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Electron Microscopic Observations of Hepatic and Subcutaneous Hemangiosarcomas Induced in Mice Exposed to Vinyl Chloride Monomer

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Both subcutaneous and hepatic hemangiosarcomas were induced in mice exposed to vinyl chloride monomer, and for the first time, the ultrastructure of hemangiosarcomas related to vinyl chloride exposure has been described. The subcutaneous hemangiosarcoma developed in a mouse's ear 29 weeks after exposure to 10 ppm vinyl chloride for 4 weeks, and the hepatic hemangiosarcoma was found in a mouse's liver at necropsy 56 weeks after exposure to 600 ppm vinyl chloride for 4 weeks. Both tumors showed a localized, cystic nodular appearance with a dark red tone. Histologically, an angiomatous architecture, the presence of neoplastic mesenchymal cell aggregates, focal necrosis, and hemorrhagic foci were common. Ultrastructurally, two neoplastic cell types, a mesenchymal and a well-differentiated endothelial cell type, were identified in both tumors. In addition, in the hepatic tumor, a pericyte-like neoplastic cell was also present. The incidence of hemangiosarcoma was much lower than that of lung tumors (alveologenic tumor) in mice exposed to vinyl chloride at the same concentrations for the same duration. This study suggests that there is no basic difference in ultrastructure between the vinyl chloride-induced hemangiosarcomas and hemangiosarcomas not related to this chemical exposure.

Key words: vinyl chloride, hepatic hemangiosarcoma, subcutaneous hemangiosarcoma, electron microscope studies of hemangiosarcoma

INTRODUCTION

It has been known that human hepatic hemangiosarcoma is characteristically induced among workers exposed to vinyl chloride monomer at vinyl chloride polymerization plants [Falk et al, 1974; Creech and Johnson, 1974; Lange et al, 1974; Lee and Harry, 1974; Thomas et al, 1975; Mark et al, 1976]. In addition, arsenic and thorotrast are also known to induce this otherwise rare malignant tumor [Popper et al, 1978; Underwood and Huck, 1978; Baxter et al, 1980].

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Maltoni and Lefemine [1974, 1975] have undertaken extensive studies on the oncogenicity of vinyl chloride using various experimental designs, animals, and methods. In addition to hepatic hemangiosarcoma, they have induced other tumors, such as Zymbal gland carcinomas, nephroblastomas, skin cancer, and extrahepatic hemangiosarcomas in rats; lung adenomas, mammary carcinomas, and extrahepatic hemangiosarcomas in mice; and skin trichoepitheliomas, lymphomas, and forestomach papillomas in hamsters. They have suggested that the vinyl chloride-induced tumors were dose related.

Recently, we have completed an experimental study in mice on the neoplastic effects of vinyl chloride at low doses (1, 10, 100, 300, and 600 ppm) and with a short exposure (four weeks). In addition to a high incidence of lung tumors (alveologenic tumor of mouse), we have found, much less commonly, subcutaneous and hepatic hemangiosarcomas.

In these tumors, we have had the advantage of conducting an ultrastructural study.

MATERIALS AND METHODS

Two hundred and twenty C.D.1. male mice (five to six weeks old) were exposed to vinyl chloride at 0 ppm (60 mice, control), 1 ppm (30), 10 ppm (30), 100 ppm (30), 300 ppm (30), and 600 ppm (40) for four weeks.* Inhalation experiments were performed under supervision of Dr. M. J. McKenna at Toxicology Research Laboratory, Health and Environmental Science, USA Dow Chemical.

A large majority of the animals were sacrificed at three different stages, (1) *Immediately after the exposure* (a total of 70 mice: 10 of the 600, 300, 100, 10, and 1 ppm groups, and 20 of the 0 ppm group), (2) *12 weeks after exposure* (a total of 61 mice; 9 of 600, 9 of 300, 6 of 100, 9 of 10, 10 of 1, and 18 of 0 ppm), and (3) *40 or 41 weeks after exposure* (a total of 58 mice: 7 of 600, 7 of 300, 9 of 100, 9 of 10, 9 of 1, and 17 of 0 ppm). *Between the first and second sacrifices,* 14 mice (6 of 600, 1 of 300, 4 of 100, 1 of 10, and 2 of 0 ppm) were found dead or killed. Eleven mice (4 of 600, 3 of 300, 1 of 100, 1 of 10, 1 of 1, and 1 of 0 ppm) were found dead or killed *between the second and third sacrifices.* A small number of the animals (4 of 600 ppm and 2 of 0 ppm) were allowed a long-term post exposure recovery period (42–65 weeks) prior to sacrifice or death.

All animals were systematically autopsied. After recording of gross anatomical findings, various organs such as brain, lungs, kidneys, stomach, liver, spleen, adrenal, testis, intestine, and omentum were taken for histological examinations. Sections were stained with hematoxylin and eosin, PAS, Masson's trichrome, and Gomori's silver staining. Electron microscopic specimens of these tissues were also prepared. Electron microscopic observations were made with Siemens 101 and Hitachi 11 D-S electron microscopes.

RESULTS

Hemangiosarcoma

Subcutaneous hemangiosarcoma. A mouse of the 10 ppm group developed a tumor in the subcutaneous connective tissue of the left ear, 29 weeks after the exposure. The tumor was dark red, relatively soft, and measured over 1 cm in diameter.

