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Comparative Electron-Microscopic Studies of Benign Hepatoma and Icterus in Patients on Oral Contraceptives

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Summary. The author compared the electron-microscopic picture of 6 benign hepatomas and 3 specimens of icteric liver tissue in female patients on oral contraceptives. The majority of ultrastructural changes seen were identical in the tumor and non-tumor cases. It is noteworthy that there is an accumulation of filamentous substance of unknown origin in the bile canaliculi and in the Disse's spaces associated with a damage to the cell membrane. The electron-microscopic picture of the sinusoidal endothelial cells suggested an increased protein producing activity. The most conspicuous feature of the tumours was formation of continuous capillaries at the site of the sinusoids.

The importance of systematic electron-microscopic investigation in studying the effect of oral contraceptives on the liver cells is stressed by the author.

Key words: Bile canaliculi – Cell membrane – Endothelial cell – Disse's space – Continuous capillary.

In the literature in recent years, special attention has been devoted to liver disease in young female patients on oral contraceptives. A possible association between oral contraceptives and hepatic changes has been confirmed by clinical observations and by an accumulation of cases but the pathogenesis has not been elucidated. Most tumors are benign but malignancy may occur (Baum et al., 1973; Brander et al., 1976; Christopherson et al., 1975; Christopherson et al., 1977; Grabowski et al., 1975; Kamber et al., 1977; Knowles et al., 1976; Mays, 1976; Roschlau, 1977; Stauffer et al., 1976). In other cases, jaundice (Adlercreutz et al., 1970; Drill, 1974; Feldmann, 1976; Larsson-Cohn et al., 1967) and peliosis hepatis (Bagheri et al., 1974; Winkler et al., 1975) have been reported. Compared with the number of patients on oral contraceptives, liver disease can be considered to be infrequent (Christopherson et al., 1977; Kamber et al., 1977; Mays, 1976) but it is still important to clarify any correlation since the effects of

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the drugs can be cumulative and consequently, liver disease can be manifest after both prolonged administration and following a long interval after stopping the drug (Perez et al., 1969; Porte et al., 1977). An increase in the number of cases is therefore to be expected.

Fatal complications may also occur. Both the richly vascularized tumours and peliosis hepatis may result in spontaneous liver rupture with death of the patient by bleeding (Christopherson et al., 1977).

There have been different approaches to possible associations between oral contraceptives and liver damage (Palmer et al., 1977; Roe, 1976) but only a few electron-microscopic studies (Balázs, 1976; Feldmann, 1976; Horvath et al., 1972; Kay et al., 1971; Larsson-Cohn et al., 1967; Perez et al., 1969; Porte et al., 1977). Comparative studies on the ultrastructural characteristics of different hepatic changes have not been published.

In our earlier reports, light- and electronmicroscopic examination of a case of liver cell adenoma (Balázs, 1976), one of primary liver carcinoma (Balázs et al., 1977) and two focal nodular hyperplasias due to oral contraceptives (Balázs et al., 1978) were presented. In this paper, a comparison will be drawn between the electronmicroscopic pictures of 6 benign liver tumours and 3 specimens of icteric liver tissue. Our attention was directed to the characteristic features of tumors and non-tumorous live tissue. Clinicopathological analyses of the same cases will be presented elsewhere.

Materials and Method

The benign liver tumours of 6 female patients on oral contraceptives for 2 to 7 years were surgically removed. In one case only excision was performed. On light-microscopic examination the tumours were classified as: 1 case of benign liver cell adenoma and 5 cases of focal nodular hyperplasia. Of the 6 cases, 3 have been included in our previous reports (Balázs, 1976; Balázs et al., 1978), 3 further tumours were detected in the preceding year. The hepatocellular carcinoma we have previously reported (Balázs et al., 1977) has been omitted from our present study, since the possible association between malignant liver tumours and oral contraceptives has not yet been proven.

In 3 patients on oral contraceptives jaundice occurred. In 2 of them needle biopsy was performed. In one case, liver tissue was excised during gallbladder surgery.

The 1 mm^3 pieces of liver tissue were fixed in 1 percent Palade buffered osmium tetroxide, dehydrated in graded ethanol and embedded in araldite. The sections were prepared by Reichert ultramicrotome and photographed by the JEM 100 B electron microscope. For orientation 0.5-micron semithin section were made stained with toluidine blue.

Results

Changes in the Bile Canaliculi and the Peribiliary Zone

Changes were identical in both the tumor and icteric cases. The lumen of the bile canaliculi was usually dilated with an accumulation of filamentous substance (Figs. 1, 2). In the bile canaliculi filled with this material the microvilli were destroyed (Fig. 3a). In some places, the surface was flattened, in other regions, oedema of microvilli was present (Fig. 3b). The peribiliary cytoplasm contained several micro-filaments.

Changes in the Liver Cell Organelles

Changes in cytoplasmic organelles were identical in character and severity in both tumor and non-tumor liver cells.

