Virchows Arch [Pathol Anat] (1982) 396:155–166

Virchows Archiv A

Myofibroblasts in Focal Nodular Hyperplasia of the Liver

Francesco Callea, Jos Mebis, and Valeer J. Desmet

Laboratorium voor Histochemie en Cytochemie, Departement Medische Navorsing, Katholieke Universiteit Leuven, Minderbroedersstraat 12, B-3000 Leuven, Belgium

Summary. Myofibroblasts have been identified by electron microscopy in a case of Focal Nodular Hyperplasia (FNH) of the liver associated with oral contraceptives.

The contractile fibroblasts were observed in the immediate vicinity of, or in close contact with, the proliferating bile ductules and in the recesses between parenchymal cells.

Transitional forms between fat-storing cells (Ito cells), fibroblasts and myofribroblasts were observed.

Transition of fat-storing cells to myofibroblasts, possibly under the influence of oestrogens, may be responsible for the fibrosis and retraction in FNH.

Key words: Myofibroblast – Fat storing cell – Liver fibrosis – Focal nodular hyperplasia – Oestrogens – ultrastructure

The myofibroblast (MF) is a cell with contractile properties, resembling the fibroblast and smooth muscle cell structurally and functionally (Majno 1971).

Originally described as the principal cellular component in granulation tissue of healing wounds (Gabbiani et al. 1971), the MF has been found in various pathological conditions (Seemayer et al. 1980). Although this cell is not found in the normal liver (Seemayer et al. 1980), it has been described in both human (Bathal 1972; Rudolph et al. 1979) and experimental (Irle et al. 1980) cirrhosis.

The purpose of this paper is to document the finding of contractile fibroblasts and to discuss their possible origin in a further pathological process: Focal Nodular Hyperplasia (FNH) of the liver.

Material and Methods

A 31 year old female patient underwent laparatomy for a hypochondrial mass. She had been taking oral contraceptives for one year. The mass was located in the right lobe of the liver and

