Fine Vasculature of Hepatoma in Chinese Hamsters (*Cricetulus griceus*); Scanning Electron Microscopy of Resin Casts of Blood Vessels

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ABSTRACT. Scanning electron microscopy of resin casts was carried out on the vascular system of hepatoma in Chinese hamster. Casts of capillaries in the hepatoma were thin and fragmentary and formed a fine randomly arranged network in contrast to those in the liver with radial arrangement and central veins in centrilobular regions. Capillaries in the cancer merged into superficial venules corresponding to central veins while those in the border region merged into sinusoids of adjacent liver tissue. The superficial venules connected to tributaries of hepatic veins to drain into the posterior vena cava. Neovascularization did not occur by sprouting and anastomosing to form hair-pin loops of capillaries but by division of pre-existing vessels into smaller vessels, thus increasing the number of capillaries. The hepatic artery feeding the cancer was seemingly intact. There was no indication of arterio-portal shunts in the hepatoma. KEY WORDS: hepatoma, microvasculature, resin cast.

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The treatment of patients with hepatic tumors includes radical surgery, radiation or drug therapy. Recently, regional chemotherapy by perfusion of a hepatic vascular bed has come into use for patients with hepatic neoplasms [9]. This therapy could perhaps be made even more effective if the blood supply to the developing liver cancer were understood in greater detail.

Controversy still exists as to the final drainage channels of hepatoma. Wright [11] and Nakashima [6] maintain that the main venous drainage system from the cancer merges into the portal vein rather than the hepatic vein. Breedis and Young [1], however, consider blood from the cancer to drain into the hepatic vein. The possible presence of arterio-portal shunts has been indicated by an angiographic study on human hepatoma [8]. The correlation of angiographic appearance with the histology of a cancer had yet to be clearly determined.

This paper describes findings on the vascular system of Chinese hamster hepatoma obtained by a new technique, scanning electron microscopy of vascular resin casts.

Hepatoma: A cancer was detected in the necopsy of each of three male Chinese hamsters, 32-40 months old, used in an aging study.

Specimen preparation for histological diagnosis: A small piece (4 mm) of the cancer was removed from each of two of the three animals. It was fixed in 10% formalin, dehydrated in ethanol and embedded in paraffin. The neoplastic lesion of another animal was used only for histological examination of the vasculature. Both tissue specimens were treated similarly and sectioned serially at 4 μm.

Scanning electron microscopy: Following biopsy for histological diagnosis of the two animals, the livers were perfused with Ringer's solution at 37°C. Retrograde injection through the superior vena cava was conducted since the hepatic artery and portal vein were too small to permit insertion of a catheter. A mixture of methyl methacrylate monomer and 50% Mercox (Dainippon Ink Co., Ltd., Tokyo) was injected via the superior vena cava. Cast replicas were microdissected under a binocular microscope. Each piece of a cast was mounted on an aluminium stub and spattered with gold for scanning electron microscopic observation.

Gross appearance of the hepatoma: The cancers were large solitary masses often with multiple small nodules. The nodular masses were lighter in color than the surrounding liver tissue. Branching blood vessels could often be seen on the surface of the tumors (Fig. 1. arrow).

Histology of the hepatoma: The tumors consisted of tightly packed cells traversed by thin capillaries with slits. The neoplastic cells resembled well differentiated liver cells with large round or oval nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. The cells no longer appeared in a normal cord orientation or lobular arrangement (Fig. 2) and they were frequently found to invade the dilated sinusoidal spaces accompanying endothelial cells (Fig. 3. arrows). The cancer masses were sheathed by a single layer of endothelium. No significant changes could be detected in the wall structure of the portal or hepatic vein. Vascular channels in the cancer were analogous to sinusoidal spaces but extremely narrow, with slits. In cancers larger than 2.0 mm, a necrotic center was present, consisting of amorphous material without blood vessels. In the interlobular space through which tributaries of the portal vein and hepatic artery ran there were lymphatic spaces. The vascular wall of the hepatic artery within the tumor showed no sign of significant degenerative change. Well developed and dilated veins corresponding to central veins and terminal tributaries of the hepatic vein were frequently observed just under the fibrous capsule. The walls of the veins were quite thin, each consisting of a single layer of endothelial cells and the fibrous capsule covering the liver (Fig. 2. arrow).