*Six hours per day, 5 days per week. Total vinyl chloride exposure days were 21 days for 1 ppm, 300 ppm, and 600 ppm groups, 22 days for 10 ppm group and 23 days for 100 ppm group. The controls were exposed to 0 ppm vinyl chloride for 21 days (40 mice), 22 days (10 mice), and 23 days (10 mice). One of the 10 of the 600 ppm group was found dead on the day of the first sacrifice.

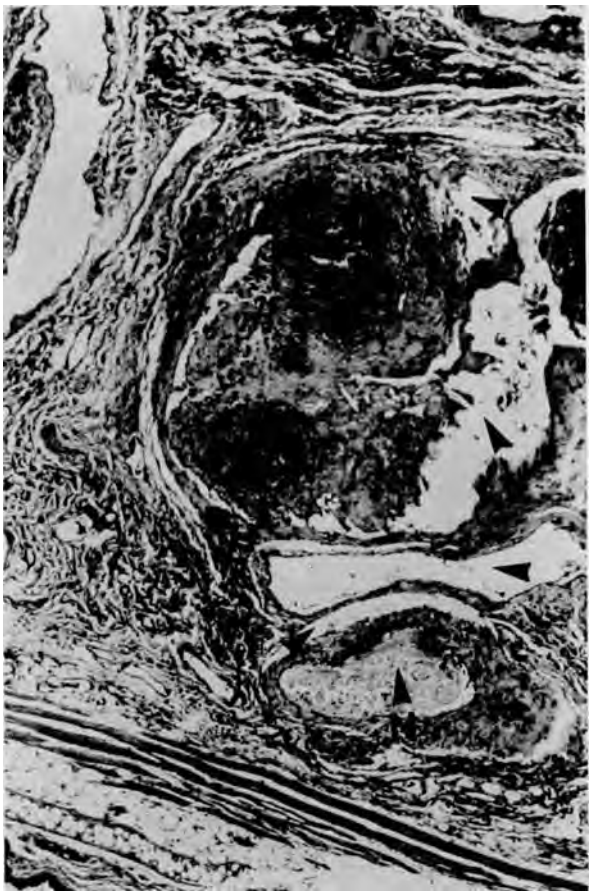

Fig. 1. A subcutaneous hemangiosarcoma (low power). Arrows indicate blood channels. Necrosis (arrow—N) and hemorrhage (arrows—H) are seen. H&E, ×74.

Since the gross anatomical appearance was suggestive of hemangiosarcoma, the animal was sacrificed to clarify the nature of the tumor. The cut surface was bloody and cystic. Gross anatomical study failed to detect any other than the ear tumor.

Histologically, as shown in Figure 1, the tumor was localized in the subcutaneous connective tissue of the ear, and it formed blood channels (arrows) that seemed to be freely communicated. Neoplastic cells that directly lined the blood cavities were generally attenuated (Fig. 2, arrows with E). Occasionally, small projections of the tumor tissue extended into the blood cavities (Fig. 2, arrows with P). In addition to the attenuated cells, solid cellular masses consisting of round mesenchymal cells (Fig. 2, 3 arrows with S), were observed in the tumor tissue. Focal necrosis (Fig. 1, arrow with N) and hemorrhage (Fig. 1, arrow with H) were frequently observed. Hemosiderin deposition was also observed in the stroma of the tumor.

The ultrastructure of the attenuated neoplastic cells was quite similar to that of capillary endothelium in the connective tissue (Fig. 4). Polarization, tight junctions, Weibel-Palade's bodies, intracytoplasmic filaments, and pinocytotic vesicles were seen in

Fig. 2. Part of the wall of the blood channel seen in the subcutaneous hemangiosarcoma. Attenuated neoplastic cells (arrows—E), projections of the tumor tissue in the blood cavity (arrows—P), and mesenchymal cell masses (arrows—S) are shown. Spindle-shaped neoplastic cells and sclerotic stroma are seen in the wall of the channel. H&E, ×380.

the cells. However, interdigitation between two adjacent cells, lack of both a continuous basement membrane, and the pericyte-like cell as well as the presence of large, pale mitochondria of irregular shape were not of normal capillary endothelium in the subcutaneous connective tissue. Neoplastic cells localized beneath the attenuated cells resembled mesenchymal cells rich in rough-surfaced endoplasmic reticulum and lysosomal granules (Fig. 4). Some neoplastic cells participated in new blood channel formation (Fig. 5 arrows). Fibrin deposition was seen in the stroma that suffered from necrosis.

Hepatic hemangiosarcoma. One mouse of the 600 ppm group developed an hepatic hemangiosarcoma approximately 65 weeks after exposure. A small, dark red tumor was found on the surface of a lobe of the liver several millimeters in diameter. The cut surface showed a cystic neoplastic nodule filled with blood. In addition to the hepatic tumor, multiple pulmonary tumors (alveologenic tumor) were observed in the animal.