Offprint requests to: F. Callea at the above address

0340-1227/82/0396/0155/$02.40

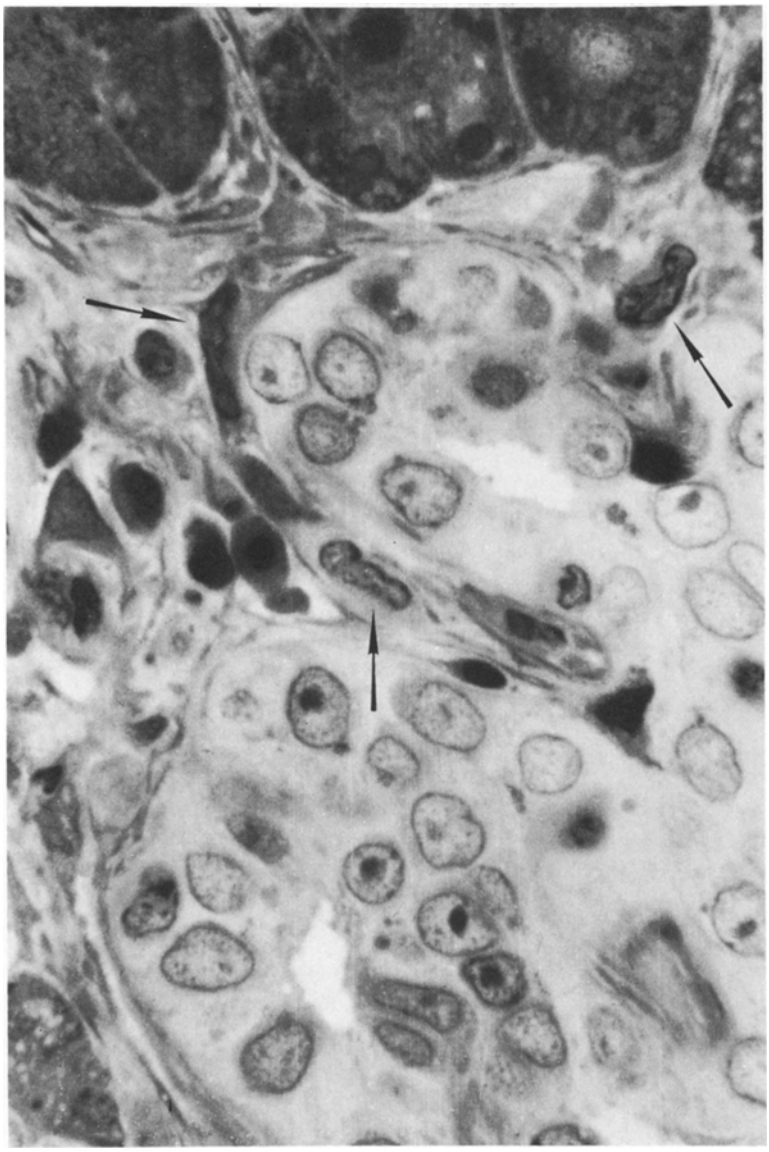

Fig. 1. Proliferating bile ductules. Several elongated cells with indented nuclei are lying in close contact (arrows). Semithin section. Methylen blue. × 1,550

excision of the tumor was performed. Some distance from it, a subcapsular haemangioma was discovered and resected.

The patient is well after 16 months follow-up.

Resection specimens of the tumor were fixed in Bouin's fluid and 10% formalin and paraffin embedded. Conventional 4 micron sections were stained with HE, PAS with and without diastase digestion, Van Gieson, Masson's trichrome, Laidlaw's reticulin.

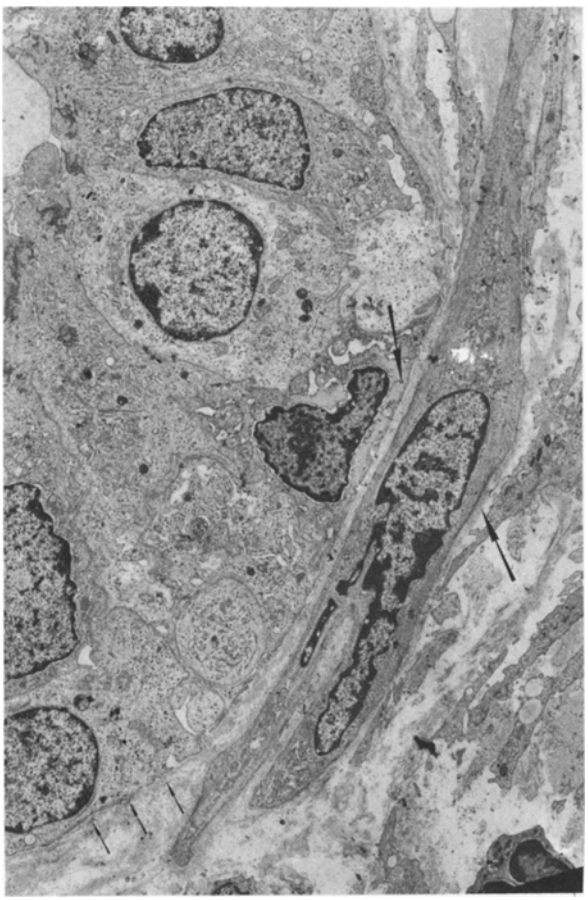

Fig. 2. Electronmicrophotograph showing a myofibroblast in contact with a bile duct. The cell has long cytoplasmic extensions and a prominent microfilamentous system. The endoplasmic reticulum is rather scanty. Note also cytoplasmic processes of other cells with similar features, well developed RER and some lipid inclusions. *Arrows* point to the basement membranes between the bile duct and the myofibroblast and along the opposite surface of the myofibroblast. × 5,500

Several 1 mm pieces were immediately fixed for 1 h in 2.5% glutaraldehyde, 0.1 M phosphate buffer pH 7.2, followed by phosphate buffer rinse overnight.

These specimens were postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated in graded alcohol solutions and embedded in Epon 812. Semithin and ultrathin sections were cut with a Reichert Ultramicrotome OMU2. Semithin sections were stained with methylen blue. Ultrathin sections were stained with uranyl acetate and 1% lead citrate and examined in a Zeiss EM 10 electronmicroscope.

Results

Gross Macroscopy

The tumor mass (10 cm diameter), grey in colour, was very well demarcated, although not encapsulated.

