Intrahepatic Portal-Venous Changes in Dogs with Dirofilariasis: Scanning Electron Microscopy of Resin Casts of Vasculature

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ABSTRACT. Intra hepatic portal-venous changes in dogs with dirofilariasis, in which characteristic cavernous transformation has been histologically observed, were studied by scanning electron microscopy (SEM) of resin casts of vasculature. Changes in the central and sublobular veins were the most significant. The venous channels were extremely dilated, thin-walled and showed cavernous structure. SEM observation showed multi-saccular formation with beads-like, helical and extremely dilated configuration. The conducting venules, i.e. terminal tributaries of the portal vein, showed a rapid taper just before the point of merging into sinusoids. Neither vascular proliferation nor collateral circulation around the portal channels could be detected in the present resin cast study.—KEY WORDS: dirofilariasis, liver vasculature, resin cast.

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In heartworm disease, chronic dirofilariasis generally leads to pulmonary arterial lesions and right-sided heart failure. Histopathologically, severe liver damage in addition to the cardiovascular lesions in dogs with dirofilariasis has been extensively studied [1, 3–6]. Lesions of the liver frequently seen are variable degrees of cavernous transformation of intrahepatic vessels, centrilobular necrosis and fibrosis, bile stasis, centrilobular congestion, microfilariae in the sinusoids and veins, and aggregation of leukocytes and histiocytes. Cavernous changes in the central veins were first reported by Lichtenberg *et al.* [5] as being suggestive of a cavernous capillary hemangioma, but not as an entity in themselves.

This paper describes findings on the intrahepatic portal-venous changes in dogs infected with dirofilariasis, in which characteristic cavernous transformation has been histologically observed.

Three adult dogs with typical signs of chronic dirofilariasis were studied. Of these, two (dogs 2 and 3) showed typical signs of the vena cava syndrome. A clinically normal dog served as control. Laboratory findings included hemolysis, hemoglobinuria, low packed cell volume and elevated blood urea nitrogen (BUN). Before the dogs were put down with barbiturates, Knott's test was performed. The dogs were of various breeds and were 1.5 (dog 1), 3.5 (dog 2) and 4 (dog 3) years of age, respectively. For histologic study, a fragment of the liver of each dog was obtained from the apex of the papillary process before administration of the resin injection. Each fragment was fixed in 10% formalin, embedded in paraffin and stained with hematoxylin and eosin and Azan Mallory stain. For SEM observation, all liver specimens were perfused with Ringer's solution at 37°C via the portal vein. A mixture of methacrylate methylester monomer with 50% Mercox (Dainippon Ink Co., Ltd.) was then injected via the portal vein. The injection was done with a syringe under manual pressure. In dogs 2 and 3, injection was continued until the superior vena cava was filled with the perfused resin, whereas dog 1 and the control dog, only just enough resin (15 m*l*) was injected to show the terminal ends of the portal vein, i.e. the conducting venules just before the point of merging into the sinusoids. The injection was continued until the resin appeared on the cut surface of the papillary process. 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.

Macroscopic findings: Each of the afflicted animals was found heavily infected with 40 to 100 adult worms present in the venae cavae, either the right atrium or ventricle, and pulmonary artery. Marked right ventricular enlargement and tricuspid valvular insufficiency by caused interference of heartworms were characteristic in dogs 2 and 3. Marked villose or rugose appearance of the pulmonary artery was observed as well. The liver had a pronounced lobular pattern in the two same animals. Dog 1 showed a relatively low parasitemia. No ascites could be detected in any animal.

Histologic findings: The present discussion is confined to the hepatic lesions. The livers of dog 2 and 3 revealed similar histopathological changes and are the basis for this report. Widespread centrilobular congestion was noted. Bile stasis, microfilariae in the sinusoids and veins and numerous interstitial foci containing leukocytic and histiocytic cells and basophilic inflammatory cell debris were present in the hepatic parenchyma. Additionally, the parenchyma was well defined by proliferative interlobular fibrous tissue. Central and sublobular veins were extremely congested. The veins were highly dilated and showed cavernous changes involving multiple saccular dilations (Fig. 1). No significant lesions in the intra-hepatic artery or its major sub-branches was observed.

