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ONE-YEAR TIME-SEQUENCE INHALATION TOXICITY STUDY OF VINYL CHLORIDE IN RATS. III. MORPHOLOGICAL CHANGES IN THE LIVER*

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SUMMARY

Wistar rats were exposed to atmospheres containing 0 (control) or 5000 ppm vinyl chloride monomer (VCM), 7 h/day, 5 days/week, for a period of 52 weeks. After 4, 13, 26 and 52 weeks each time 10 rats/sex/group were killed and subjected to extensive examinations. The present paper describes the morphological changes found in the liver.

The major parenchymal changes comprised swelling and malformation of mitochondria, an increased amount of smooth endoplasmic reticulum, necrosis, nuclear and cellular polymorphism of hepatocytes, "foci of cellular alteration", neoplastic nodules and hepatocellular carcinomas. A reduced glucose-6-phosphatase activity in hepatocytes and a strong sinusoidal activity of alkaline phosphatase were found within "foci of cellular alteration".

The non-parenchymal alterations included focal dilatation of sinusoids, focal proliferation of atypical sinusoidal cells and multicentric angiosarcomas.

The effects of VCM on the hepatic parenchyma seemed to precede those on the hepatic stroma.

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Abbreviations: MFO, mixed function oxidase; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; VCM, vinyl chloride monomer.
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TABLE I

TYPE AND INCIDENCE OF VCM-RELATED HISTOPATHOLOGICAL LIVER CHANGES OBSERVED IN RATS KILLED AFTER 13, 26 OR 52 WEEKS[a]

Type of lesions	Incidence of lesions			
	Males		Females	
	VCM	(ppm)	VCM	(ppm)
	0	5000	0	5000
Animals killed after 13 weeks				
Number of animals examined	10	10	10	10
Foci of cellular alteration:				
(a) Clear cell foci				
1. A few foci	0	1	0	0
Animals killed after 26 weeks				
Number of animals examined	10	10	10	10
Foci of cellular alteration:				
(a) Clear cell foci				
1. One or a few	0	5	0	6
2. Several	0	3	0	0
(b) Basophilic foci				
1. One or a few	0	2	0	2
2. Several foci	0	1	0	0
Animals killed after 52 weeks				
Number of animals examined	10	9	10	10
Foci of cellular alteration:				
(a) Clear cell foci				
1. One or a few	2	0	2	0
2. Several	0	2	0	4
3. Many	0	5	0	1
(b) Basophilic foci				
1. One or a few	0	3	0	4
Neoplastic nodule	0	0	0	1
Hepatocellular carcinoma	0	1	0	1
Proliferation of atypical sinusoidal cells only	0	1	0	1
Angiosarcoma[b]	0	3	0	6
Extensive necrosis	0	1	0	0
Distended sinusoids	0	3	0	4

[a] In rats killed after 4 weeks no treatment-related histopathological liver changes were observed.

[b] In 3 cases anaplastic carcinoma could not be excluded.

indications were found for a neoplastic response of the liver. In others, however, the liver contained large and highly malignant tumours. Apart from neoplastic liver cell nodules 2 major types of tumours were found in the liver: hepatocellular carcinoma and angiosarcoma (Table II). The liver cell lesions were classified according to Squire and Levitt [28]. The angiosarcomas were multicentric and often contained fairly large foci of necrosis,

TABLE II

TYPE AND INCIDENCE OF LIVER TUMOURS FOUND IN 2 GROUPS OF RATS, EACH INITIALLY CONSISTING OF 62 MALES AND 62 FEMALES, WHICH WERE EXPOSED TO 0 AND 5000 PPM VCM RESPECTIVELY[a]

Type of tumours	Incidence of tumours			
	Males		Females	
	VCM	(ppm)	VCM	(ppm)
	0	5000	0	5000
Neoplastic nodule	0	0	0	3
Hepatocellular carcinoma	0	1	0	2
Angiosarcoma[b]	0	6	0	16

[a] 10 rats/sex/group were killed after 4, 13, 26 and 52 weeks (only 9 males and 10 females of the test group were still alive in week 52). The remaining controls were all killed in week 53.