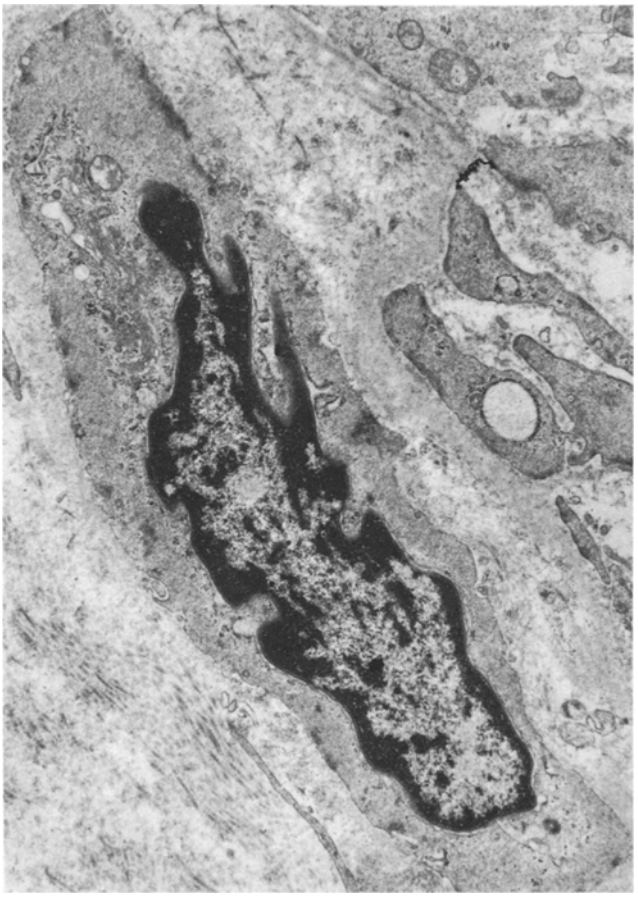

Fig. 3. Myofibroblast showing an indented nucleus with nuclear bodies, abundant cytoplasmic microfilaments, subplasmalemmal densities. The endoplasmic reticulum is scanty, the Golgi apparatus prominent. ×17,000

A central scar was present. Radiating septa divided the periphery of the mass in to multiple, variable sized nodules, simulating a pattern of focal cirrhosis. No areas of haemorrage or infarction were observed. The haemangioma specimen showed multiple cyst-like cavities filled with blood.

Light Microscopy

The bosselated and lobulated appearence was due to areas of fibrosis, which was most marked centrally.

Vascular changes similar to those described by Mays et al. (Mays et al. 1974) and bile ductular proliferation analogous to that described by others (Knowles and Wolff 1976) were seen.

Single small bile ductules were frequently observed within parenchymal nodules, without any relation to the intranodular scars. Cells with elongated, cross