The mitochondria showed a great variation in shape and size, containing a great number of paracrystalloid inclusions (Fig. 4a). The external double membrane of some mitochondria was partially or totally missing. In some places the mitochondrial matrix seemed to be discharged into the hyaloplasm due to absence of the membrane (Fig. 4b).

The smooth endoplasmic reticulum was aggregated and cystic in some places; the rough endoplasmic reticulum was regular. In the peribiliary zone several dense bodies were present.

The Vascular Pole of the Liver Cells

On the sinusoidal cell surface, oedema of the microvilli (Fig. 5a) and in some places, herniation of the cytoplasm (Fig. 5b) could be seen, in both the tumour cases and those with jaundice. In addition, there seemed to be an accumulation of granular and filamentous substance in Disse's space. In some areas, the plasma membrane was missing.

Sinusoidal Cells

The number of, and relationship between the mesenchymal elements lining the sinusoids differed in the tumor and nontumor cases.

In cases with jaundice, the sinusoidal endothelial cells were hyperplastic (Fig. 6). They had a large nucleus, and their cytoplasm was widened, being rich in organelles, particularly in rough endoplasmic reticular lamellae and free ribosomal granules (Fig. 7a). Surrounding them on all sides a basement membrane was observed (Fig. 7b). There were also a great number of Kupffer cells containing pigment granules of varying structure (Fig. 8).

In case with tumours, continuous capillaries were formed at the site of sinusoids (Fig. 9), lined with hyperplastic endothelial cells, the cytoplasm of which contained several vesicles, vacuoles and a particularly large number of rough endoplasmic reticulum and free ribosomal granules. The endothelial cells were connected by cell binding structures, with several layers of basement membrane (Fig. 10). In some areas, pericytes also occured.

Bile Ductules

Changes in the cell membranes lining the lumen of bile ductules were observed everywhere, with partial destruction and occasional oedema of the microvilli (Fig. 11). Beneath the surface there were several microfilaments. In the cytoplasm of the cells there was a plexiform arrangement of microfilaments in many places (Fig. 12).

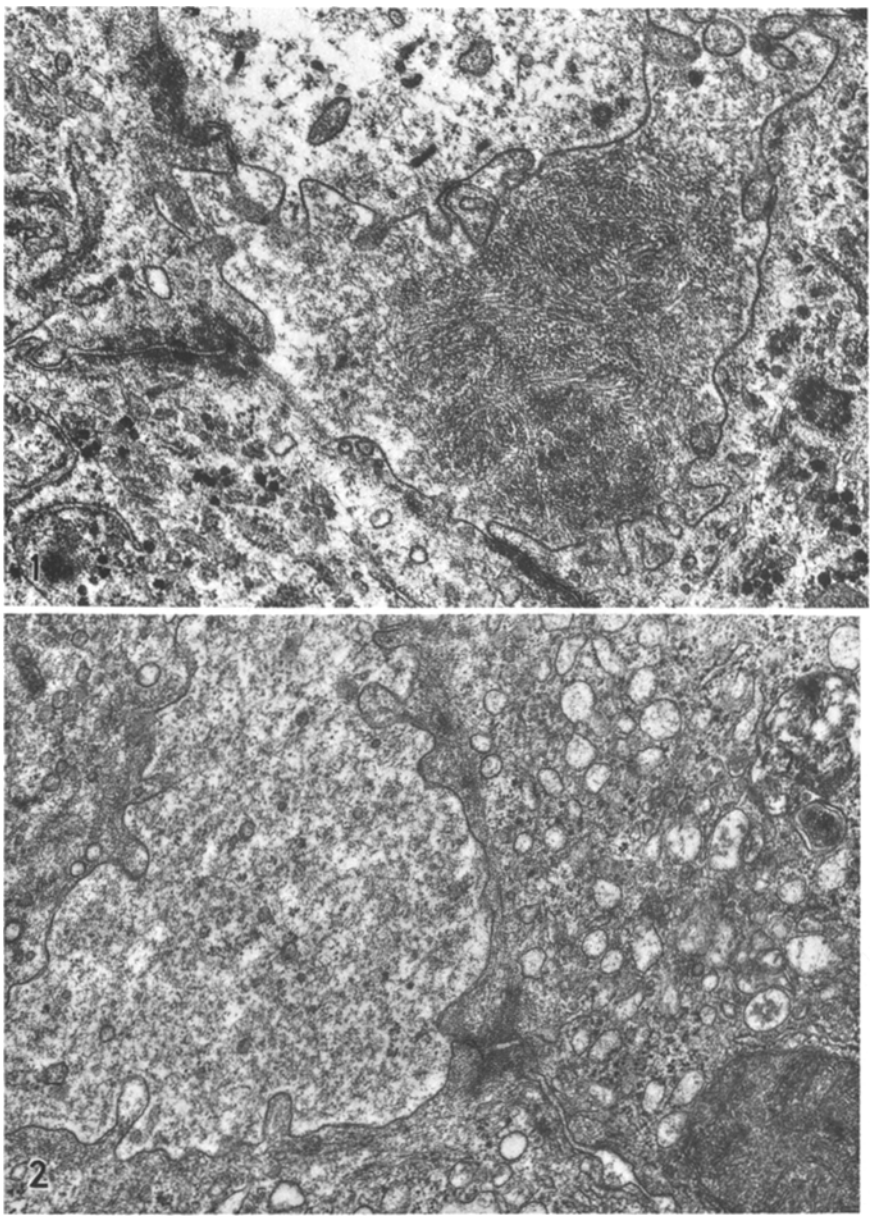

Fig. 1. Electron-microscopic picture of the benign liver cell adnoma of a 31-year-old female patient. The figure shows a dilated bile canaliculus, its lumen being filled with filamentous substance ($\times 17{,}500$)