Histologically, the hepatic tumor showed formation of cystic blood channels, and the stroma of the tumor was frequently sclerotic (Fig. 6). The neoplastic tissue contained

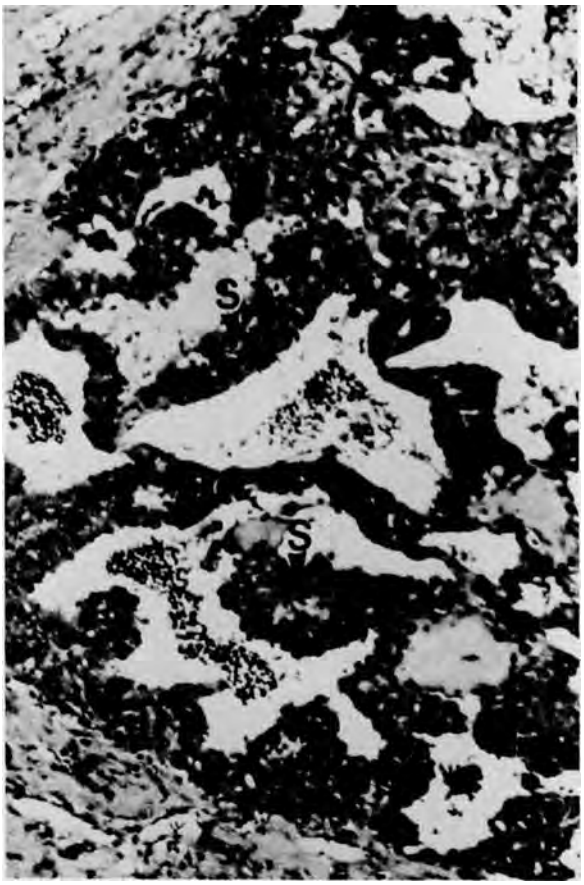

Fig. 3. Subcutaneous hemangiosarcoma. A large number of mesenchymal neoplastic cells are present in the walls of the blood cavities. H&E, ×180.

degenerated hepatic parenchyma (Fig. 7 arrow with H). The neoplastic cells lining the blood cavity were attenuated (Fig. 7 arrows with E) compared with normal sinusoidal endothelium.

Foci of necrosis were observed in the hepatic tumor (Fig. 6 arrows with N). The sclerotic neoplastic tissue showed the presence of small, narrow channels (Figs. 7, 8). Spindle-shaped neoplastic cells were also observed beneath the attenuated endothelium-like tumor cells. Sinusoidal dilatation was not observed in liver tissue unaffected by the neoplasm.

Electron microscopically, attenuated cells similar to endothelium (Figs. 9, 10, arrows with E), pericyte-like cells (Fig. 9 arrows with P), and mesenchymal neoplastic cells (Fig. 10 arrows with M) were identified among neoplastic cells. Cytoplasmic projections, tight junctions, and pinocytotic vesicles were frequently observed in the attenuated cells (Figs. 9, 10).

Pericyte-like cells were characterized by the presence of pinocytotic vesicles and dense bodies beneath the cell membrane. However, unlike the normal pericyte, the cells were not completely invested by a basement membrane. In hepatic hemangiosarcoma,