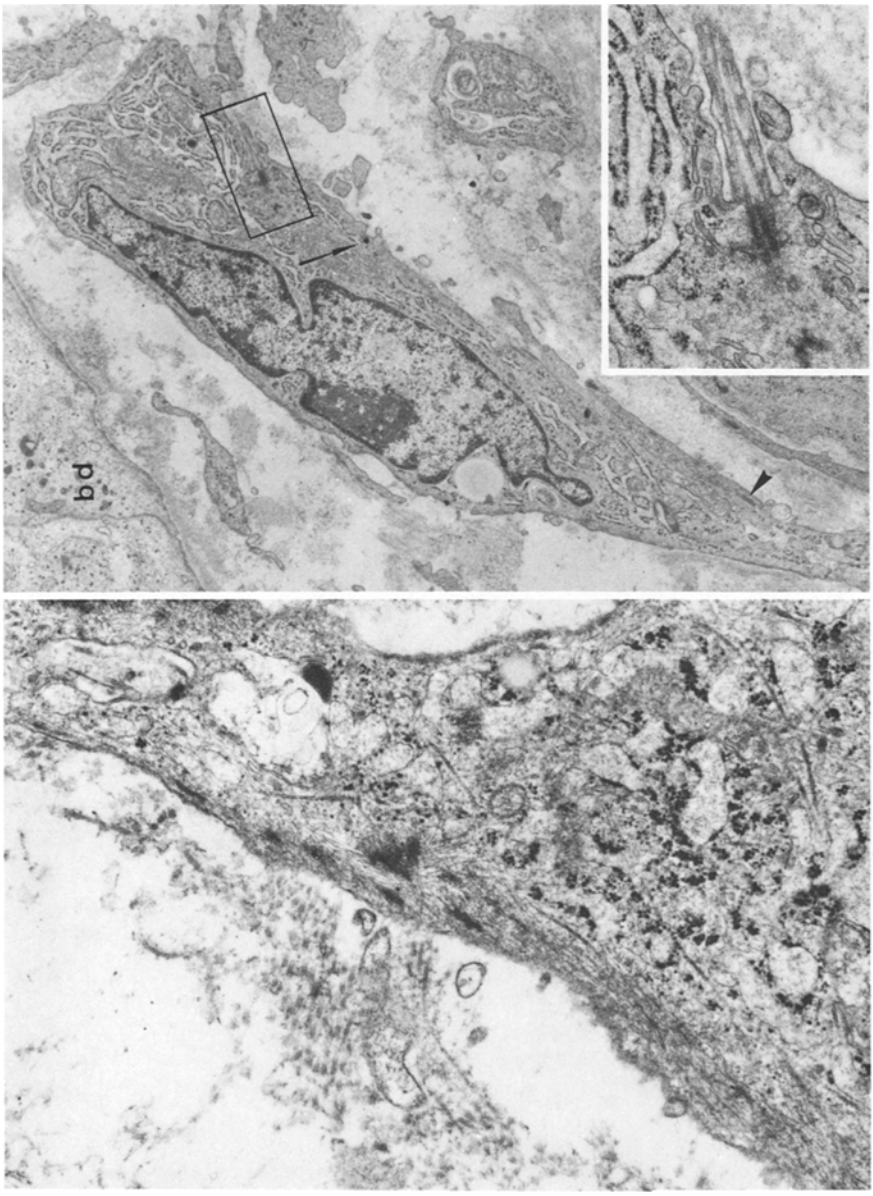

Fig. 4. Cytoplasmic extension of a myofibroblast showing microtubules and dense bodies distributed along bundles of microfilaments. ×37,000

Fig. 5. Cell with indented nucleus and nuclear bodies, well developed RER and Golgi apparatus. A single cilium emerges into the extracellular space (*Inset*). *Arrow* points to an early cilium formation, *arrowhead* points to a desmosome between this cell and the cytoplasmic process of another cell. A lipid inclusion and a few subplasmalemmal densities are also shown. A bile duct (*bd*) is present to the bottom left. ×10,000. *Inset* ×37,000

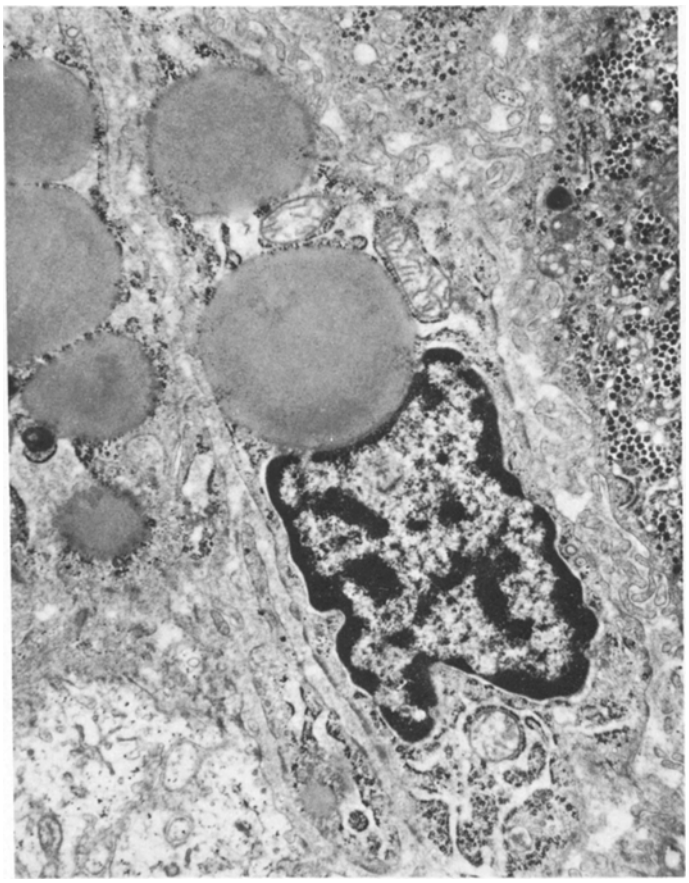

Fig. 6. Electron microphotograph of two typical fat-storing cells with large lipid inclusions and a well developed RER. ×16,000

banded nuclei were in close vicinity of the newly formed ductules. Inflammatory and fibroblast-like cells were seen in the fibrous connective tissue septa.

On semithin sections, cells with crenated nuclei, isolated or in indian file, were lying in close contact with the proliferating bile ductules even when the latter were merging with the liver cell plates (Fig. 1).

