory activity and increase considerably in cardiac failure [1]. Fluctuations in hepatic blood volume may cause repeated expansion and contraction in the peripheral capacitance vessels, more specifically in the central veins. Vascular connective tissue components such as collagen and elastic fibers provide the stabilizing framework for vascular structures [3, 8, 9]. This framework must be sturdy enough to maintain the patency of the peripheral vascular structures, yet flexible enough to permit the expansion and contraction of circulatory movements. The connective tissue arrangement in veins has been studied in several organs by light microscopy of serial sections [3, 8–10]. However the three-dimensional organization of the collagen fibers has not been satisfactorily demonstrated even by serial section microreconstruction. Recently, cell-maceration/scanning electron microscopy (SEM) which allows a three-dimensional view of individual collagen fibers in their natural form and location has been introduced [5–7, 12]. This study examines the connective tissue architecture of the hepatic venules and develops a concept of the functional anatomy of these structures.

MATERIALS AND METHODS

The liver tissue used was obtained from six healthy male dogs (range 4–6 presumptive years of age) that were euthanaized at an animal shelter in Kanagawa prefecture. No abnormality of the liver was detected on visual examination. Additional livers with congestive cirrhosis were obtained from two male dogs (5 and 7 years of age) which had suffered from dirofilariasis to compare vascular configuration in the congestive state. In each case, the caudal lobe was removed for histologic study and cell-maceration/SEM, and the remaining hepatic lobes were used for resin cast study of vascular vessels.

Serial sections: Blocks of the liver tissue were routinely processed, fixed in 2.5% glutaraldehyde, paraffin embedded, serially sectioned at 4 μm, and stained with hematoxylin and eosin, Azan-Mallory and elastica van Gieson stains.

Cell-maceration/SEM: Samples for SEM were processed identically with those for light microscopy through the fixation stage. Following fixation, liver tissue for SEM use was immersed in 2N NaOH for 5–7 days, followed by maceration in distilled water until it became a transparent whitish color. The preparations were immersed in 0.1% tannic acid for 2–3 hr, then rinsed in distilled water for 2–3 hr. After dehydration through graded concentrations of ethanol, the preparations were critical point-dried (CP-5A, Topcon, Tokyo, Japan) using liquid CO_2. All samples were mounted on aluminum stubs with silver paste, spattered with gold in an ion coater (IB-3, Eiko Engineering, Tokyo, Japan) and observed by SEM (ABT-32, Topcon, Tokyo, Japan).

Resin casts study: After removal of the caudal lobe, the remaining hepatic lobes were irrigated by manual perfusion with Ringer's solution at 37°C through the portal vein until the flushing medium appeared free of blood. A mixture of methacrylate methylester monomer with Mercox (Dainippon Ink & Chemicals, Tokyo, Japan) was then injected via the portal vein and/or hepatic artery until the caudal vena cava was filled with the perfused resin. In 2 of the dogs, only just enough resin (15 m*l*) was injected via the portal vein and/or hepatic vein to show the terminal tributaries of the

two veins. After the injected resin was polymerized, the liver tissue was macerated in a 20% NaOH solution at 40°C. Cast replicas were then micro-dissected under a binocular microscope. Each piece of cast was mounted on an aluminum stub and spattered with gold for SEM observation.

RESULTS

Light microscopic findings

The wall of the terminal hepatic venules, involving the central, intercalated and collecting veins, consisted of a layer of endothelium surrounded by a thin layer of longitudinally or circumferentially directed collagenous fibers and fibroblasts. In longitudinal sections, some collagenous fibers were observed to integrate into thick bundles and to form ridges (Fig. 1). These ridges were composed of circularly oriented collagen fibers with a few smooth muscle cells and a few fibroblasts. No elastic fibers appeared within the connective tissue sheaths of the venules.

The wall of the portal venules consisted of endothelium, several layers of smooth muscle cells and prominent connective tissue. The portal venules did not have any collagenous ridges as seen in the terminal hepatic venules. The wall of the hepatic arteriole also did not show any collagenous ridges.