SEM findings: The whole course of the portal vein, from its main trunks to the terminal sub-branches of interlobular veins, was undulating and showed smaller diameter in the affected dogs (Fig. 3) than in the normal liver (Fig. 2). The terminal sub-branches of interlobular veins and conducting veins showed a U-turn arrangement (Fig. 3, arrows), with diameter abruptly reduced from 100 μm to 42 μm on average at the points of merging into sinusoids (Fig. 4, arrow). The casts of sinusoids were 7–12 μm in diameter and appeared to be slender compared with

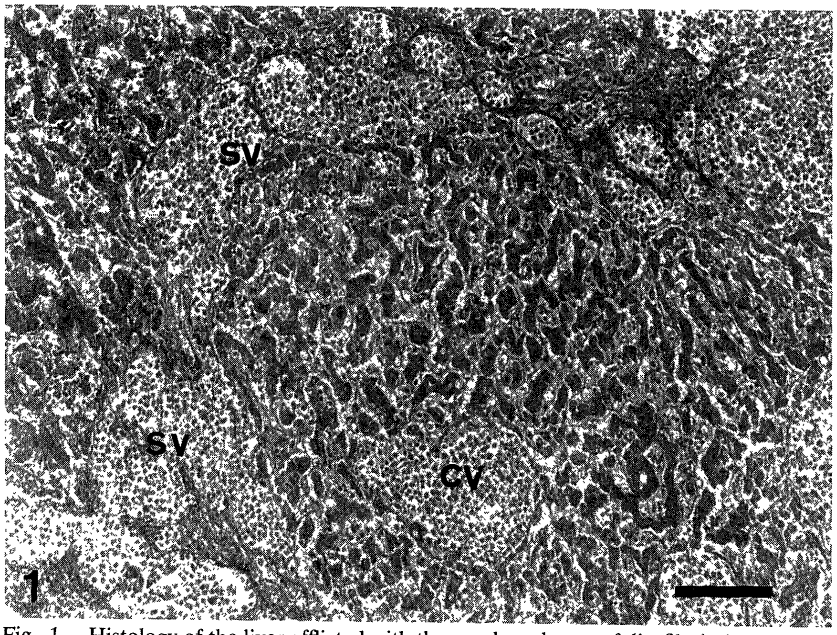

Fig. 1. Histology of the liver afflicted with the caval syndrome of dirofilariasis. Note the extremely dilated and congested central (cv) and sublobular (sv) veins with multisaccular dilations. (× 25) Azan Mallory stain. Bar=100 μm. Dog 3.