Fig. 2. Liver tissue of an icteric female patient, aged 38 years. The dilated bile canaliculus contains partly granular, partly filamentous substance. In the peribiliary zone, the microfilaments are accumulated ($\times 12{,}500$)

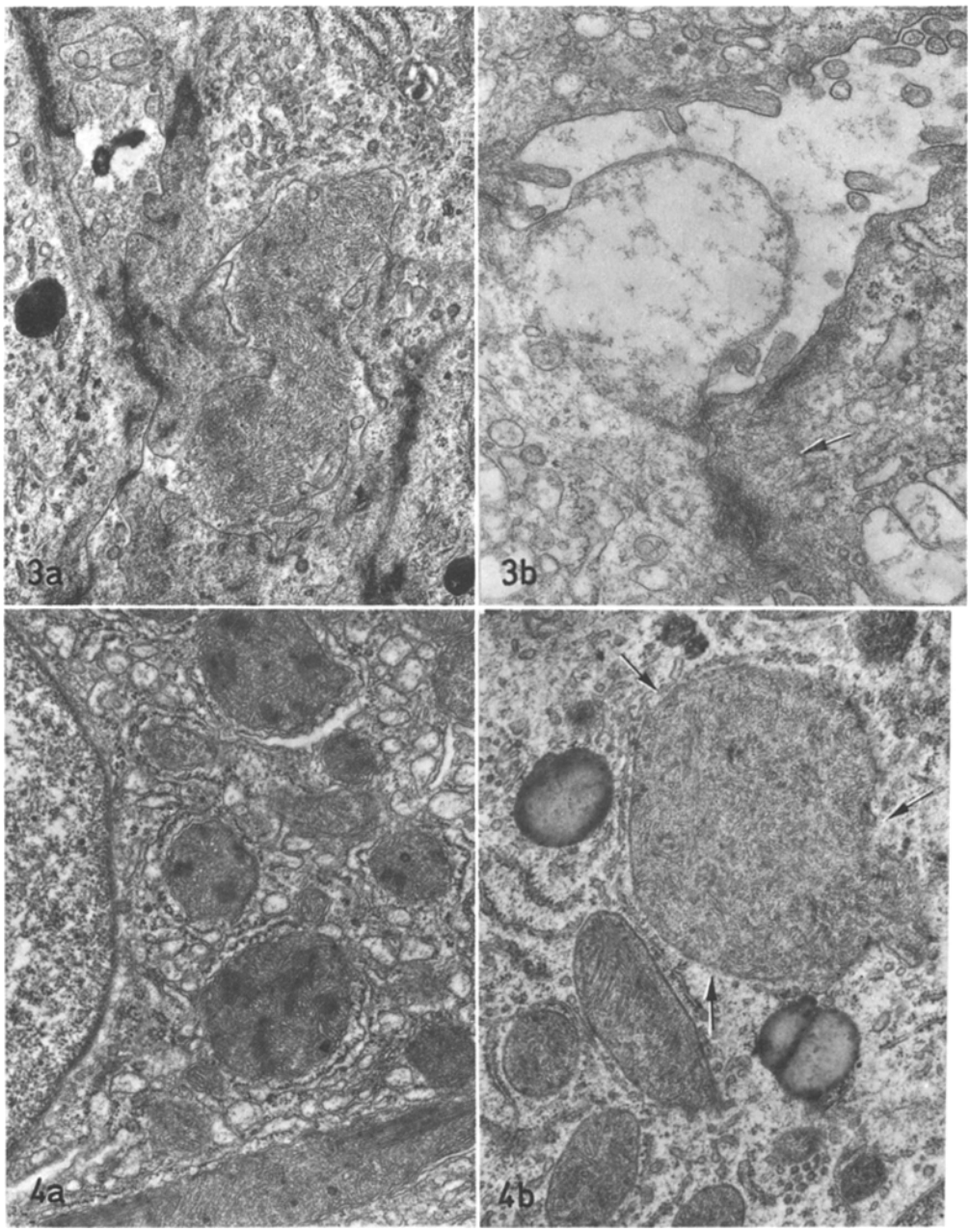

Fig. 3a. Focal nodular hyperplasia of a female patient, aged 28 years. The cell membrane lining the bile canaliculus containing filamentous substance is flattened and destroyd in some places (× 11,250)