Vascular system of the liver: The portal vein runs along the portal canal and its terminal tributaries branch into peripheral sinusoids of a lobule. The sinusoids consists of richly anastomotic fine vascular beds arranged around the central veins at a centrilobular location (Fig. 4). The central veins connect to each other to drain into larger venules as terminal tributaries of the hepatic vein. The veins pour into the posterior vena cava.

Vascular arrangement of hepatoma: The gross appearance of the liver injected with resin through the hepatic vein is shown in Fig. 1.

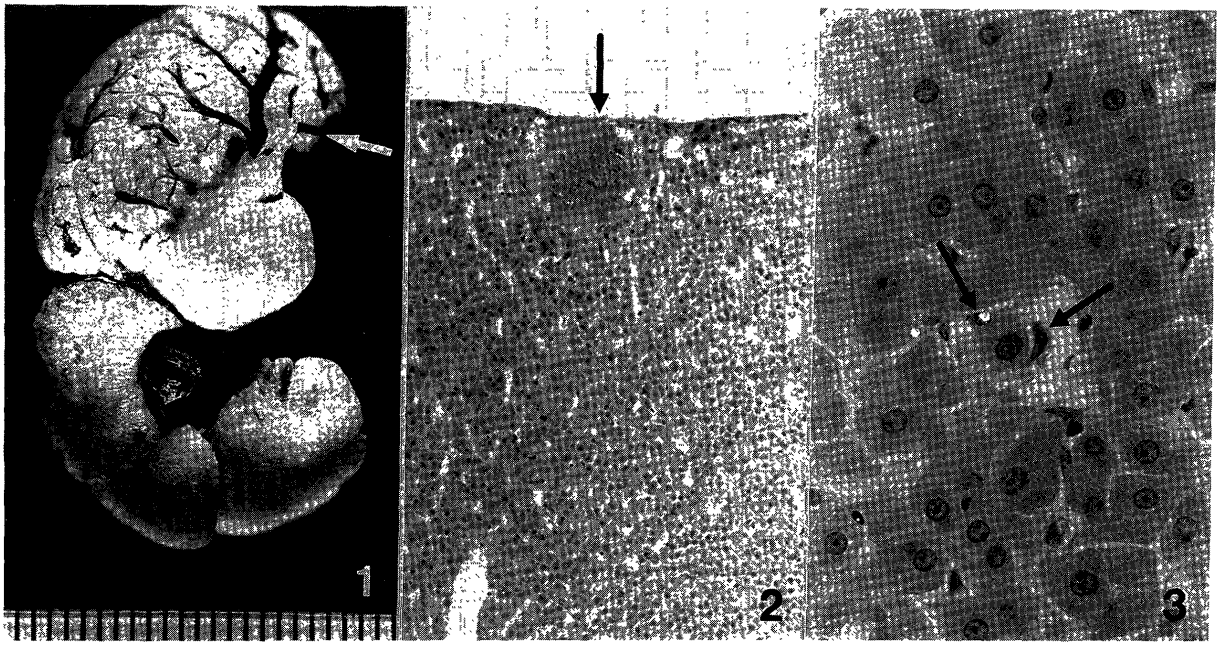

Fig. 1. Gross appearance of resin casts of Chinese hamster hepatoma. Well developed superficial veins are clearly seen in the right lobe (arrow). (× 2.5)

Fig. 2. Histology of Chinese hamster hepatoma. Note superficial veins with walls consisting of a single layer of endothelial cells and the fibrous capsule of the liver (arrow). (× 100) H & E stain.

Fig. 3. Histology of Chinese hamster hepatoma, showing a cancer cell and endothelial cells (arrows) invading the sinusoidal space. (× 400) H & E stain.