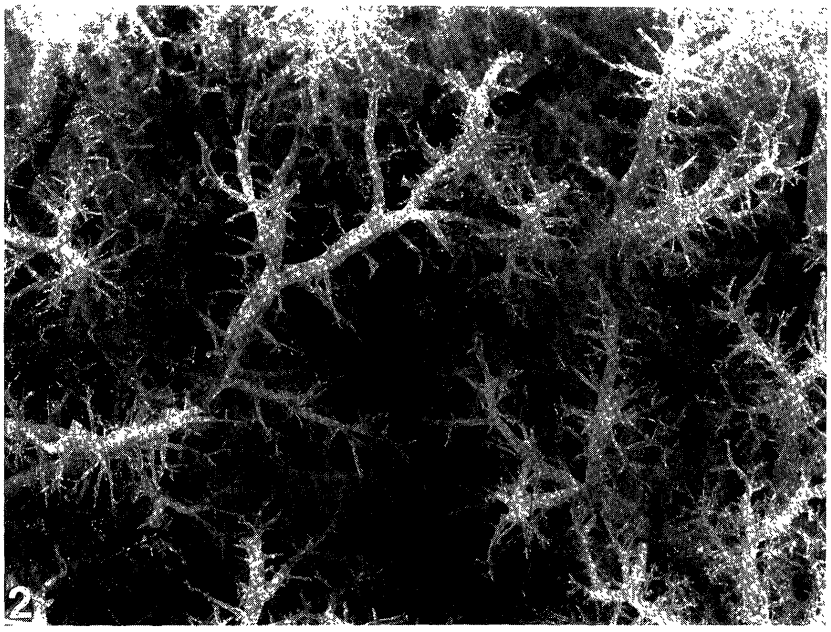

Fig. 2. Resin cast of interlobular veins of a clinically normal dog. Note the veins running straight. (× 1.3)

normal sinusoids. The centrilobular arrangement of sinusoids which was found in the normal dog liver was sporadically lost. Vascular replicas of the central veins receiving sinusoids and sublobular veins collecting central veins showed distinctive and characteristic morphological alterations, helical, bellows-like or beads-like in appearance (Figs. 5, 6). The veins were greatly dilated, with luminal diameter ranging from 197 μm to 256 μm (average 237 μm), whereas mean diameter of central veins in the normal liver was 53 μm. The casts of terminal subbranches of the hepatic vein, which collected sublobular veins, did not show the beads-like configuration. Instead they showed a slightly undulating course and were reduced in diameter to 140 μm on average. No vessels representing collateral circulation of the portal vein or vascular proliferation around the vein could be detected in this study. Nor did the present study find any evidence of sphincteric structures at the point of merges into sinusoids

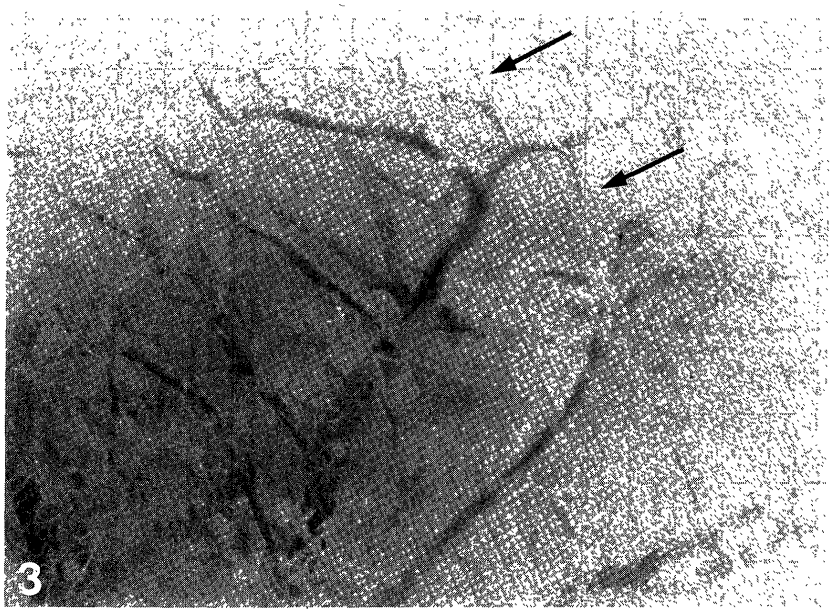

Fig. 3. Resin cast of interlobular veins of a dog with the caval syndrome. Note the veins showing undulating and slender (arrows). (× 1.3) Dog 1.