Fig. 3b. Liver biopsy material of a female patient, aged 38 years. A great part of the canalicular microvilli are absent. One is oedematous and intrudes into the lumen of the canaliculus. In the region marked by arrow there are many microfilaments (× 12,500)

Fig. 4a. Picture from an icteric female patient, aged 30 years. The cytoplasm of the liver cell contains mitochondria of varying shape and size with several paracrystalloid inclusions. The smooth endoplasmic reticulum is aggregated, the rough endoplasmic reticulum is regular (× 10,000)

Fig. 4b. Female patient, aged 32 years, with focal nodular hyperplasia. The figure shows a detail of the cytoplasm of a tumorous liver cell. The membrane of the mitochondrium (*marked by an arrow*) is missing (× 18,500)

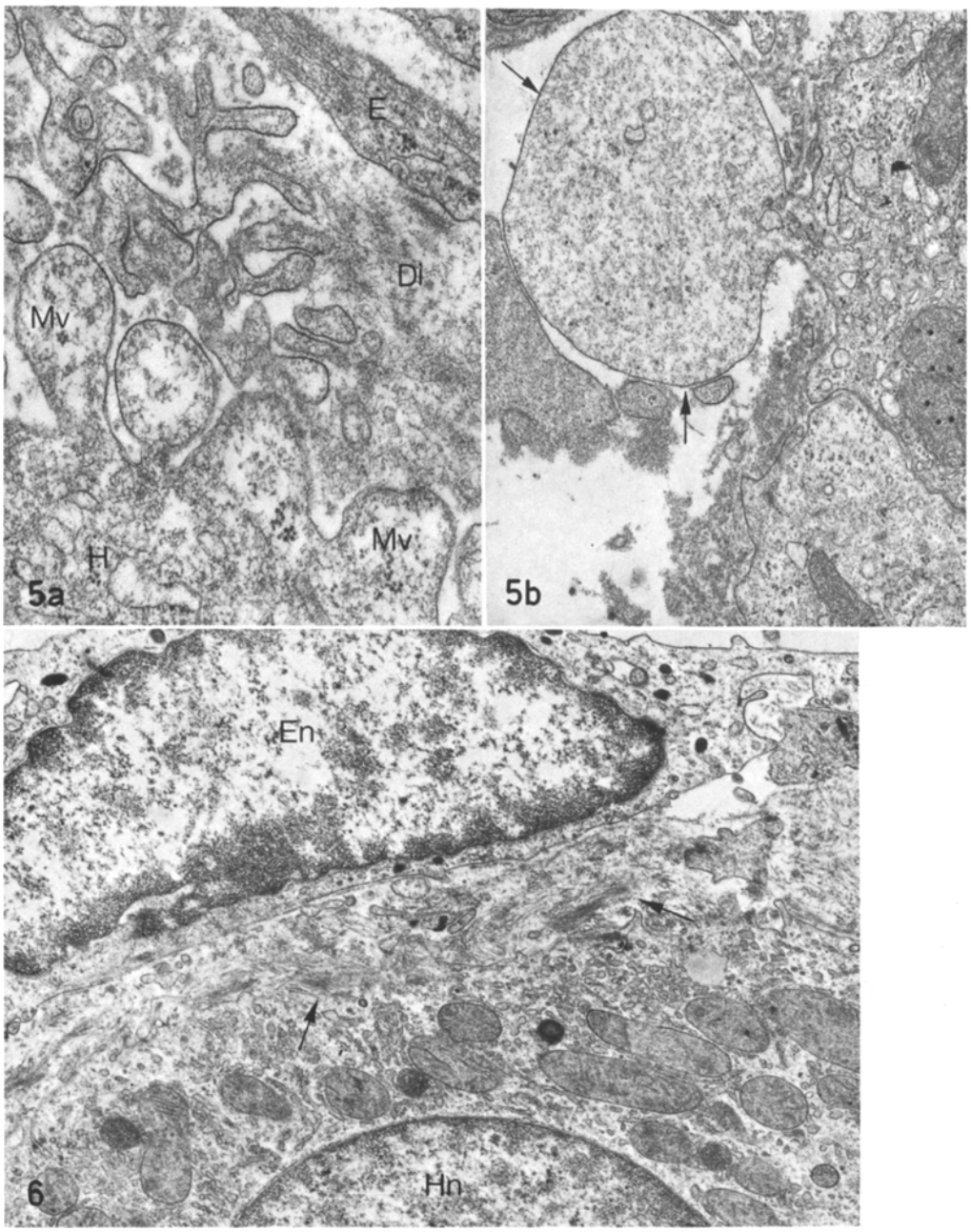

Fig. 5a. 38-year-old icteric female patient. The figure shows the vascular pole of a liver cell (H) with oedematous microvilli (*Mv*). Disse's space *(Di)* is filled with filamentous substance (× 18,550)

Fig. 5b. Previous case. Picture of an other region shows herniation of the cytoplasm (*arrows*) of the liver cell (× 6,250)