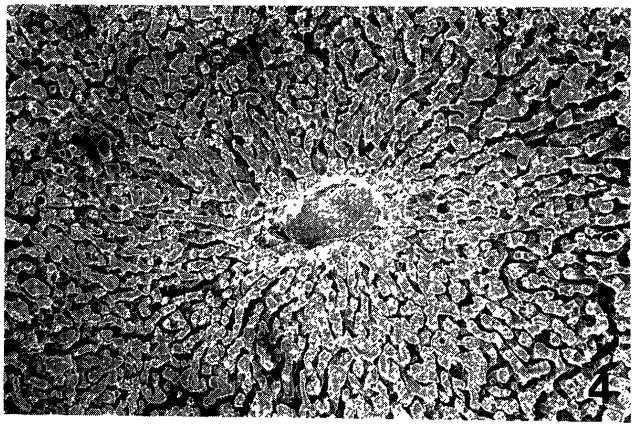

Fig. 4. Scanning electron micrograph (SEM) of resin casts of capillaries in normal liver tissue. Note sinusoids radially arranged about the central vein. (× 100)

Casts of capillaries in the neoplastic tissue had sinusoid-like features analogous to those of sinusoids of normal liver tissue, but were thin and fragmentary. They extensively anastomosed to form a randomly arranged fine capillary network (Figs. 5, 6).

Hardly any neoplastic sinusoidal capillaries extended toward the centrilobular location, most, rather, toward surface regions to drain into superficial venules corresponding to the central vein of the liver (Fig. 5). Capillaries in the border region merged into sinusoids surrounding the cancer to form numerous capillary connections between neoplastic tissue and adjacent normal tissue (Fig. 7). Neither capillaries with sprouts nor capillaries forming hair-pin loops could be seen in the hepatoma. Venules receiving capillaries from the neoplastic tissue were not limited to the smallest terminal branches of the superfical venules but extended to the hepatic vein which was quite large in diameter. The venules merged into larger veins corresponding to central veins. These veins also ran over the surface of the tumor and connected to the hepatic vein. Most of the hepatic venous tree was thus superficially located in the hepatoma (Figs. 1, 2, 5) and drained into

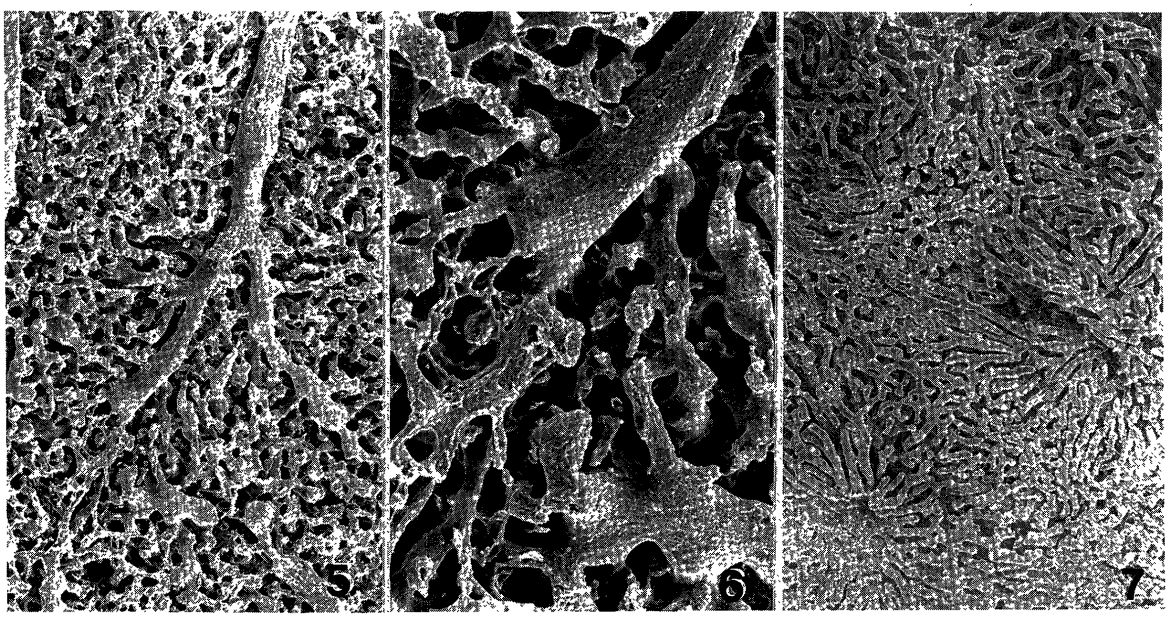

Fig. 5. SEM of surface vasculature of a hepatoma showing superficial veins receiving capillaries from the neoplasm. (× 100)
Fig. 6. Closer view of Fig. 5 showing neoplastic capillaries which are thin and fragmentary without any sinusoidal structure. (× 300)
Fig. 7. SEM of the border region between neoplastic (upper left) and normal (lower right) tissues. Note both tissues are tightly connected by capillaries. (× 100)