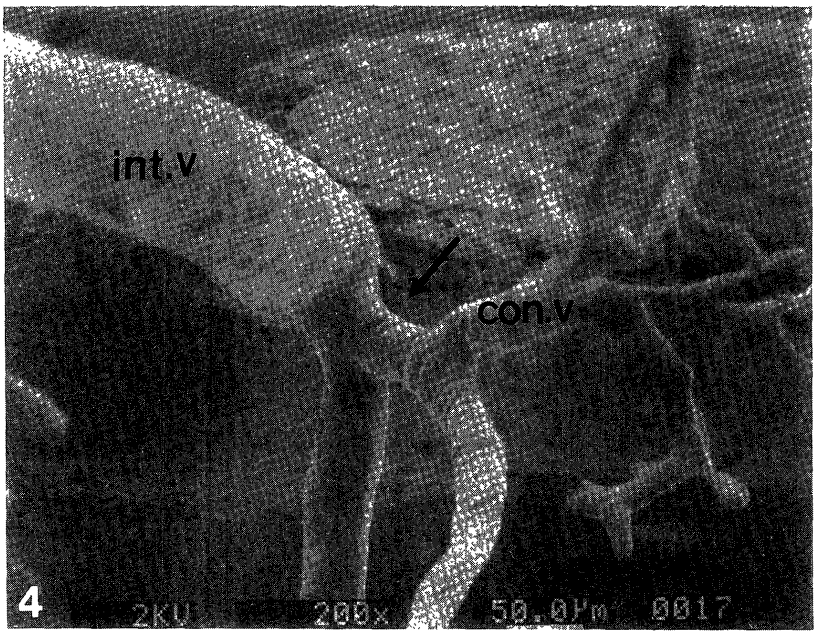

Fig. 4. Scanning electron micrograph (SEM) of resin casts of conducting venules (con.v), i.e. terminal tributaries of the interlobular vein (int.v), showing a drastic reduction in diameter (arrow). (× 200) Dog 1.

in either the affected or normal liver vasculature.

The present histological observations corroborate the findings of Lichtenberg *et al.* [5], who observed hepatic lesions in dogs afflicted with the caval syndrome. In the present study, cavernous configuration of the tributaries of hepatic veins and centrilobular fibrosis were the most conspicuous changes. Cavernous transformation has been reported in human portal hypertension as well and has been considered to relate to collateral channels of terminal vessels of the portal vein [7]. The present study, however, clearly indicated that cavernous transformation derives not from proliferation of vessels or collateral circulation but rather from venous multi-saccular dilation. Multi saccular dilation of vessels may produce a cavernous appearance in tissue section, as shown in Fig. 1.

The pathogenesis of multi-saccular dilation and helical configuration in intrahepatic veins is unknown. Regurgitation through the right atrio-ventricular valve associated