Scanning electron microscopic findings

1. Collagen fiber arrangement of vessels

In the terminal hepatic venules, most of the fascicles of collagen fibers showed a predominantly oblique spiral-like arrangement, while others stretched circularly around the vascular lumen. These fascicles intermingled with each other to form the architectural skeleton outlining the venules. Some of these fascicles further intermingled to form thick bundles corresponding to the ridges in histological sections. The thick bundles were approximately 45 μm in diameter, protruded into the vascular lumen and assumed the form of ridges with a cylindrical spiral rotating around the venules (Fig. 2). Additional evidence for the helical arrangement of venules was seen in the vascular resin casts. The distances between the thick collagen bundles were 100–200 μm. In contrast to the terminal hepatic venules, the collagen fibers of the portal venule and hepatic arteriole did not show a cylindrical spiral arrangement (Fig. 3). The sheaths of the terminal venules, i.e. central, intercalated and collecting veins, also possessed round or oval pores having diameters of approximately 9 μm (Fig. 2–2). The walls of the terminal hepatic venule have a rather rough meshwork of collagen fibers in comparison to those of the portal venule and hepatic arteriole.

2. Resin casts of vessels

Images of the spiral configuration of the venules obtained by SEM of resin casts correlated well with those obtained by cell-maceration/SEM. Vascular replicas of the terminal hepatic venule, especially the central and small-sized veins (histologically, the intercalated and collecting veins) showed distinctive and characteristic morphological conformations, either helical or bead-like in appearance (Figs. 4 and 6). In the liver with congestive cirrhosis the venules were greatly dilated, with luminal diameters ranging from 197 to 256 μm (average 237 μm), whereas the mean diameter of central veins in the normal liver was 53 μm (Fig. 6). As the terminal hepatic venule increased in caliber to become large hepatic veins, the spiral structure steadily disappeared. Neither the portal venule nor the hepatic arteriole showed any spiral spring-like shape (Figs. 5 and 6).

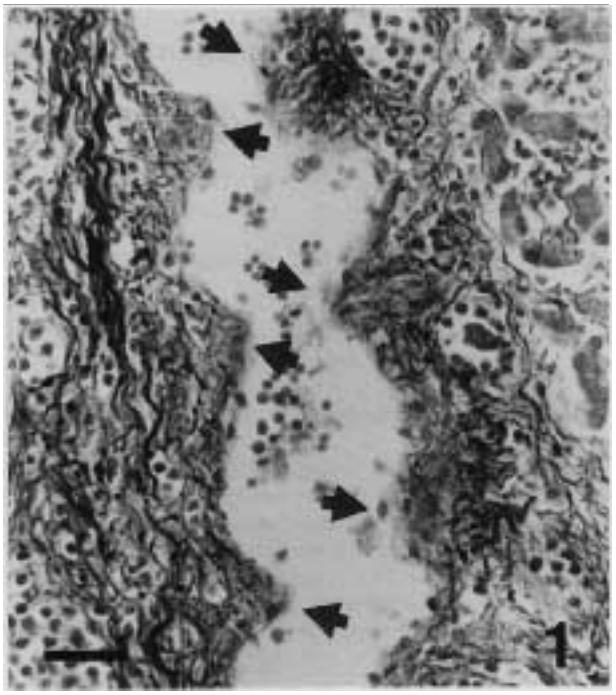

Fig. 1. Light micrograph of a terminal hepatic venule (collecting vein). Note ridges (thick arrows) composed of circumferentially directed collagen fibers, fibroblasts and a few smooth muscle cells. Liver with congestive cirrhosis. Azan-Mallory stain. Bar=30 μm.

DISCUSSION

Spiral collagen arrangement within venous wall has been reported in the splenic veins [10], cerebral veins [8], saphenous veins [3], internal jugular veins [8] and penile cavernous spaces [4]. The authors who studied these veins concur that this disposition of collagen fibers within the venous walls gives to these veins a common property of parietal lengthwise expansion and contractability in order to regulate blood circulation.

In the present study, we found that some of the collagen fibers observed formed thick bundles with an elongated spiral configuration within the wall of the terminal intrahepatic venules. These spiral bundles have not been reported previously, to our knowledge. The structural role of such bundles in the venule wall is probably to create relatively inflexible, constrained regions in comparison to the more flexible areas between the spring-like spirals in

