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The Ultrastructure of Primary Hepatocellular Cancer in Man

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Summary. The authors carried out an electronmicroscopical study of material obtained by needle biopsy from 12 human hepatocellular carcinomas. Pleomorphism of the cell nuclei and the occurrence in great numbers of cytoplasmic infoldings into the nuclei were striking features. The nucleoli were enlarged and nucleoli of a peculiar target-like form were observed. The endoplasmic reticulum had a vesicularized, fragmented appearance and the membranes were degranulated. The frequent occurrence of fingerprint-like formations of endoplasmic reticulum were also observed. The cristae of the mitochondria were diminished and other degenerative changes were also regularly present. In one case peculiar electron-dense inclusions were seen in the mitochondria. The amount of glycogen granules in the tumour cells seemed to be considerably diminished. No significantly great changes were seen in the Golgi complex. Bile canaliculi as well as desmosomes were regularly found: usually as many as five or six participated in the formation of the canaliculi. Changes were seen also in the microvilli of the biliary and vascular poles.

The relation of the tumour cells to the sinusoids proved in many respects to be similar to that in normal liver. It was a frequent finding, however, that the tumour cells formed plates consisting of several cell rows; furthermore, that a basement membrane had developed around the sinusoids, which thus became capillarized, while the sinusoid endothelium became multilayered and the Disse space filled by a fibrin-like substance.

The authors compare the alterations observed in human hepatocellular carcinomas with the ultrastructural alterations found in other pathological conditions of the liver and in experimental liver tumours. Based on this comparison they offer conclusions concerning the functional cytopathological significance of the various alterations on the one hand, and the possible pathogenesis of hepatic tumours on the other.

The ultrastructure of tumours of the liver experimentally induced by various carcinogens has been studied extensively (Driessens *et al.*, 1959; Fawcett and Wilson, 1955; Kendrey, 1965; Kendrey, 1968; Leduc and Wilson, 1959; Rouiller, 1964; Salomon, 1962; Thoenes and Bannasch, 1962; etc.).

Investigations on the ultrastructure of primary liver tumours in man, however, have been few (Creemers and Jadin, 1968; Ghadially and Parry, 1966; Ito, 1969; Ma and Blackburn, 1966; Ruebner *et al.*, 1967; Tanikawa, 1968; Theron and Mekel, 1964; Theron *et al.*, 1962; Toker and Trevino, 1966). Our observations are recorded in this paper.

One of the aims of our investigations was to compare the electron microscopical alterations observed in human hepatomas with the published accounts of the ultrastructure of experimental hepatomas induced in various ways. As the pathogenesis of experimental hepatomas has been clarified more extensively than that of hepatic tumours in man, we hoped that such a comparison would provide information on the pathogenesis and morphogenesis of human liver carcinoma.

Further, as there are some contradictions between different workers' published observations on the ultrastructure of human hepatomas, we hoped to be able to clarify these, at least in part, by investigation of a larger number of cases.

Finally, as it is often difficult with the light microscope to distinguish between primary and secondary tumours in material obtained from the liver by needle biopsy, we studied the question whether electron microscopic investigations could help in differential diagnosis in this field.

Material and Methods

Among almost one thousand liver biopsy specimens received in our institute in the course of four years, 43 proved cases of primary liver cancer were found. This diagnosis was confirmed by operation in 4 cases and at post mortem in 39 cases. We have studied 14 cases electron microscopically: in 12 of these the tumour proved to be a hepatocellular carcinoma and in two a cholangiocellular carcinoma. In the following account it is with the 12 hepatocellular carcinomas only that we are concerned.

The liver biopsy specimens were obtained with the Menghini needle. The specimens for light microscopy were fixed in 4% formaldehyde and embedded in paraffin. Sections, 5–7 μ thick, were stained with haematoxylin and eosin and by Mallory's method. The periodic-acid Schiff reaction, with and without prior digestion by diastase, and the Gomori technique were also used in all cases.

Specimens for electron microscopy were fixed in 2% osmium tetroxide buffered with Veronal acetate to 7.4 at 4° centigrade for one hour. Following dehydration in alcohol the specimens were embedded in Araldite. Ultra-thin sections were cut on a LKB ultramicrotome, stained with uranylacetate and by the method of Karnovsky (1961), and examined with a SEM 3–1 electron microscope at an accelerating voltage of 60–80 kW.