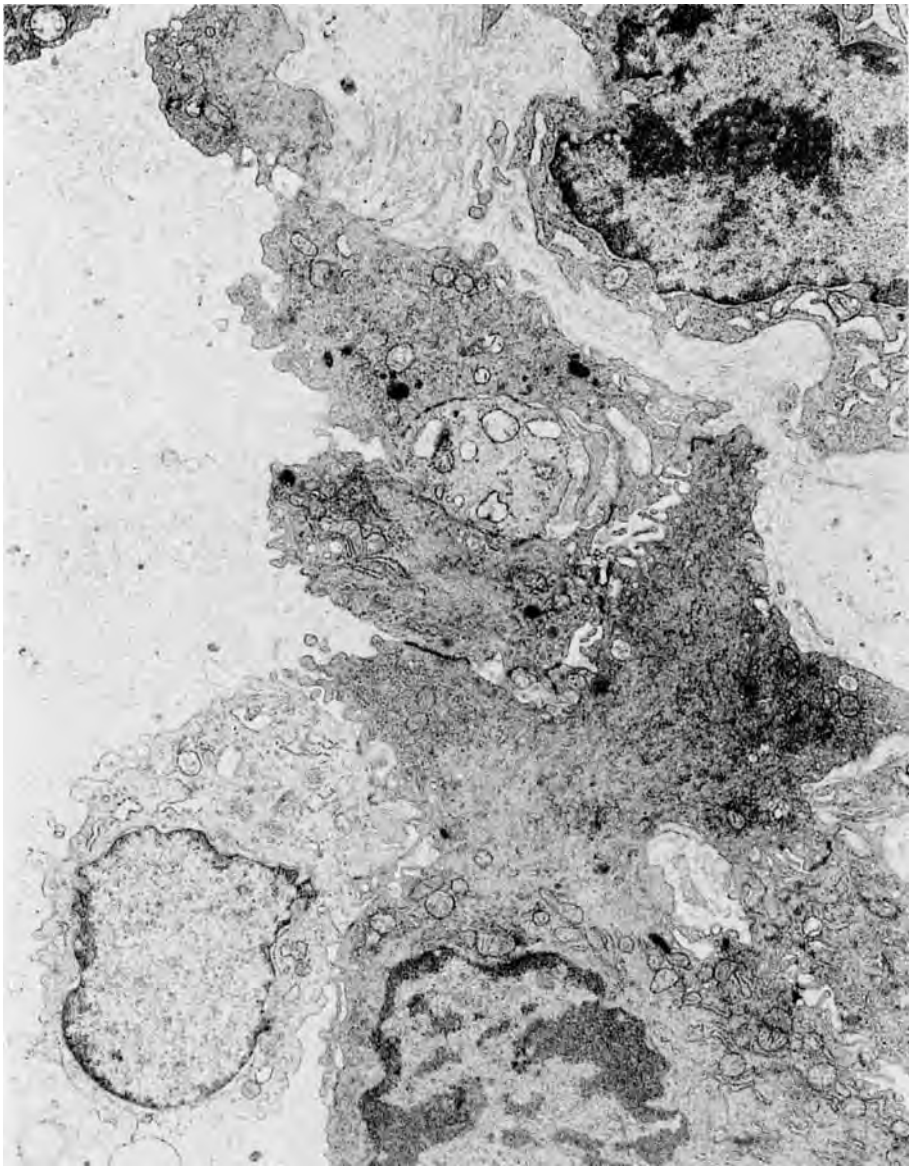

Fig. 4. Ultrastructure of the endothelium-like neoplastic cells seen in the subcutaneous hemangiosarcoma. ×11,300

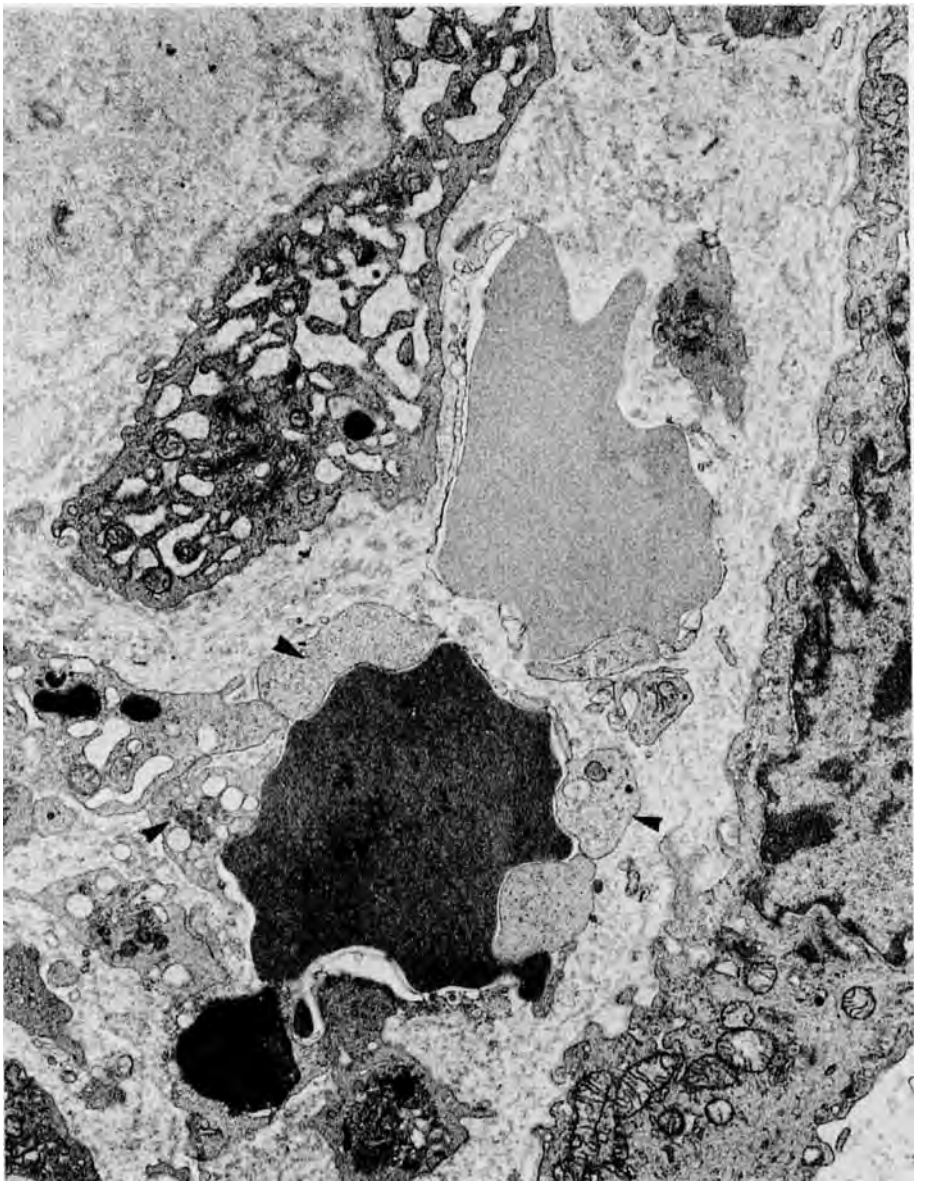

Fig. 5. Electron microscopy of the subcutaneous hemangiosarcoma, showing transformation of the mesenchymal neoplastic cells into endothelium-like cells (arrows). ×14,300