the posterior vena cava. Casts of the veins were thick and flattened. No characteristic structures on the surface of any vein could be detected and neither was there indication of arterio-portal shunts connecting sinusoidal spaces of liver tissue surrounding the hepatoma and terminal branches of the hepatic artery. No threads and streaks, which indicate tumor forci in the major trunks of the portal vein in angiography in the case of a human hepatoma [12], could be detected in the Chinese hamster hepatoma in this study.

The present results on Chinese hamster clearly indicate that venous blood from the cancer flows into twigs of the hepatic vein. This finding is consistent with the view of Breedis and Young [1] but at variance with the study of Wright [11] and Nakashima [6], indicating that capillaries from the cancer merge into tributaries of the portal vein. The possible existence of arterio-portal shunts between terminal branches of the hepatic artery feeding the cancer and those of the portal vein has been suggested [8]. A cancer and adjacent normal tissue are opacified by contrast medium when the medium is injected via the hepatic artery. Such opacification is said to be due to drainage of the medium into marginal tissue through arterio-portal shunts. In agreement with Breedis and Young [1], histologic and scanning electron microscopic observation of the resin casts failed to disclose the presence of such shunts. It is evident, however, from the present study, that there are profuse sinusoidal capillary connections between neoplastic sinusoids and those in marginal tissue. Although the injected contrast medium drains primarily into superficial venules after reaching neoplastic sinusoids, some may possibly drain into adjacent tissue through the capillary connections after entering neoplastic tissue to cause ring-like opacification about the cancer.

The process of capillary growth has been observed in hamster neurilemmoma [2], human carcinoma of the larynx [5] and rat mammary adenoma [7]. The neovascularization of these cancers is characterized by capillary sprouts which indicate the generation of new capillary loops, each giving rise to an elaborate capillary network. By scanning electron microscopy, the sprouts can be seen as spike-like or knob-like protrusions in hair-pin loops. Resin casts of capillaries in Chinese hamster hepatoma do not possess such sprouts or loops but show thin capillaries with slits. In hepatoma, cancer and endothelial cells proliferate together in dilated hepatic sinusoids, the portal vein and hepatic vein so that, consequently, these pre-existing vascular channels divide into finer vascular spaces analogous to sinusoidal spaces. It would thus appear that neovascularization in hepatoma may depend not on the sprouting or anastomosing of new capillaries, but rather on the division of pre-existing vessels into smaller ones by proliferating tumor cells. This possibility is strongly supported by findings in human pathology [4]. The thin resin casts with slits of hepatoma capillaries may be an indication of this vascularization in a cancer.

Well developed superficial veins which receive capillaries from tumor tissue may be characteristic of hepatoma. These veins apparently develop from pre-existing hepatic sinusoids just under the fibrous capsule. With tumor growth, the requirement for blood increases and conse-

quently blood from neoplastic tissue starts to drain through sinusoids adjacent to neoplastic tissue in superficial regions since superficial vascular spaces are not distorted or compressed by tumor tissue growth, and growth will, of course, follow the path of least resistance. Such growth has been observed by Schoefl [10] in a study on vascular response in wound healing. Eddy and Casarett [2] and Gullino and Grantham [3] noted a pre-existing vascular net to be initially essential for development of the final net. The walls of superficial veins on the surface of a cancer are extremely thin, each consisting only of a single layer of endothelial cells and the fibrous capsule of the liver. The thin wall structure may be easily torn by the impact of an outside body or congestion. Patients with hepatomas bleed easily in the abdominal cavity and this leads to sudden death. The vulnerable wall structure of a vein in a hepatoma may partly explain this lethal bleeding.

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