Results

Light Microscopy. Of the 12 hepatocellular carcinomas, 6 were identified as trabecular and 6 as trabeculo-tubular. Hyperchromasia of the tumour cell nuclei and marked nuclear polymorphism were found. Nuclear inclusions were frequently present. The cytoplasm of the tumour cells was more basophilic than that of normal liver cells. Glycogen was demonstrated in the tumour cells by the PAS method. Evidence of bile production was also found in the tumour.

Electron Microscopy. There was much variation in the shape of the tumour cell nuclei, from rounded and oval shapes to extremely pleomorphic, bizarre configurations (Figs. 1, 2a, 4a, 5a). The surface of the nuclei appeared markedly scalloped and uneven. Cells with 2 or 3 nuclei were quite frequent. The nuclei were of moderate electron density, with chromatin mainly marginal.

Cytoplasm had formed indentations or inclusions in some nuclei. Most of the inclusions were surrounded by double membranes and contained various cytoplasmic organelles (endoplasmic reticulum, ribosomes, glycogen granules, lipid droplets, etc.) (Fig. 1). Other inclusions were not separated by a distinct membrane from the nuclear substance: most of these were seen to be lipid droplets (Fig. 1b).

The nucleoli also showed much variation. They were considerably larger than those of normal liver cells (Figs. 1b, 2, 8b). Two or even more nucleoli of various sizes were quite often seen in a single nucleus. In the substance of the nucleoli areas of lower electron density were observed and were identified as karyoplasmic