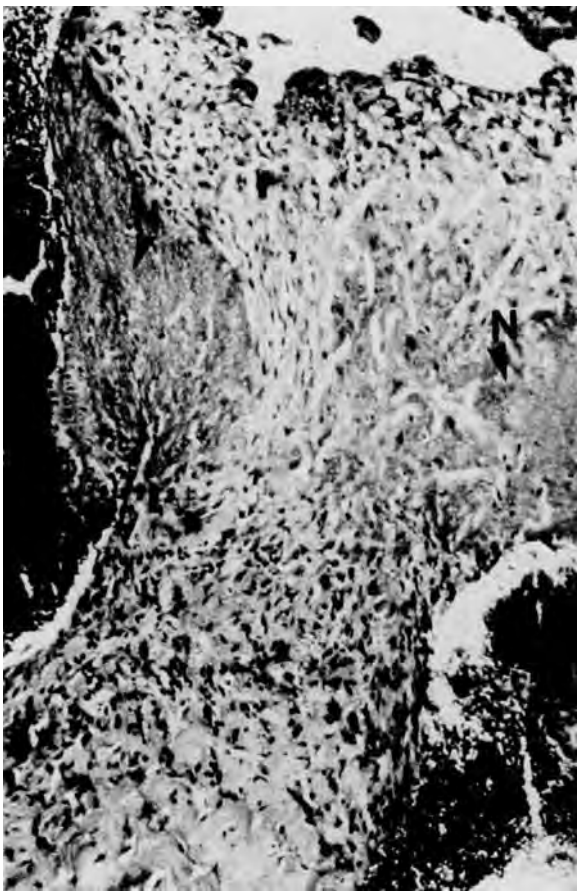

Fig. 6. Hepatic hemangiosarcoma. Necrosis (arrows—N) is seen in the sclerotic neoplastic tissue. Mesenchymal cell types are also found in the neoplastic tissue. H&E, ×170.

formation of a basement membrane was poor, and no continuous basement membrane was found beneath the attenuated endothelium-like cells nor around the pericyte-like cell. Mesenchymal cells (Fig. 10 arrows with M) were similar in ultrastructure to fibroblasts: rough, surfaced endoplastic reticulum was well developed in the cell cytoplasm. Some of the mesenchymal cells seemed to be transformed into the endothelium-like neoplastic cells (Fig. 11). The transforming mesenchymal cells were rich in free ribosomes with occasional appearance of Weibel-Palade bodies and the adjacent cells formed a narrow space (Fig. 11 arrows) that might be the early stage of a blood cavity. Stroma of the tumor was replete with collagen fibers.

It was interesting that pericyte-like cells were identified in the hepatic hemangiosarcoma because under normal conditions the pericyte does not exist in Disse's space of the mouse, although fat storage cells are present.

Alveologenic Tumor

In addition to the two hemangiosarcomas, alveologenic tumors were induced at a high rate in the mice 12 weeks and longer after exposure. There were 23 of the 24 mice in the

Fig. 7. Attenuated neoplastic cells (arrows—E), degenerating hepatic parenchyma (arrows—H), and narrow blood channels are shown in the hepatic hemangiosarcoma. H&E, ×430.

600 ppm group, 12 of 19 in the 300 ppm group, 7 of 16 in the 100 ppm, 3 of 20 in the 10 ppm, 2 of 20 in the 100 ppm, and 2 of 38 in the 0 ppm (control) group. Details of those alveologenic tumors will be reported elsewhere.

COMMENTS

Since 1974, the occurrence of hepatic hemangiosarcomas among workers exposed to vinyl chloride monomer has been well documented [Falk et al, 1974; Creech and Johnson, 1974; Lange et al, 1974; Lee and Harry, 1974; Thomas et al, 1975; Mark et al, 1976]. It is generally accepted that there is a specific relation between hepatic hemangiosarcoma and vinyl chloride exposure because this tumor is extremely rare in the general population, and prior to the discovery of the tumor among workers, identical tumors had been induced by Maltoni and Lefemine [1974, 1975] in various laboratory animals exposed to the monomer.

To date, increased incidence of human neoplasms other than hepatic hemangiosarcomas due to vinyl chloride exposure has not been clearly documented. However, animal experimentation has shown that, in addition to the liver, various other organs such as the