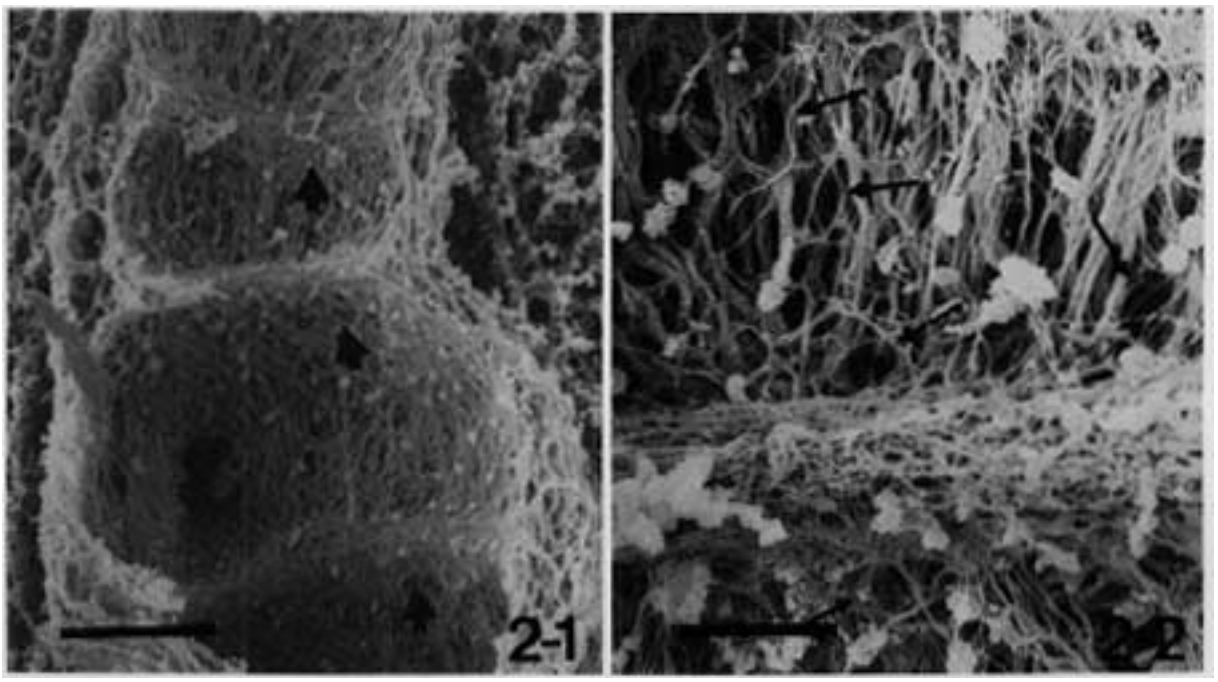

Fig. 2. Cell-maceration/SEM view of a terminal hepatic venule. 2–1. Spirally arranged collagen bundles (thick arrows) running around the circumference of the venule. Bar=140 μm. 2–2. A closer view of Fig. 2–1. The collagen fibers in the venule show a fabric with a looser weave than those in the portal venules. Many pores (thin arrows) are also seen in the collagenous sheath. Compare with Fig. 3–2. Bar=34 μm.