[b] In 5 cases anaplastic carcinoma could not be excluded.

haemorrhages and thrombi. In most of the tumours the neoplastic cells tended to line spaces, the wall of which was thickened by fibrous tissue. The neoplastic cells were polymorphic and had hyperchromatic, pleomorphic nuclei. The amount of fibrous connective tissue in the neoplasms varied widely. The direct extension of some of the more angiomatous tumours to the adjacent liver tissue was seen to occur in a destructive way, whereas other more solid tumours extended to the adjacent tissue by infiltration between preserved liver cords, thus gradually causing atrophy of the liver parenchyma. In view of their growth pattern, the tumours were considered to have developed from sinusoidal cells, most probably endothelial cells, though other sinusoidal cells could not be fully excluded.

Many of the rats bearing hepatic angiosarcoma also showed focal distension of sinusoids and focal proliferations of either relatively normal or highly atypical sinusodial cells.

Enzyme histochemistry of the liver

In VCM-exposed rats an increased activity of acid phosphatase was seen in Kupffer-cells (or other sinusoidal cells) located in the periphery of the hepatic lobules. In areas where the sinusoids were distended, sinusoidal cells did not show any activity of acid phosphatase, indicating Kupffer-cells being either absent or inactive with regard to this enzyme. Moreover, "foci of cellular alteration" or neoplastic nodules exhibited much less pericanalicular acid phosphatase activity than normal liver tissue. In comparison with normal liver cells the hepatocytes in these foci of altered liver tissue showed a reduced glucose-6-phosphatase activity. It was interesting to find a strong sinusoidal activity of alkaline phosphatase within the foci of cellular alter-

ations. An increased sinusoidal activity of this enzyme was also observed in areas showing distended sinusoids. No difference in adenosine monophosphatase activity was found between livers of test and control animals.

*Electron microscopy of the hepatic parenchyma**

Light microscopy of semi-thin sections showed small foci of hepatocytes, containing a finely "vacuolized" cytoplasm in most of the VCM-exposed rats killed after 4, 26 and 52 weeks (Fig. 1). No such "vacuolized" liver cells were encountered in any of the controls.

Ultrastructurally, the "vacuoles" appeared to represent swollen mitochondria with a pale augmented matrix and relatively short cristae arranged radially and restricted to the periphery, thus leaving a fairly large central area free from cristae (Fig. 2). Hepatocytes with swollen mitochondria were occasionally found to contain myelin-like figures localized in lipid droplets or mitochondria.