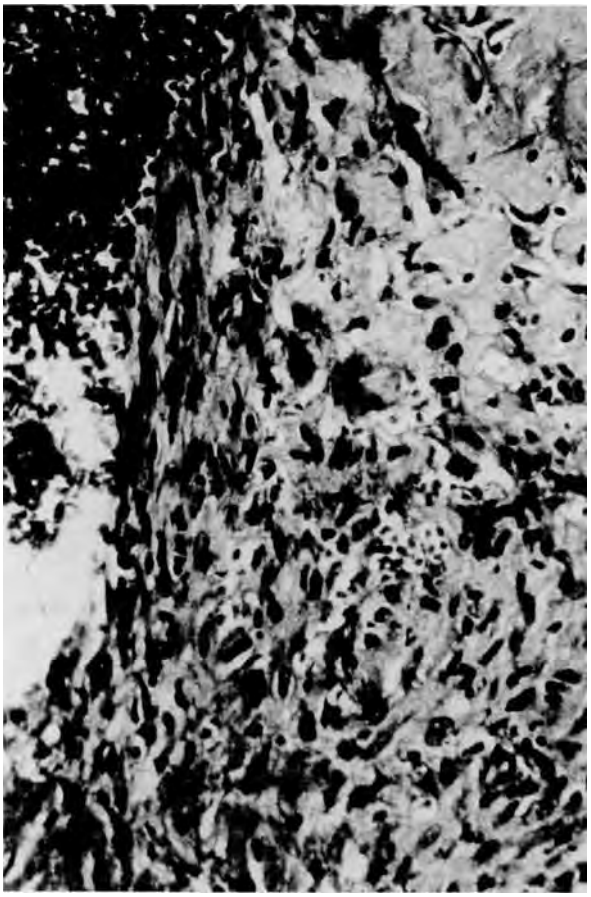

Fig. 8. A large number of spindle-shaped neoplastic cells are seen. In addition, the formation of new blood channels is recognized. H&E, ×430.

mammary gland, lung, kidney, brain, and skin may be primary sites for vinyl chloride tumor induction [Maltoni and Lefemine, 1974, 1975; Viola et al, 1971; Keplinger et al, 1975; Lee et al, 1977, 1978; Holmberg et al, 1976; Suzuki, 1978].

In addition to hepatic hemangiosarcomas, extrahepatic hemangiosarcomas have been known to occur in animals exposed to the monomer [Maltoni and Lefemine, 1974, 1975; Lee et al, 1977; Holmberg et al, 1976]. According to Holmberg and his associates [1976], the incidence of the extrahepatic hemangiosarcomas in a subperitoneal and subcutaneous adipose tissue was higher than that of the hepatic hemangiosarcomas in mice exposed to vinyl chloride at 50 ppm (for 52 weeks) and 500 ppm (for 26 weeks).

Oncogenicity of vinyl chloride has been explained in terms of metabolism of the chemical in the hepatic microsomes. It has been strongly suggested that vinyl chloride itself is not carcinogenic but rather that metabolites of the chemical, including chloroethylene oxide and chloracetaldehyde, are responsible as the ultimate carcinogens for the induction of the chemical-induced neoplasms [Antweiler, 1976; Hathway, 1977; Berk et al, 1976].

Formation of metabolites in the hepatic cell, the discharge of the metabolites into the sinusoidal endothelium and malignant transformation of the endothelium have been suggested as the mechanism of induction of hepatic hemangiosarcomas [Schaffner et al, 1976].

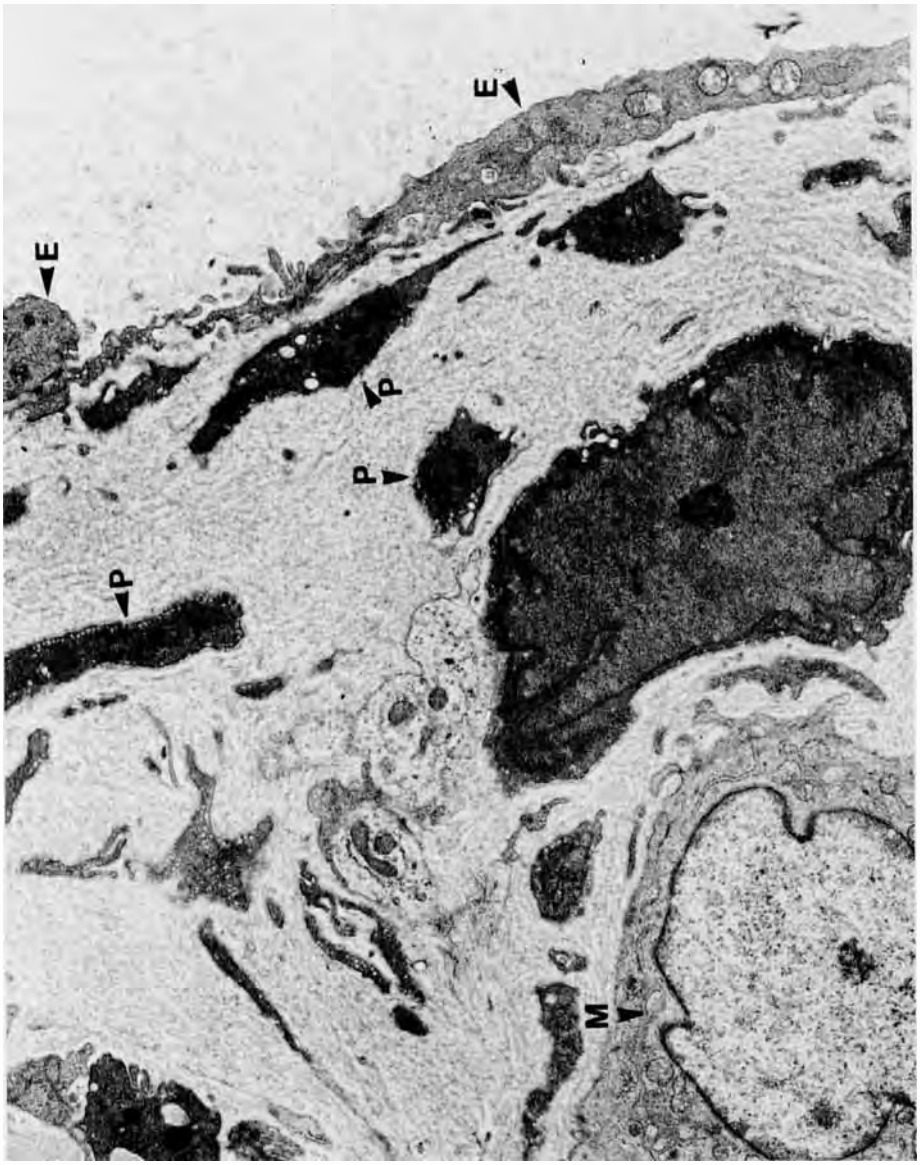

Fig. 9. Ultrastructure of the hepatic hemangiosarcoma. The endothelial-like cell (arrow—E), the pericyte-like cell (arrow—P), and immature mesenchymal cells (arrow—M) are shown. ×13,000.