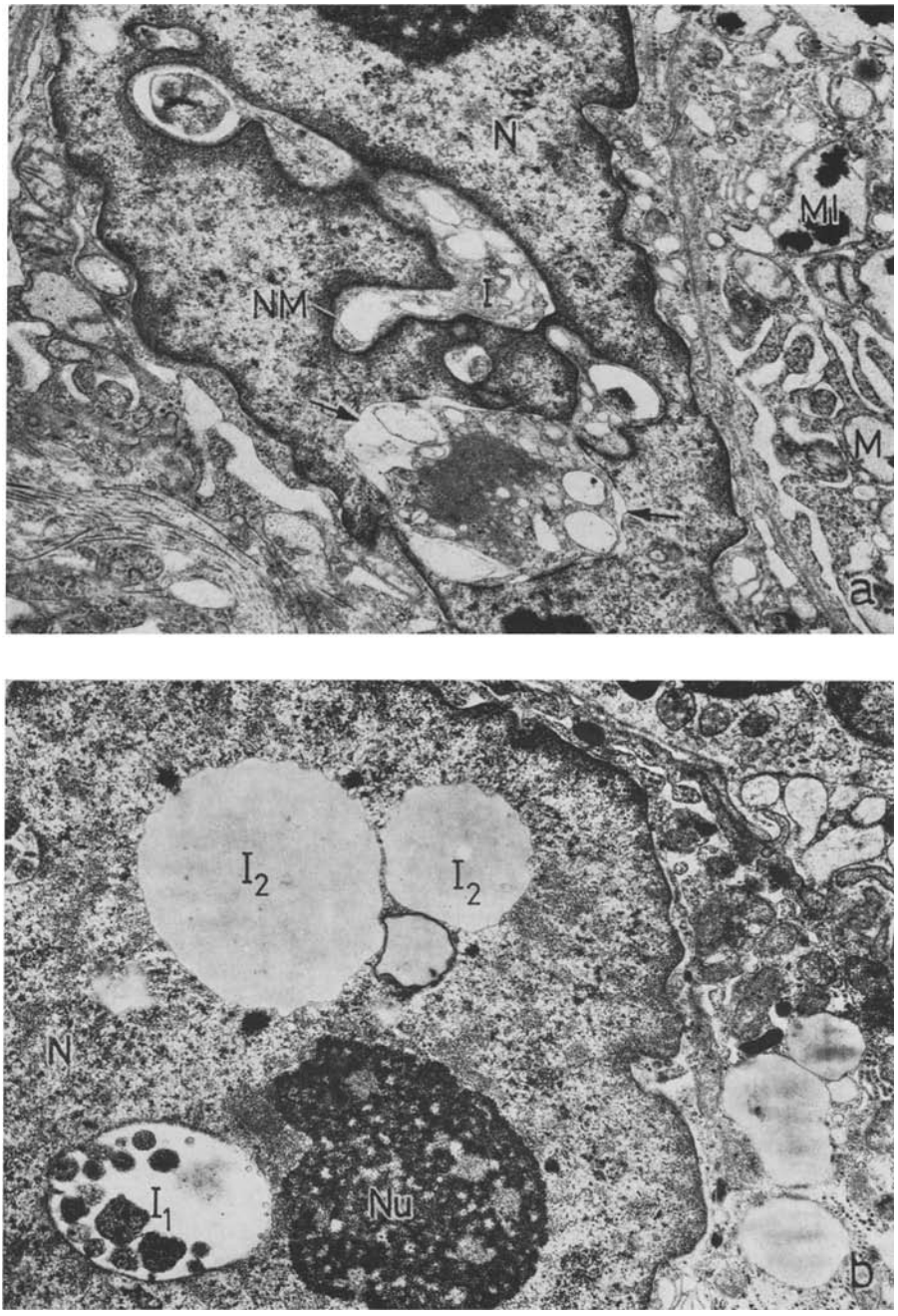

Fig. 1. a Irregular nucleus N of hepatoma cell with surrounding cytoplasm. The inclusion I contains partly degenerated cytoplasmic components, surrounded at places by a nuclear membrane NM. Elsewhere the structure of the nuclear membrane cannot be recognized →. In the mitochondria M homogenous, osmophilic inclusions MI may be seen. × 12200. b Irregular nucleus N of a hepatoma cell, with surrounding cytoplasm. Nucleolus Nu; inclusion within nuclear membrane I_1; inclusion not separated from nuclear substance by any membrane I_2. × 7500