The second lesion showed the typical features of a cavernous haemangioma.

Electron Microscopy

Typical fibroblasts were identified by their elongated or star-like shape, the slender fusiform shape and relatively smooth outline of the nucleus, the well developed RER and Golgi apparatus, the scattered mitochondria and the absence or scanty presence of cytofilaments.

A large number of elongated cells exibited the characteristic morphology of modified contractile fibroblasts (MF). They showed a variable amount of

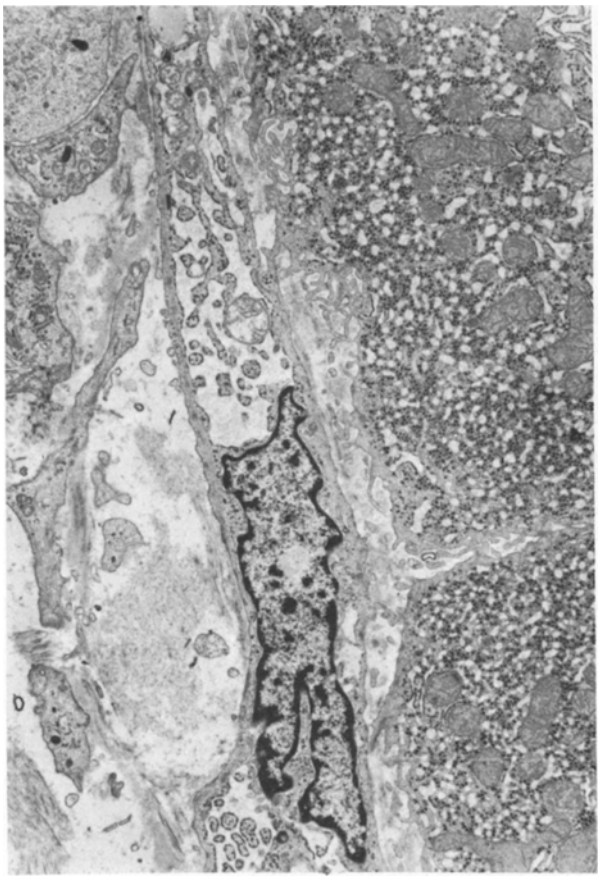

Fig. 7. A fat-storing cell transforming into fibroblast. Note a single residual lipid inclusion. The cisternae of RER are dilated and contain a fluffy material. Discontinuous basement membrane-like material is present in the Disse space. × 10,000

intracytoplasmic microfilaments, arranged in longitudinal bundles, usually parallel to the long axis of the cell (Fig. 2, 3 and 4) predominantly located beneath the plasma membrane. Among the bundles of microfilaments, numerous electron-dense bodies and attachment sites (Rhodin 1962) were present (Fig. 4). Many of these structures were located just beneath the plasmamembrane, and together with subplasmalemmal linear densities resulted in hemidesmosome-like complexes. A striking microtubular system was observed in some of these cells. The microtubules ran parallel to the filament bundles or crossed the cell at long angles (Fig. 4). The remaining cytoplasm contained packed cisternae of RER, Golgi apparatus and a few mitochondria. The nuclei showed multiple indentations and rod-like structures.

Intercellular connections between modified fibroblasts were frequently observed; they were mostly of the macula-adhaerens type.