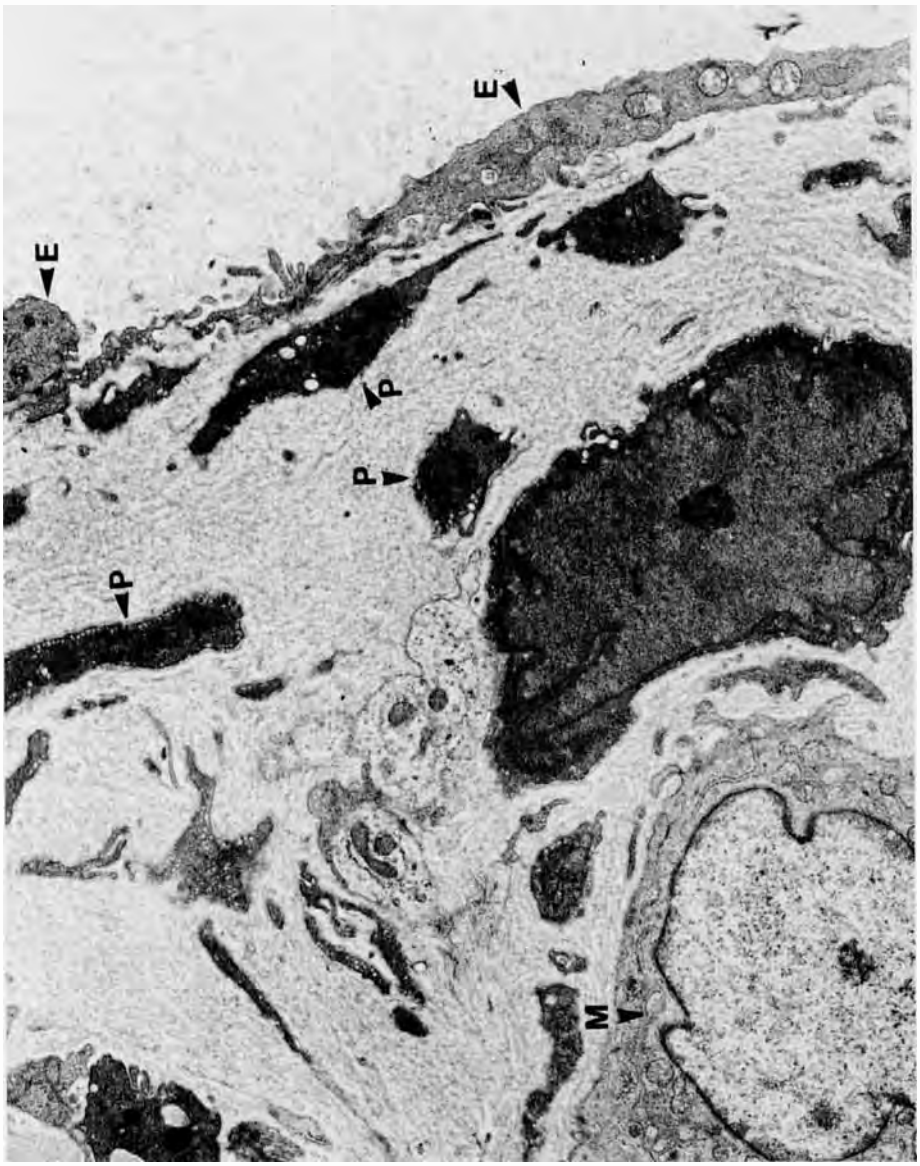

Fig. 10. Ultrastructure of the hepatic hemangiosarcoma. The endothelial-like cell (arrow—E), the pericyte-like cell (arrow—P), and immature mesenchymal cells (arrow—M) are shown. ×13,000.