Fig. 6. Female patient with jaundice, aged 32 years. The figure shows detail of the nucleus of the liver cell *(HN)* and the endothelial cell *(E)*. The nucleus of the endothelial cell *(EN)* is conspicuously large. Disse's space contains filamentous substance *(arrows)* (× 7,500)

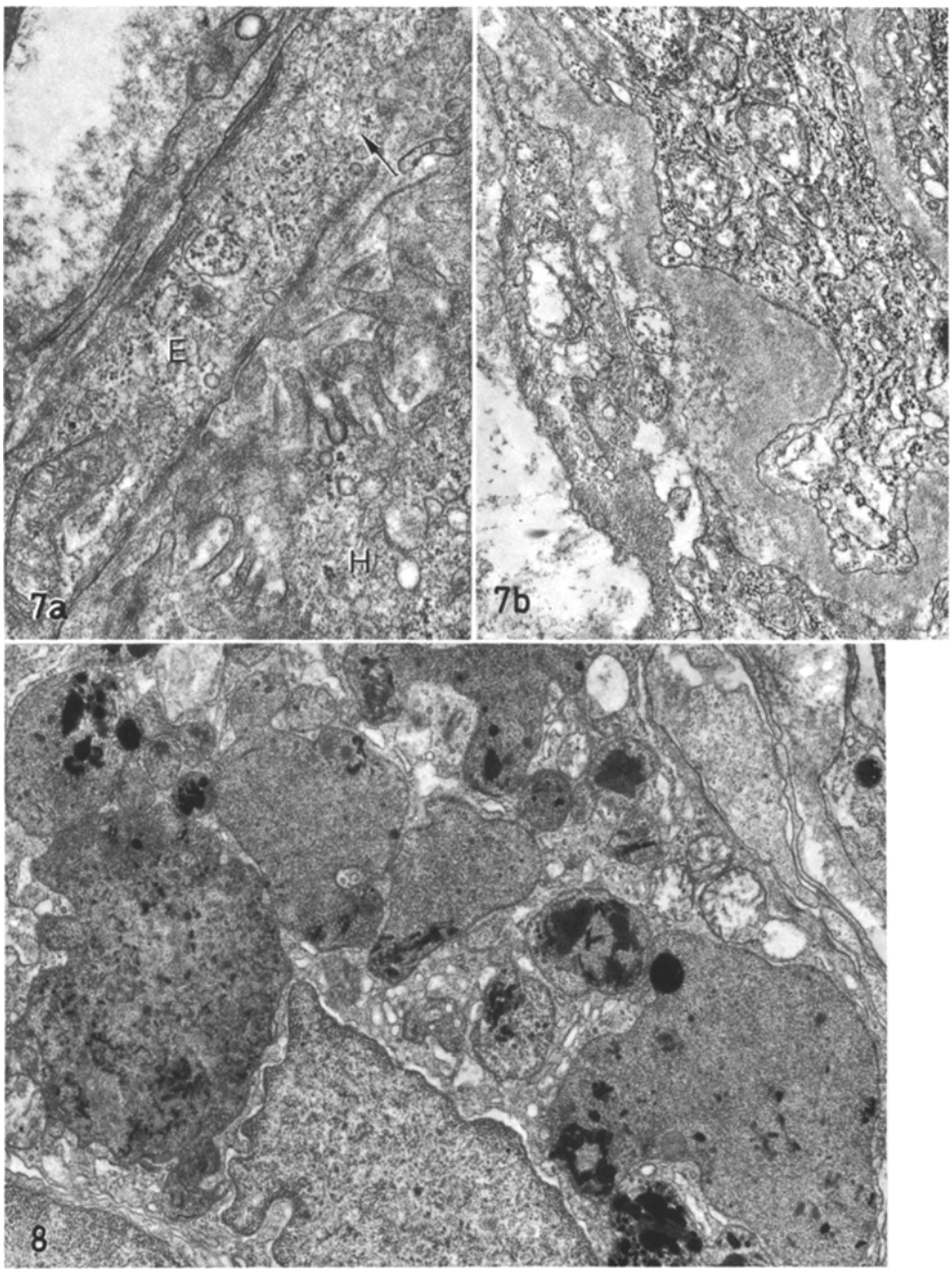

Fig. 7a. Liver biopsy material of a female patient with icterus, aged 30 years. On the right: vascular pole of the liver cell *(H)*. The cytoplasmic process of the endothelial cell *(E)* is widened containing a large number of ribosomal granules and rough endoplasmic reticulum. In the region indicated by an arrow, the cytoplasm of the endothelial cell contains a filamentous substance ($\times 12{,}500$)

Fig. 7b. Another picture of the previous liver specimen. The cytoplasm of the endothelial cell is rich in organelles, mainly in rough endoplasmic reticulum, surrounded by a basement membrane of varying width ($\times 10{,}000$)

Fig. 8. Liver tissue of icteric female patient, aged 38 years. The figure shows Kupffer cell with autophagic vacuoles of varying shape and composition ($\times 6{,}250$)