After 26 weeks swollen mitochondria were often empty or deprived of

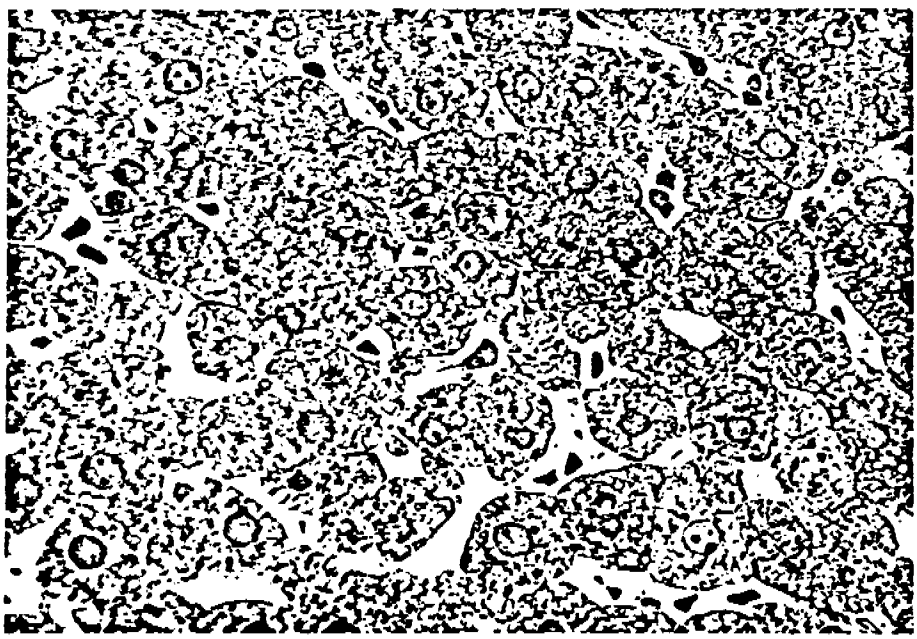

Fig. 1. Focus of "vacuolized" hepatocytes. Female rat exposed to 5000 ppm VCM for 4 weeks. One-micron Epon section, Paragon, × 640.

*** The ultrastructure of VCM-induced hepatic angiosarcoma in rats will be the subject of a separate paper.**

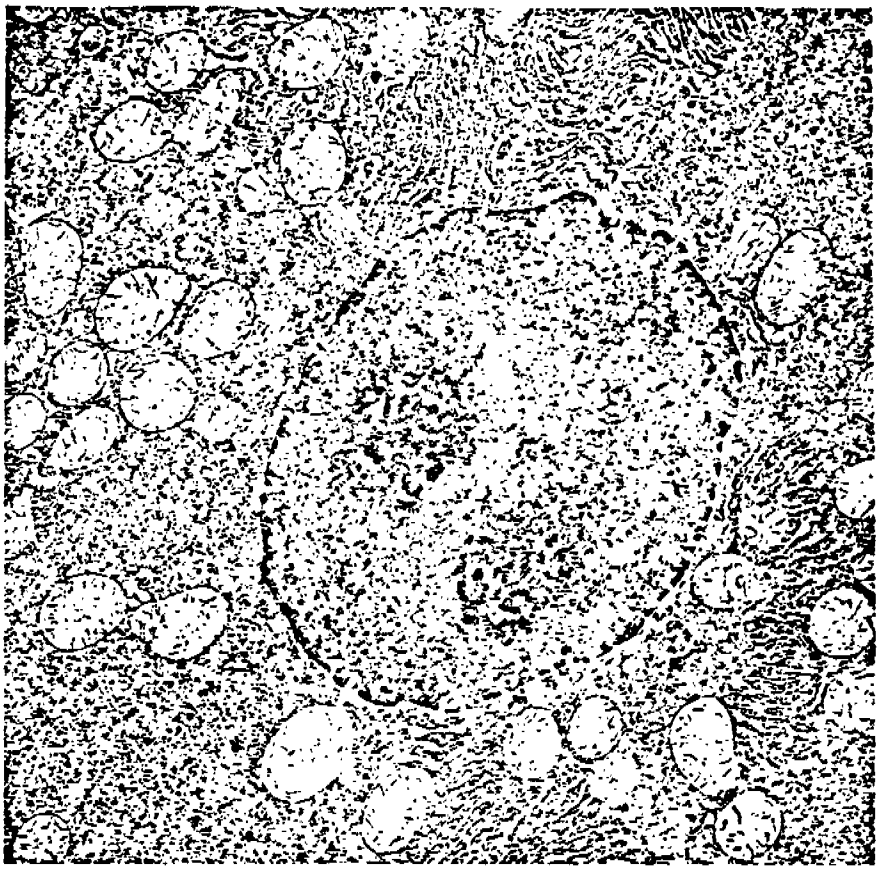

Fig. 2. Hepatocyte. Swollen mitochondria with pale matrix and short cristae radially arranged along the periphery. Note nucleus with 2 pronounced nucleoli. Male rat exposed to 5000 ppm VCM for 4 weeks. Uranyl acetate and lead citrate, x 10 500.

cristae. Irregularly shaped mitochondria with a heterogeneous matrix (light- and dark-grey areas) was a frequent finding in hepatocytes not containing swollen mitochondria. In these hepatocytes the RER consisted of clearly shortened packets of wavy lamellae, and mitochondria were often surrounded by single cisternae of RER. In general, no glycogen particles were present. Occasionally, small areas not showing the normal hepatic architecture were found in the liver. The liver cells in these regions were joined together in an unusual way (Fig. 3).