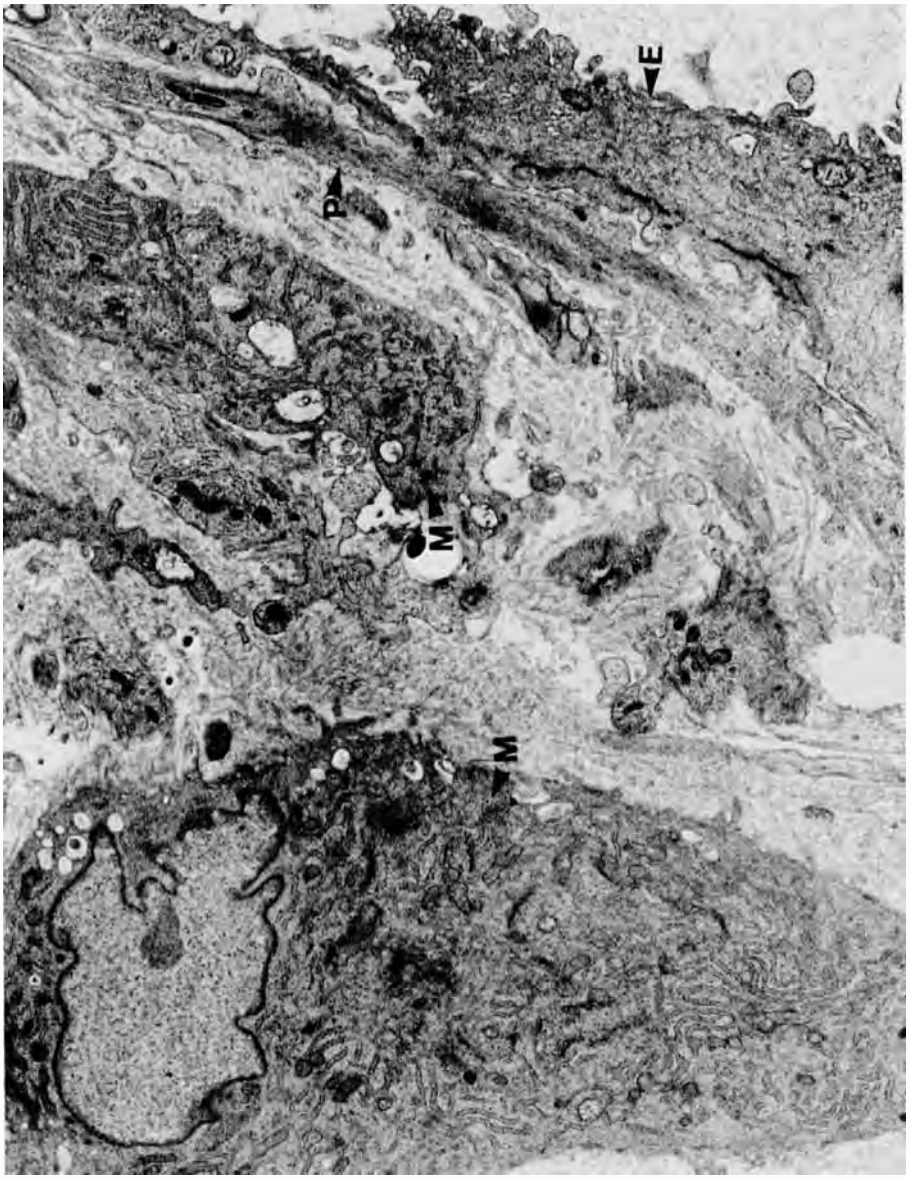

Fig. 11. Ultrastructure of immature mesenchymal cells (arrows—M), which seemed to be transformed into endothelial cell types. Arrows indicate narrow intercellular gaps (the early stage of blood channel formation), seen in the hepatic hemangiosarcoma. ×11,400.

It is not known whether cell types other than the hepatocyte can metabolize vinyl chloride monomer and produce these metabolites. However, Holmberg et al [1976] have suggested that the capillary endothelium has such capacity; the most reasonable explanation for the oncogenesis of the extrahepatic hemangiosarcoma in adipose tissue was the production of the metabolites by capillary endothelium, followed by malignant transformation of the endothelium.

Human and animal hemangiosarcomas of various sites have been studied by electron microscopy. It has been emphasized that electron microscopy is a useful method for differentiation of hemangiosarcoma from other vascular tumors, as well as from mesenchymal tumors [Tomec et al, 1976; Ash and Loutit, 1977; Rosai et al, 1976; Spence and Rubinstein, 1975; Jacobiec et al, 1976; Chandhry et al, 1978; Yang et al, 1981]. Electron microscopically, three cell types, the attenuated endothelium-like cell, the pericyte-like cell, and the immature mesenchymal cell have been identified in hemangiosarcomas by some investigators [Jacobiec et al, 1976; Chandhry et al, 1978; Yang et al, 1981]. To the present, however, ultrastructure of hemangiosarcoma related to vinyl chloride exposure has not been reported.

Our present study suggests that there is no basic difference in ultrastructure between the vinyl chloride-induced hemangiosarcomas and hemangiosarcomas not related to this chemical exposure. We interpret our data as supporting the concept [Chandhry et al, 1978] that the mesenchymal cell is the stem cell of the neoplastic cells in hemangiosarcoma.

A dose-response relation has been suggested by others in the induction of the vinyl chloride-related neoplasms [Maltoni and Lefemine, 1974, 1975]. In the present study, it was not possible to evaluate such a relationship since there were too few induced tumors. The range of doses (1, 10, 100, 300, and 600 ppm) and the duration of chemical exposure, or the cumulative doses were insufficient to induce adequate numbers of tumors.

In contrast, unlike hemangiosarcoma, a large number of alveologenic tumors were induced in the present study. The induction of these alveologenic tumors was clearly dose related.

It is concluded that in the mouse, the alveolar epithelium (the original cell type of the alveologenic tumors) was much more sensitive than the capillary endothelium to the carcinogenicity of vinyl chloride.

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