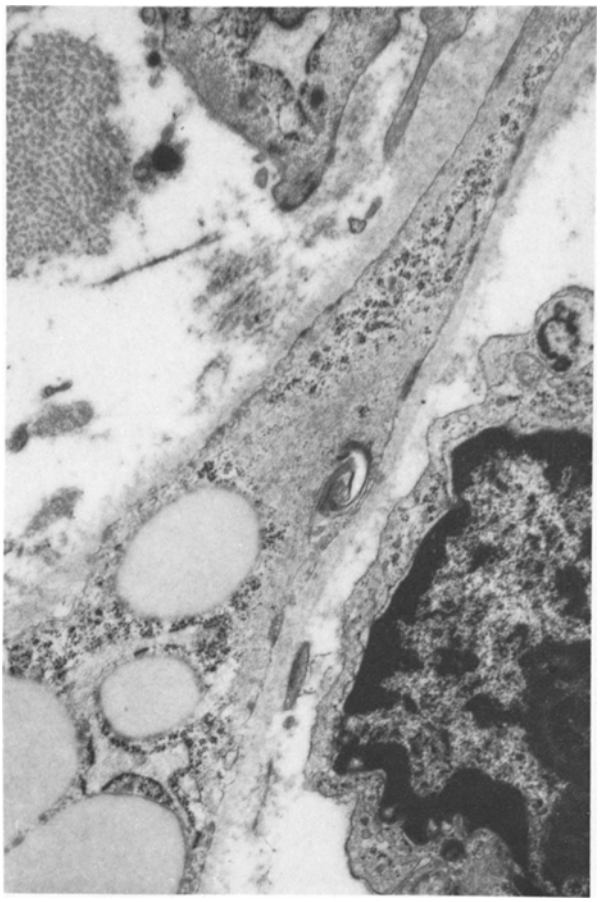

Fig. 8. High power of a cytoplasmic extension with large lipid inclusions and RER, characteristic of the Ito cell. A microfilamentous system runs parallel to the cell process, and subplasmalemmal densities appear. A basement membrane-like material is present all around the cell process. × 29,000

Basement membrane-like material of amorphous or finely fibrillar type (Figs. 2 and 3), at times contiguous with dense collagen fibres was observed in close proximity to these cell types.

Cells were found with well developed cilia emerging into the extracellular space, very close to the newly formed ductules (Fig. 5). These cells showed indented nuclei with a prominent nucleolus and nuclear bodies. The cytoplasm contained a well developed RER, numerous Golgi complexes and a few lipid inclusions. Desmosomes were seen between these cells and the cytoplasmic extensions of other cells (Fig. 5).

Typical fat-storing cells occurred in recesses between parenchymal cells; they were identified on the base of the intracytoplasmic fat droplets (Fig. 6).

Most perisinusoidal cells showed a large, very bizarre cell body with enormous cytoplasmic extensions, reduction or disappearence of fat droplets together with an increase of RER.

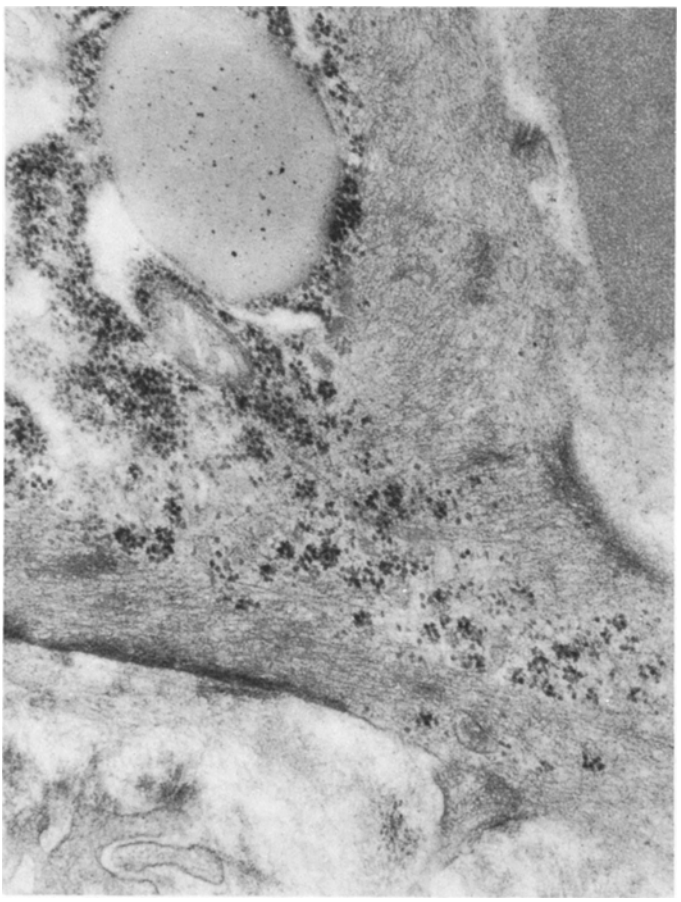

Fig. 9. Detail of a fat-storing cell-myofibroblast. A lipid inclusion is still present. Dense areas are scattered among a pronounced microfilamentous system and attached to the plasma membrane. × 50,000

The endoplasmic reticulum cisternae were usually dilated and contained fluffy material (Fig. 7). Golgi complexes were prominent. Centrioles were frequently seen migrating towards the cell periphery, forming a basal body with or without early cilium formation.