There was a remarkable cell demarcation, invariably seen as a thin layer of condensed, somewhat filamentous cytoplasm adjacent to the plasma membrane. Bile canaliculi were nearly always present, but sinusoids or sinusoid-like spaces occurred only sporadically in these areas. The amount

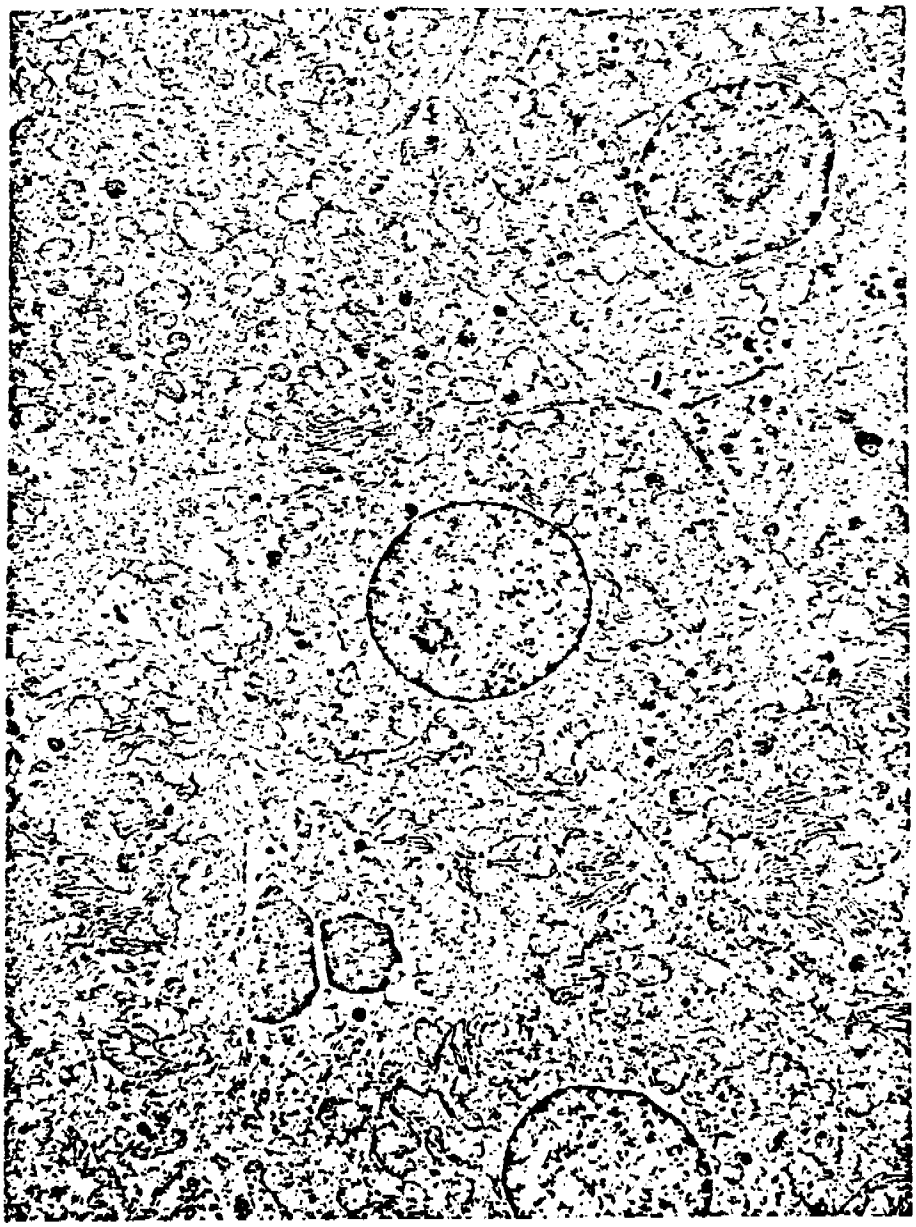

Fig. 3. Hepatocytes joined together. Sinusoids are almost completely absent. Note damaged mitochondria and increased tubular SER. Female rat exposed to 5000 ppm VCM for 26 weeks. Uranyl acetate and lead citrate, x 4500.

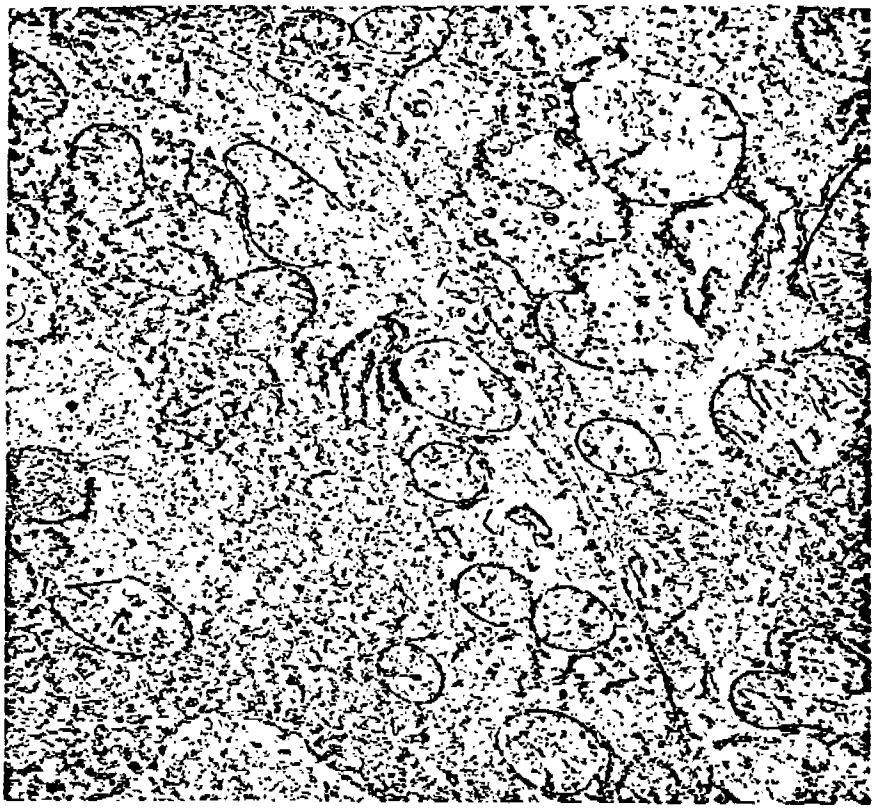

Fig. 4. Hepatocytes containing whorl-like bands of tubular SER. Female rat exposed to 5000 ppm VCM for 26 weeks. Uranyl acetate and lead citrate, x 17 000.

of tubular smooth endoplasmic reticulum (SER) was increased, whereas the amount of RER, generally occurring as short stacks around the mitochondria, was decreased. In some cases, liver cells were filled with whorl-like bands of tubular SER (Fig. 4).

The liver changes found after 52 weeks were similar to those seen after 26 weeks, but generally much more pronounced. Remarkable findings were cells tightly packed with swollen mitochondria often filled with a kind of flocky material, RER showing loss of ribosomes, absence of glycogen particles and accumulation of free ribosomes (Fig. 5).