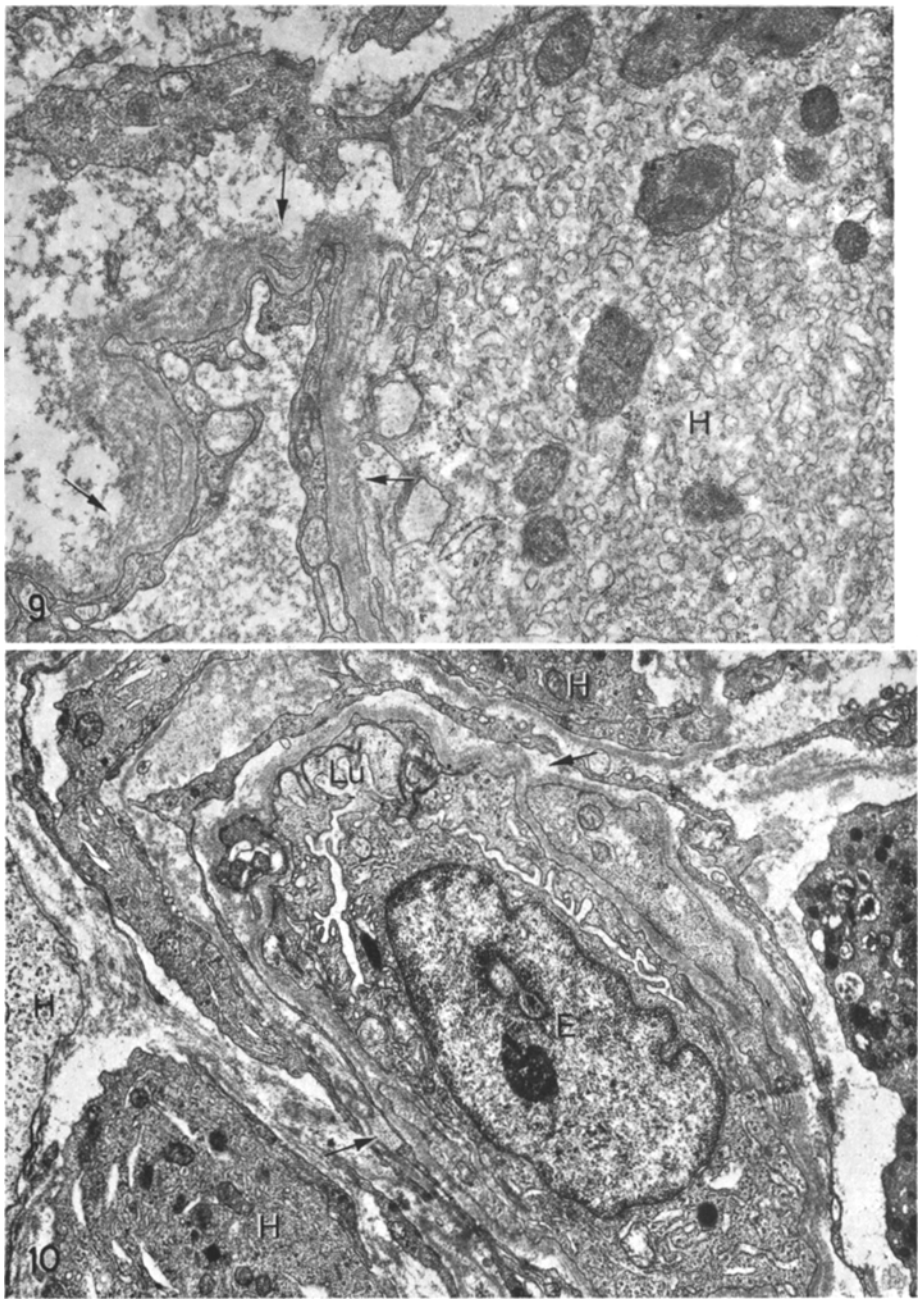

Fig. 9. 27-year-old female patient with focal nodular hyperplasia. In the liver cell *(H)* there is an accumulation of smooth endoplasmic reticulum and alteration of mitochondria. In the site of the sinusoid, continuous capillaries can be seen (× 6,250)

Fig. 10. Picture of another region of the previous case. In between the liver cells *(H)*, there is a capillary with narrow lumen *(Lu)*. The nucleus of the endothelial cell *(E)* lining the capillary is large, its cytoplasm is wide and rich in organelles. Around the capillaries there is a basement membrane of varying width, in some places in several layers *(arrows)* (× 5,000)