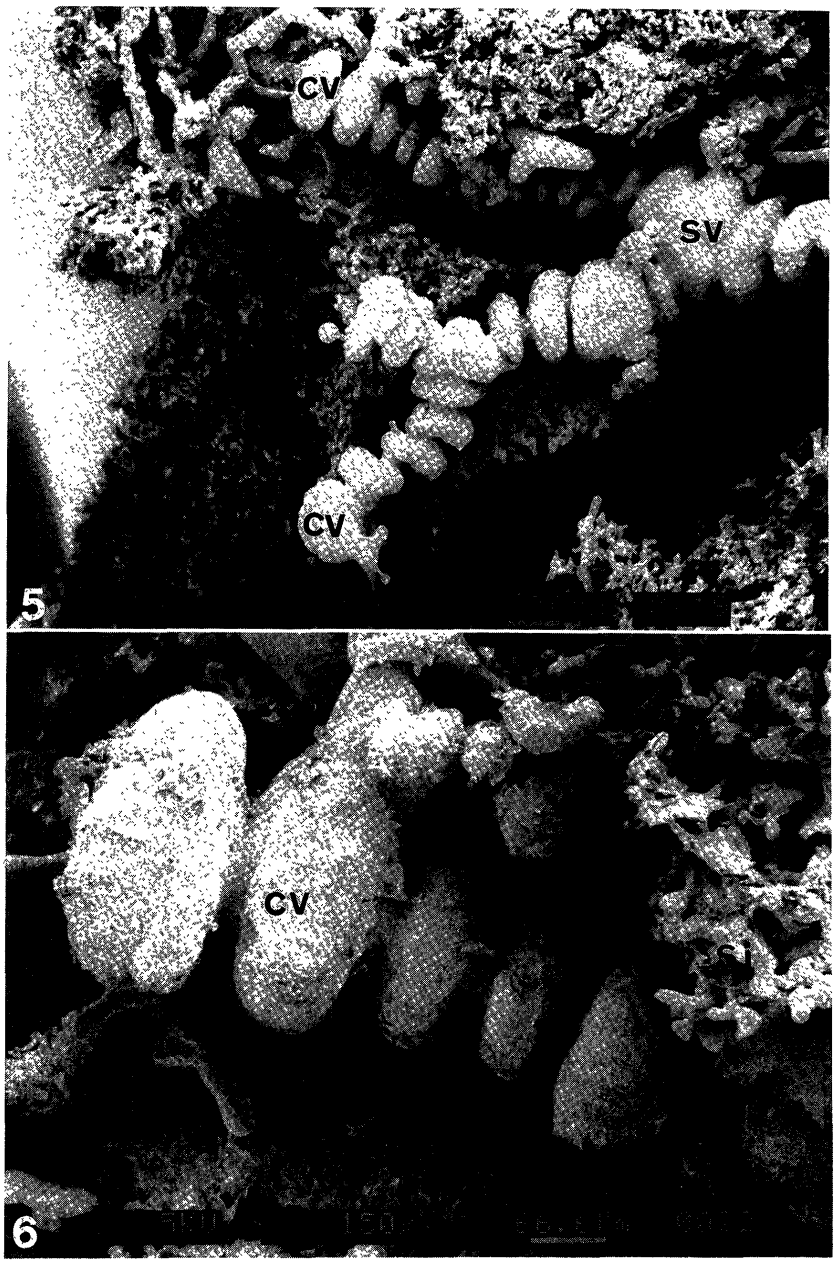

Fig. 5. SEM of resin casts of central (cv) and sublobular (sv) veins. Note the helical and beads-like appearance. (× 40) Dog 3.

Fig. 6. High power magnification of Fig. 5 showing beads-like venules, also showing sinusoids (si). (× 150)

with valve interference due to filariae and/or expansion of the valve annulus may be predisposing factors of severe liver congestion. Severe chronic passive liver congestion and reverse flow of blood upon atrial contraction could well be the reasons for dilation of the veins, since the wall of the veins comprises only one layer of endothelial cells.

It is of interest that terminal branches of the portal vein showed a marked narrowing from 100 μm to 42 μm in average diameter just before merging into sinusoids. According to Poiseuille's law [2], blood draining into sinusoids should be restricted by 93%, when vessel diameter is reduced by 60%. Drastic reduction in blood flow into sinusoids appears to occur in the liver. Such severe and advanced blood flow disturbance may be one reason for progressive interlobular fibrosis and liver functional disorders in dirofilariasis. Additionally, the constriction of terminal branches of the portal vein may cause portal hypertension in case of cirrhosis further afflicted by filariasis. More direct evidence is required to determine the real mechanism of blood flow disturbance in the liver affected with dirofilariasis.

REFERENCES

1. Adcock, J. L. 1961. *Am. J. Vet. Res.* 22: 655–662.
2. Burton, A. C. 1966. pp. 523–542. *In*: Physiology and Biophysics (Ruch, T. C. and Patton, H. D. eds.), Saunders, Philadelphia and London.
3. Eaton, K. A. and Rosol, T. J. 1989. *J. Am. Vet. Med. Assoc.* 195: 223–224.
4. Jackson, R. F., Lichtenberg, F., and Otto, G. F. 1962. *J. Am. Vet. Med. Assoc.* 141: 117–121.
5. Lichtenberg, F., Jackson, R. F., and Otto, G. F. 1962. *J. Am. Vet. Med. Assoc.* 141: 121–128.
6. Liu, S-K., Yarns, D. A., Carmichael, A. A., and Tashjian, R. J. 1969. *Am. J. Vet. Res.* 30: 1723–1735.
7. Ohta, G. 1982. pp. 45–55. *In*: Metabolism and Disease, Nakayama Shoten, Tokyo (in Japanese).