DISCUSSION

Degenerative, hyperplastic and neoplastic alterations of the liver parenchyma have been reported to occur in several animal species and also in man following exposure to VCM. The degenerative changes varied from fatty

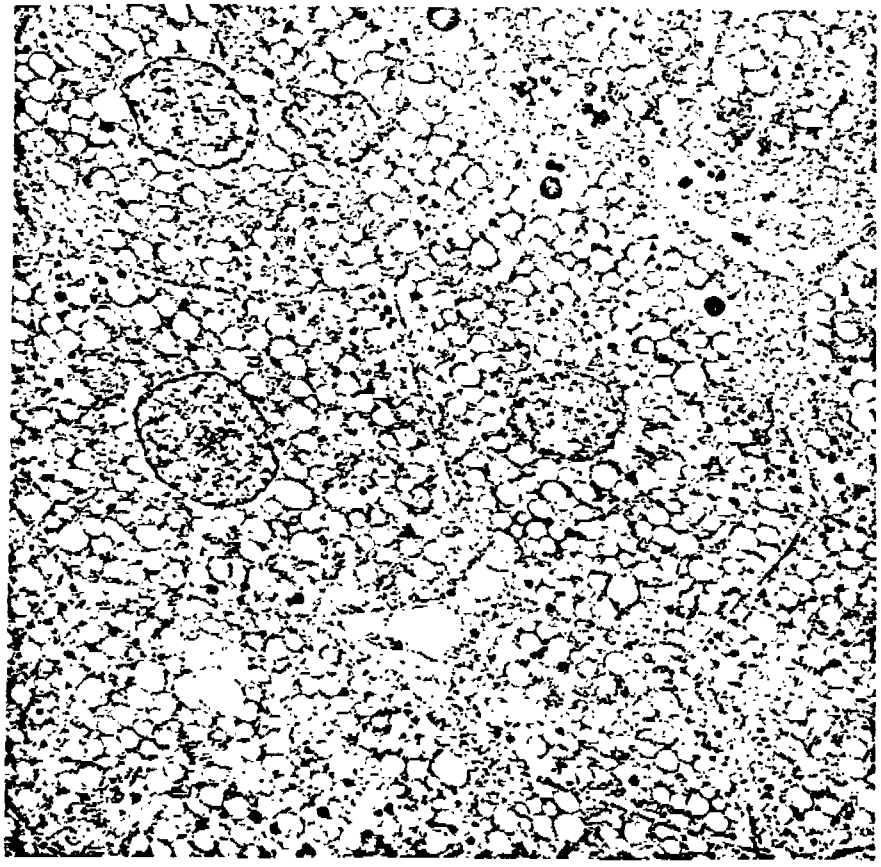

Fig. 5. Hepatocytes tightly packed with swollen mitochondria. Male rat exposed to 5000 ppm VCM for 52 weeks. Uranyl acetate and lead citrate, x 2600.

infiltration [18,20], hydropic swelling with or without vacuolisation [12,20] isolated-cell necrosis [20,24] and centrolobular vacuolisation [9] to severe chronic hepatitis [32]. Hyperplasia was seen as "proliferation of hepatocytes" [2] and enlarged polymorphic parenchymal cells with big hyperchromatic nuclei containing several nucleoli [13,20]. A few hepatomas have been found in rats exposed to VCM for a prolonged period of time [15,16,29,33].

The results of the present study in rats were in full agreement with the observations reported in the literature mentioned, and likewise produced convincing evidence of VCM adversely affecting the hepatic parenchyma.

Swelling of mitochondria in foci of parenchymal cells was the earliest change observed and mitochondrial alterations remained characteristic features of the VCM-damage seen at later stages. Slight swelling of hepato-

cytic mitochondria has been reported to occur in a VCM-reactor cleaner suffering from splenomegaly and non-cirrhotic portal hypertension [10].