Many of these transitional fat storing-fibroblast cells showed further cytoplasmic changes such as appearence of microfilaments running parallel to the long axis of the cell processes or the cell body, dense bodies and subplasmalemmal densities (Figs. 8 and 9). These changes occurred either at one pole of the cell or throughout the whole cytoplasm. Moreover, basement membrane-like material was seen all around the cell periphery (Fig. 9).

An increase in collagen bundles was a common finding in the Disse spaces. Among the inflammatory cells, eosinophils, monocytes, and lymphocytes were identified. Interdigitations and contacts between lymphocytes and fibroblasts were occasionally noted.

Discussion

The tumor-like lesion of this patient presents all characteristics of FNH of the liver. This is a distinct pathologic entity, usually presenting as a focal cirrhotic-like mass in an otherwise unremarkable liver (Knowles and Wolff 1976). Macroscopically, a central stellate scar area is highly characteristic. On histological and electron microscopic examination, peculiar bile ductular proliferation (Knowles and Wolff 1976) and vascular changes (Mays et al. 1974; Lough et al. 1980) are observed. Many cases have been reported in association with oral contraceptives. The myofibroblast, a unique cell with contractile properties, is not a normal constituent of the liver. However, it has been detected in the scar tissue of both human (Bathal 1972; Rudolph et al. 1979) and experimental (Irle et al. 1980) cirrhosis. On EM examination, the FNH lesion contained typical myofibroblasts. These cells were identified by the presence of a well developed intracytoplasmic microfilamentous system with typical dense bodies and attachment sites, surface differentiation with cell-cell and cell-stroma connections, indented nucleus, nuclear bodies and microtubules. The latter structures have recently been included as a prominent feature of the myofibroblast (Rudolph and Woodward 1978) and considered to be mediators of the contractile force.

The spatial orientation of MF in FNH is highly suggestive of a bracing or scaffolding function. MF's were mainly observed in the immediate vicinity of or in close contact with proliferating bile ductules, which are mostly observed in parenchymal regions. Moreover, transitional forms from typical fat-storing cells to fibroblasts and myofibroblasts were seen in the perisinusoidal spaces.

The finding of contractile fibroblasts raises questions on the origin and the significance of such cells in FNH. Since MF's are similar to both fibroblasts and smooth muscle cells, it seemed reasonable to conclude that both cells, under appropriate conditions, may become MF (Seemayer et al. 1981). However, the presence of transitional forms between fat-storing cells, fibroblasts and myofibroblasts suggest that the fat-storing cells of Ito may also be a progenitor of contractile fibroblasts. Fat-storing cells have already been shown to transform to fibroblasts and have been postulated to play a role in hepatic fibrosis (Kent et al. 1976). The transformation of Ito cells into contractile fibroblasts may be an alternative pathway in hepatic fibrogenesis, since MFs are able to synthetise collagen (Gabbiani et al. 1976). Transition of resting fat-storing cells into collagen synthetising myofibroblasts has been postulated as a general pathogenetic mechanism in all forms of liver fibrosis (Hahn et al. 1980).

Ciliated cells represent a further finding of interest in this case of FNH. The majority of these cells showed ultrastructural features of fibroblasts. However, the presence of intracytoplasmic lipid droplets is suggestive of a possible derivation from fat-storing cells. A single cilium projecting into the sinusoidal lumen has been described in fat-storing cells (Wisse 1977; Tanuma and Ito 1980). Formation of rudimentary cilia has been reported in fibroblasts and in smooth muscle cells (Sorokin 1962). Moreover, fibroblasts in culture, when treated with Cytochalasin B, develop desmosomes, microfilaments and also cilia (Ghadially 1975). Analogous ciliated cells have been observed in cellular leio-

myoma of the uterus and interpreted as recapitaluation of the early stages of myogenesis (Ferenczy 1971). However, since single cilia may be observed in a large variety of cell types (Ghadially 1975) without apparent relation to cellular movement (Sorokin 1962), the significance of this structure remains unknown, although it definitely indicates a special functional state of the cell.

The factors capable of inducing MF modulation in general remain unknown. Probably they differ in the various pathological conditions in which these cells appear. In right-sided endocardial fibrous plaques characteristic of the carcinoid syndrome, a relationship between MF and hormonal stimulation has been proposed (Muller and Siebenmann 1981). Likewise, an oestrogenic influence may be postulated for the occurrence of MF in the present FNH case associated with contraceptives. MF induction has been observed in the oestrogen-stimulated rat uterus (Ross and Klebanoff 1967). Moreover, oestrogens have been regarded as an important pathogenetic factor in the vascular changes in FNH (Lough et al. 1980). The perspective of a unifying factor (oestrogens) responsible for both MF induction and vascular lesions in FNH is attractive, although speculative at the present time.