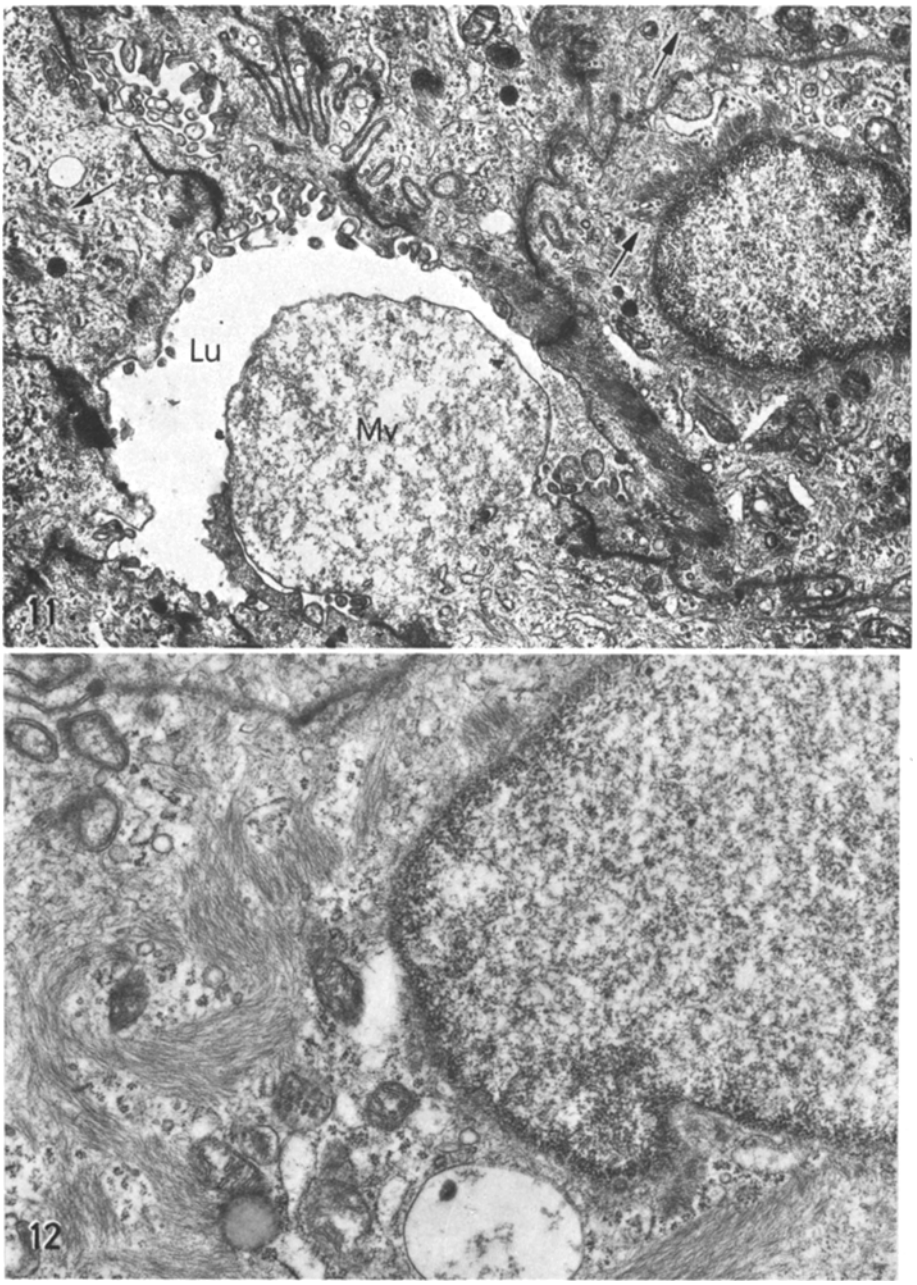

Fig. 11. 27-year-old female with focal nodular hyperplasia. Detail of a bile ductule. Part of the lumen *(Lu)* is filled by an oedematous microvillus *(Mv)*. The cytoplasm of the epithelial cell of the bile duct is rich in organelles, containing several microfilaments (× 12,500)

Fig. 12. Another region of the previous case. Microfilaments in plexiform arrangement in the epithelial cells of the bile duct (× 20,000)

Discussion

Electron-microscopic studies on the reaction of liver cells to oral contraceptives revealed that ultrastructural changes are more sensitive indicators of the lesion than liver function tests (Feldmann, 1976; Larsson-Cohn et al., 1967; Perez et al., 1977; Stauffer et al., 1976). Larsson-Cohn and Stenram (1967) have emphasized that in testing drugs electron-microscopic examination of human liver tissue should be performed routinely.

The main finding of our comparative electron-microscopic studies has been that in cases with tumors or jaundice, ultrastructural changes in the liver cells, sinusoidal cells and epithelial cells of the bile duct, were, in most respects, the same both qualitatively and quantitatively.

In the lumen of the bile canaliculi and in Disse's space, in both groups, there was an accumulation of filamentous substance. The canalicular and vascular cell membranes were seriously damaged, the lesion being most marked where filamentous substance was most abundant. On the basis of a static electron-microscopic picture, we cannot judged whether a material damaging the cell membrane is being formed, whether the damaged cell membrane is discharged into the bile ducts or into the vascular surface of the cell, following injury to the cell membrane. A lesion of the plasma membrane was observed by Feldmann (1976) in cases of jaundice related to oral contraceptives. According to him direct toxic effects of the drugs are responsible for the lesion of the membrane.