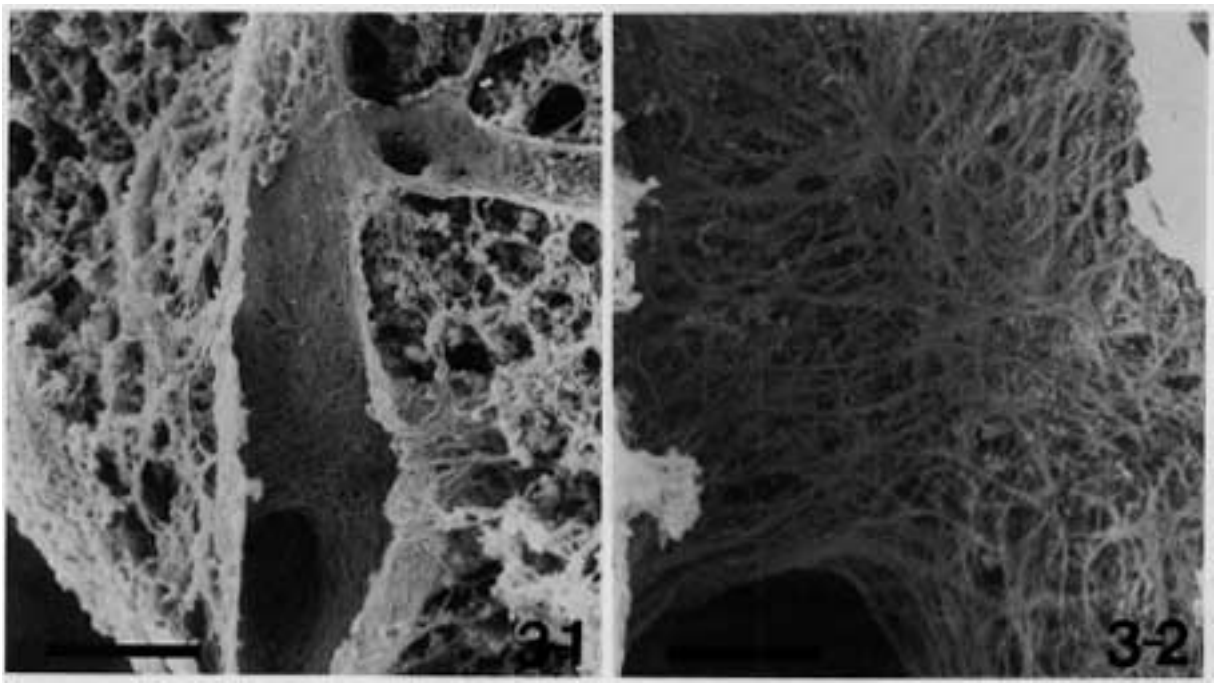

Fig. 3. Cell-maceration/SEM of a terminal portal venule. 3–1. Note a smooth inner surface structure of the venule. Bar=140 μm. 3–2. A closer view of Fig. 3–1. Bar=34 μm.

the venule wall.

The liver has extremely large vascular beds and plays an important role as a blood reservoir, receiving approximately 25% of cardiac output. Approximately 60% of the hepatic