MF induction is probably a basic phenomenon apt to occur in many tissues, regardless of the aetiological agent or the species. The mechanism and its possible mediators are unknown. In conclusion, FNH of the liver represent a further condition in which MF induction can be observed. In FNH, fat-storing cells are the apparent precursors of MF, which, in turn, by their contractile and collagen synthetising capacities, play a role in producing the retracted scarlike and nodular appearence of FNH.

Acknowledgment. The authors thank Mrs. L. Vanrykel and Mrs. Y. Piccart for technical assistance, Mr. M. Roseleers for preparing photographs.

References

Bathal PS (1971) Presence of modified fibroblasts in cirrhotic livers in man. Pathology 4:139–144

Ferenczy A, Richart RM, Okagaki T (1971) A comparative ultrastructural study of leiomyosarcoma, cellular leiomyoma and leiomyoma of the uterus. Cancer 28:1004–1018

Gabbiani G, Ryan GB, Majno G (1971) Presence of modified fobroblasts in granulation tissue and their possible role in wound contraction. Experientia 27:649

Gabbiani G, Le Lous M, Bailey AJ, Bazin S, Delauney R (1976) Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural and immunologic study. Virchows Arch [Cell Pathol] 21:133–145

Ghadially FN (1975) Ultrastructural pathology of the cell. Butterworths, London and Boston 516–518

Hanh EG, Ott U, Martini GA (1980) Die Leberfibrose. Z Gastroenterologie 18:453–469 and 507–523

Irle C, Kocker O, Gabbiani G (1980) Contractility of myofibroblasts during experimental liver cirrhosis. J Submicr Cytol 12:209–217

Kent G, Gay S, Inouye T, Bahu R, Minick OT, Popper H (1976) Vitamin A-containing lipocytes and formation of type III collagen in liver injury. Proc Natl Acad Sci USA 73:3719–3722

Knowles II DM, Wolff M (1976) Focal nodular hyperplasia of the liver. Hum Pathol 7:533–545

Lough J, Spicer P, Kinch R (1980) Focal nodular hyperplasia of the liver. An electron microscopic study of the vascular lesions. Hum Pathol 11:181–186

Majno G, Gabbiani G, Hirschel BY, Ryan GB, Statkov PR (1971) Contraction of granulation tissue in vitro. Science 173:548–550

Mays ET, Christopherson WM, Barrows GH (1974) Focal nodular hyperplasia of the liver. Possible relationship to oral contraceptives. Am J Clin Pathol 61:735–746

Muller HG, Siebenmann RE (1981) Ultrastructure of endocardial lesions in the carcinoid syndrome. Virchows Arch [Pathol Anat] 391:33–44

Rhodin JAG (1960) Fine structure of vascular walls in mammals. With special references to smooth muscle component. Physiol Rev 42 (Suppl 5) 48–81

Ross R, Klebanoff SJ (1967) Fine structural changes in uterine smooth muscle and fibroblasts in response to estrogens. J Cell Biol 32:155

Rudolph R, Woodward M (1978) Spatial orientation of microtubules in contractile fibroblasts in vivo. Anat Rec 191:169–182

Rudolph R, McClure WJ, Woodward M (1979) Contractile fibroblasts in chronic alcoholic cirrhosis. Gastroenterology 76:704–709

Seemayer TA, Lagacé R, Schurch W, Thelmo WL (1980) The myofibroblast. Pathol Ann 443–470

Seemayer TA, Scurch W, Lagacé R (1981) Myofibroblasts in human pathology. Hum Pathol 6:491–492

Sorokin S (1962) Centrioles and formation of rudimentary cilia by fibroblasts and smooth muscle cells. J Cell Biol 15:363–377

Tanuma Y, Ito T (1980) Electron microscopic study on the sinusoidal wall of the liver of the Crucian, *Carassius carassius*, with special remarks on the fat-storing cells (FSC). Arch Histol Jpn 43:241–263

Wisse E (1977) Ultrastructure and function of Kupffer cells and other sinusoidal cells. Elsevier/North-Holland Biochemical Press, Amsterdam pp 33–60

Accepted March 23, 1982