Histochemical studies have shown that protein formation is liable to change in the liver cells of women on oral contraceptives (Adlercreutz et al., 1970; Palmer et al., 1977; Porte et al., 1977). This perhaps accounts for the cumulative effect of the drug on liver cells and the fact that after prolonged administration, liver lesions occur more frequently (Perez et al., 1969), or that the liver disease is manifest following a long while after stopping of the drug (Roe, 1976; Stauffer et al., 1976). The pathological changes in liver cells in their protein forming and storing capacity can be observed in different clinical pictures, most frequently in alcoholic liver lesions, (Mallory's hyalin: Rumpelt, 1977; Sim et al., 1977) and in cases of liver tumour (Enat et al., 1973; Norkin, 1968). Most recently, accumulation of a filamentous substance similar to Mallory's hyalin in the liver cells was observed during prolonged corticoid treatment (Itoh et al., 1977). In our own cases, the filamentous substance was chiefly observed extracellularly, but its ultrastructure resembled the pathological proteins described in the above reports.

In the pericanalicular zone and in the cytoplasm of the ductular epithelial cells, there was an accumulation of microfilaments in cases with tumours and in those with jaundice. According to recent studies, microfilaments are contractile elements, containing actin, maintaining the tone of the wall of bile ducts and promoting the secretion of bile through their contraction (Badaruddin et al., 1976; Feldmann et al., 1975; French et al., 1975; Phillips et al., 1975). In cases with cholestasis, the accumulation of microfilaments is always conspicuous (Balázs et al., 1975; Lapis et al., 1977; Popper et al., 1970) but in our tumour cases there was also a striking accumulation of microfilaments without light-microscopic cholestasis.

The polymorphism of the mitochondria and the formation of paracrystalloid inclusions is a very frequent, non-specific change occuring in pregnancy, alcoholic liver disease, essential hyperbilirubinaemia, cholestasis, etc. (Balázs et al., 1975, 1977; Lapis et al., 1977; Popper et al., 1970). The phenomenon are considered to be a morphological sign of liver cell adaptation, representing an increased metabolic activity. Paracrystalloid inclusions are not observed in hepatocellular carcinoma (Schaff et al., 1971). Paracrystalloid inclusions were very frequent in the tumourous liver cells in our own 6 cases. The phenomenon indicates that tumorous liver cells also participate in the increased metabolic activity of the whole liver tissue. Similar mitochondrial inclusions were observed by Horvath et al. (1972) in benign hepatocellular adenoma induced by oral contraceptives. It seems a special reaction which is characteristic of liver tumours due to oral contraceptives.

In some places, the double membrane of the mitochondria was partially or totally absent and the matrix of the mitochondria joined with the hyaloplasm. Damage to the membrane of the mitochondria was also observed by other authors in liver changes associated with oral contraceptive ingestion (Feldmann, 1976; Perez et al., 1969).

There is only one essential difference in the structure of the sinusoidal wall, between tumor and non-tumor cases. It is known that normal sinusoidal endothelial cells are loosely connected to each other and do not contain cell binding structures or surrounding basement membrane (Balázs et al., 1977). In our jaundiced cases, hyperplasia of sinusoidal cells was conspicuous, with enlargement of nuclei and widening of the cytoplasms. The many vesicles and vacuoles seen were suggestive of an increased resorptive activity, while the large amounts of rough endoplasmic reticulum and free ribosomal granules, suggested an increased protein secretion. Formation of basement membrane was seen all round endothelial cells. Experimental studies have shown that protein forming activity of endothelial cells increases following an oestrogenic stimulus (Stauffer et al., 1976; Widmann et al., 1976) and it is reasonable to that the basement membrane around the endothelial cells is a product of enhanced secretion of the cells.

As in our previous investigations, at the site of the sinusoids continuous capillaries were found in the tumours (Balázs, 1976; Balázs et al., 1978). In between the endothelial cells cell binding structures were formed, the capillaries being surrounded by a continuous and in some places, multi-layered basement membrane. In other regions pericytes were observed.

Further investigations are neede to judge whether the changed microcirculation due to abnormal capillaries plays a role in the formation of the liver tumour resembling a circumscribed cirrhosis or the tumorous proliferation of the capillaries and liver cells are induced by the same noxa.

In conclusion the common features of the tumor bearing and icteric liver tissue are as follows:

1. Accumulation of filamentous substance of unknown origin in the bile canaliculi and in Disse's spaces.

2. Damage to the cell membrane on the vascular and biliary surface of the liver cells.

3. Accumulation of microfilaments in the pericanalicular zone of the liver cells and in the epithelial cells of the bile duct.

4. Polymorphism of the mitochondria, paracrystalloid inclusions and damaged external membrane of the mitochondria.

5. Hyperplasia of the sinusoidal endothelial cells with increased protein production.

6. Abnormal basement membrane fromation around the endothelial cells.

The important difference between the tumor and non-tumor liver tissue is the formation of continuous capillaries at the site of sinusoids.

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