Swelling of mitochondria has been reported to occur in hepatocytes under various pathological conditions, such as dietary deficiency in riboflavin [30], hypoxia [19, Kief, H. pers. comm.], and exposure to ethanol [4]. Furthermore, the mitochondrial changes described (swelling, electron-lucent matrix, shortening and partial loss of cristae) have also been found after exposure to other carcinogens [1]. Despite the extensive mitochondrial changes no increase in autophagic vacuoles containing remnants of mitochondria was found in the present study. This somewhat remarkable finding, however, seems to be normal for hepatocytic mitochondrial damage caused by carcinogens [1]. It seems, therefore, justified to conclude that the mitochondrial changes observed are far from specific for VCM-damage.

In addition to swelling of mitochondria, prolonged exposure to VCM (26 and 52 weeks) appeared to cause a decrease in RER, loss of ribosomes from the RER, while the most important observation was an increase in SER. Hypertrophy of SER has also been found in mice exposed to 2500—6000 ppm VCM [26]. These findings suggest an enhancing effect of VCM on the mixed function oxidase system (MFO-system) which has indeed been shown to be involved in the detoxification of VCM [3,25]. In this respect one might speculate that the MFO-system is in competition with other cellular functions (e.g. the mitochondrial respiration) in satisfying their need of oxygen.

The reduction in glucose-6-phosphatase activity in "foci of cellular alteration" occurring in the livers of VCM-exposed rats is a finding comparable to the decreased activity of this enzyme seen by Scherer and Emmelot [27] in "islands" induced by diethylnitrosamine in the liver of rats. These investigators consider the "islands" to represent an obligatory stage in liver carcinogenesis.

The hepatic "foci of cellular alteration" also exhibited an increased alkaline phosphatase activity and a decreased acid phosphatase activity. The physiological or toxicological significance of these observations is not clear, and we are not aware of similar reactions found in "foci of cellular alteration" induced by carcinogens.

In addition to the parenchymal alterations, the livers of the test animals exhibited focal dilatation of sinusoids, marked alkaline phosphatase activity of sinusoidal cells, focal proliferation of sinusoidal cells with or without atypia and multicentric angiosarcomas containing widely varying amounts of fibrous tissue. Although these impressive changes of the hepatic stroma are in accordance with the observations of other investigators in man and experimental animals exposed to VCM [2,7,10,13,17,20, 21,23], it might be of interest to draw attention to certain differences between the previous and the present findings. Maltoni and Lefemine [15] have reported the occurrence of hepatic and extrahepatic blood vessel ectasias and angiomas, and of angiosarcomas outside the liver in rats, mice and hamsters. VCM-induced extrahepatic angiosarcomas in mice have more

recently also been reported by Lee et al. [11] and Holmberg et al. [8]. No extrahepatic angiosarcomas were observed in the present study, with the possible exception of a primary angiosarcoma in the lungs [6].

In view of their morphology, the sinusoidal cell tumours were classified as angiosarcomas, and were thus considered to have developed from endothelial cells. In this respect, an interesting finding was the absence or inactivity (as to acid phosphatase) of Kupffer-cells in areas where the sinusoids were distended, possibly indicating Kupffer-cells not being involved in the neoplastic process.

A "fibrotic precursor stage" has been suggested to precede angiosarcoma development in humans [21—23,31]. In rats marked fibrosis has been seen only within fully developed angiosarcomas or as a reaction to extensive necrosis of liver parenchyma. Therefore, in rats such a conspicuous "fibrotic precursor stage" does not seem to exist, although electron microscopical examinations of livers from rats which were exposed to VCM by the oral route for a prolonged period of time, occasionally revealed the presence of bundles of collagen fibres in contact with angiosarcoma cells extending between liver cords (Spit, B.J., unpublished).

From the present results it appeared that the hepatocytic changes were visible earlier than those of the sinusoidal lining cells. This might be indicative of the hepatic parenchyma being attacked by VCM before the hepatic stroma. However, the relationship between the hepatocytic and sinusoidal cell alterations, if existing at all, is not yet clear and needs further study.

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