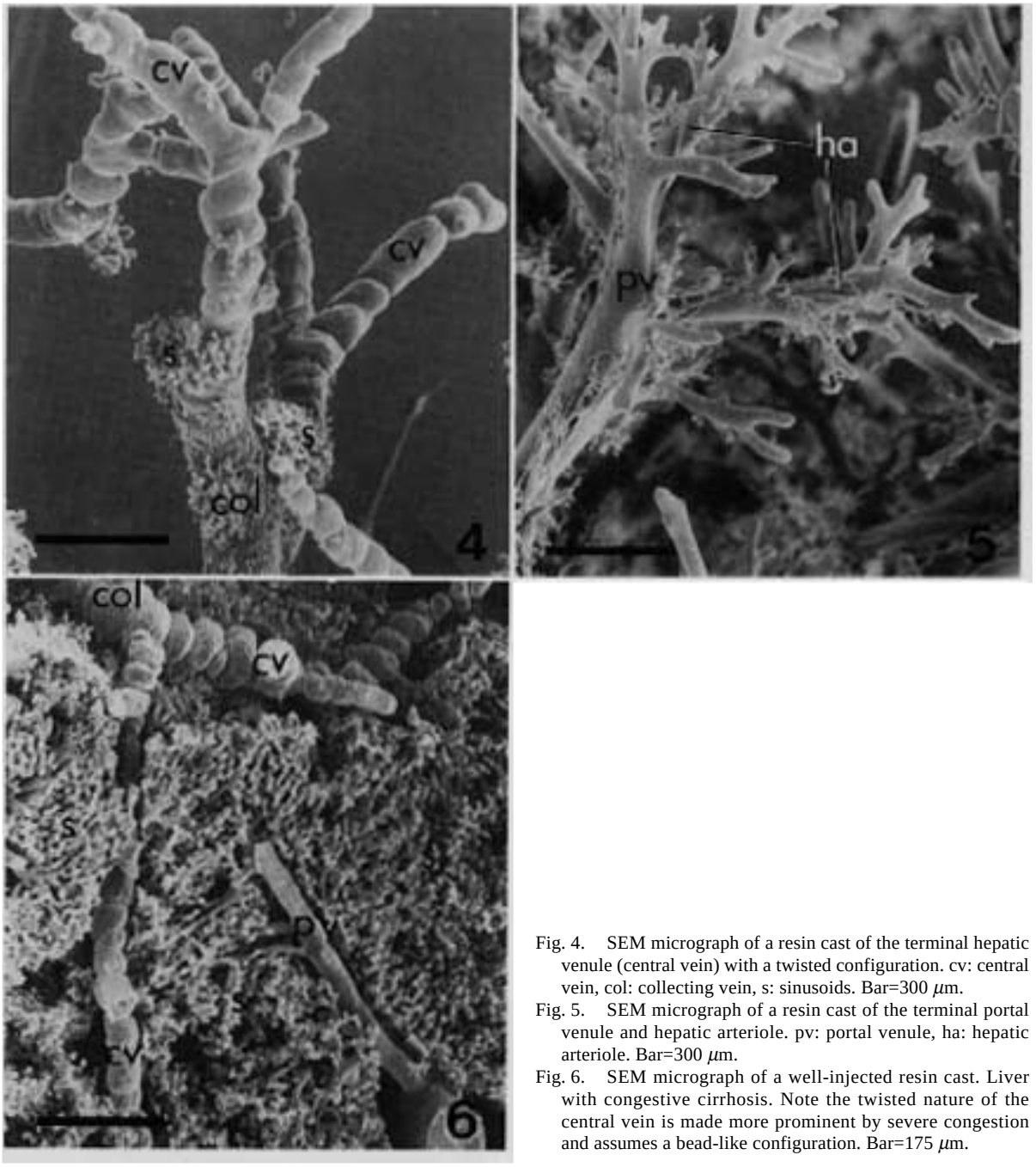

Fig. 4. SEM micrograph of a resin cast of the terminal hepatic venule (central vein) with a twisted configuration. cv: central vein, col: collecting vein, s: sinusoids. Bar=300 μm.

Fig. 5. SEM micrograph of a resin cast of the terminal portal venule and hepatic arteriole. pv: portal venule, ha: hepatic arteriole. Bar=300 μm.

Fig. 6. SEM micrograph of a well-injected resin cast. Liver with congestive cirrhosis. Note the twisted nature of the central vein is made more prominent by severe congestion and assumes a bead-like configuration. Bar=175 μm.

blood is believed to be contained in the peripheral-capacitance vessels [1]. During daily activity, hepatic blood flow varies widely in amount. In a cyclic pattern related to respiration, outflow of blood from the hepatic vein is at a maximum during expiration and decreases substantially with inspiration. Increases in central venous pressure are transmitted almost quantitatively back to the sinusoids; for a 1 mm Hg rise in hepatic venous pressure, the blood volume may increase by as much as 4 m*l*/100 g [1]. Ordinarily, the liver contains 25 to 30 m*l* blood/100 g [1]. Hepatic blood volume may expand considerably in cardiac failure, up to as much as 60 m*l*/100 g liver [1]. The volume increase is attributed to passive distention of the capacitance vessels with subsequent engorgement of the liver. It is clear that the peripheral-capacitance vessels, especially the central veins, repeatedly expand and contract. The spiral connective tissue bundles observed in this study in the terminal hepatic venules may play an important role in preserving the

integrity of the vessel walls and in resistance during the elevation of venous pressure, preventing collapse of the venules to maintain dynamic blood circulation within the liver.

Young *et al.* [13] reported spiral or helical arrangement of elastic fibers in the alveolar ducts of the lung and suggested that helical modification of connective tissue may have a role in creating relatively inflexible, constrained regions in the alveolar ducts in comparison with other more flexible areas of the alveoli. They suggested that the structural role of the connective tissue arrangement around the alveolar ducts may be to permit reversible changes in linear and circumferential dimensions in relation to respiration.

Similar to the findings of Ohtani *et al.* [7] and Ushiki *et al.* [12], who studied the arrangement of collagen fibers in rabbit Peyer's patches and rat lymph node, respectively, we have also shown the round or oval pores having diameters of approximately 9 μm in the connective tissue sheath of terminal hepatic venules corresponding to postcapillary venules. It has been reported that lymphocytes migrate from the bloodstream into the surrounding tissue through postcapillary venules. According to Ohtani *et al.*, lymphocytes pass through the pores in the sheaths of collagen fibers of postcapillary venules. The present study also showed that the walls of the terminal hepatic venules have a rather rough meshwork of collagen fibers in comparison to those of the portal vein and hepatic artery. It is well known that the walls of venules are more permeable than those of any other vessels, including capillaries, and that the venules are most susceptible to histamine and serotonin etc. known to increase vascular permeability [2, 11]. It is thus conceivable that the rough meshwork and pores observed in the connective tissue sheaths of terminal hepatic venules in the present study may play a role in ensuring high vascular permeability at these sites.

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