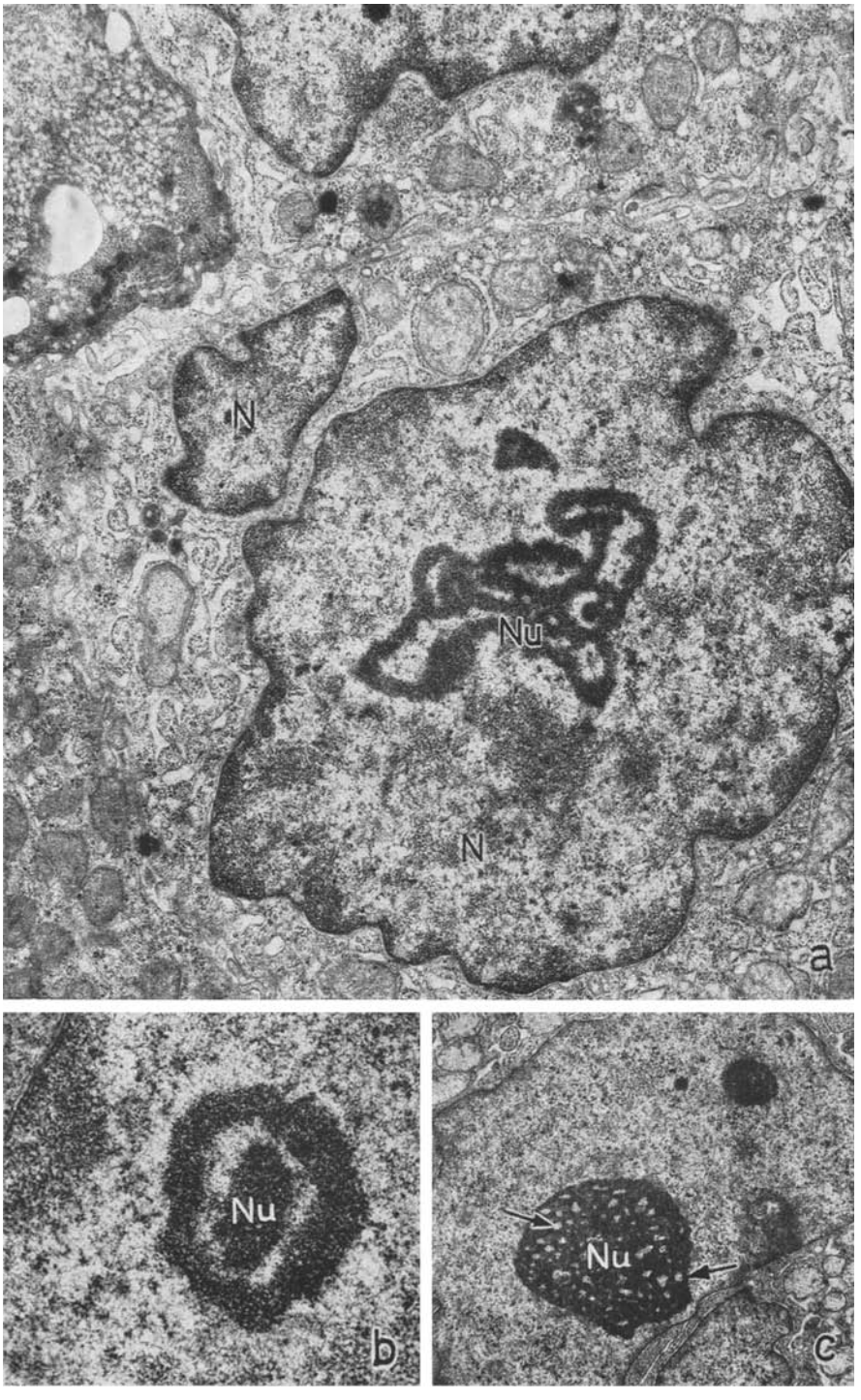

Fig. 2. a Lobulate irregularly shaped nucleus *N* of a tumour cell, with surrounding cytoplasm. The nucleolus *Nu* has a braided, woven appearance. × 8800. b Target-like nucleolus *Nu*. × 18000. c Fields of karyoplasm → in the nucleolus *Nu* of a hepatoma cell. × 3800

invaginations (Fig. 1b). Sometimes nucleoli with a strange target-form concentric structure were seen (Fig. 2b). In some instances the nucleolus had a loose, plait-like configuration, sharing a broad surface with the karyoplasm (Fig. 2c).

Among the cytoplasmic organelles it was the endoplasmic reticulum that showed the most striking alterations, having lost most of its highly organized, lamellar structure. The membranes of the rough-surfaced endoplasmic reticulum were often fragmented, degranulated and vesiculate (Fig. 3a). Free ribosomes were seen in varying amounts among the elements or vesicles of the disorganized endoplasmic reticulum. In many instances the endoplasmic reticulum formed unusually expanded, irregularly shaped cisternae, in the cavities of which there were papillary invaginations (Fig. 4a).

Besides the alterations described above, a rather frequent finding was of concentrically stratified lamellae of endoplasmic reticulum, arranged in peculiar, fingerprint-like formations (Fig. 3). These formations were sometimes found in adjacent cells and sometimes more than one in a single cell. The lamellae of these fingerprint-like formations were occasionally very close set. The cisternae often showed dilatations, reminiscent of pearls on a string. Ribosomes were rarely observed on the membranes displaying the fingerprint pattern. In the centre of the "fingerprint" formations cytoplasmic details, empty vacuoles, dense corpuscles, and occasionally heaps of glycogen granules were seen (Fig. 3). In some cells sequestration or separation of these "fingerprint" structures from the other parts of the cytoplasm was seen (Fig. 3c).

The Golgi-complex was usually well developed, being formed of sacculi and tiny vesicles (Fig. 5b).

The mitochondria presented many variations. In some of the tumour cells their number seemed to be increased and they were almost exclusively rounded, in other cells they were irregular (Fig. 5a). The number of cristae were usually diminished and most cristae were rudimentary and considerably shorter than usual. Occasionally the cristae formed parallel bundles, following the longitudinal axis in some of the mitochondria and running obliquely to it in others. Mitochondria with cristae of annular type were also frequent. In one case there were mitochondrial inclusions of a peculiar character: in this case mitochondria contained irregularly shaped crenulate inclusions, 0.2–0.3 microns in diameter, which consisted of an electron-dense, structureless material that sometimes almost completely filled the cavities of the mitochondria (Fig. 4). These inclusions could not be seen in unstained sections when electron diffraction was applied. The cristae of the mitochondria containing the inclusions were mostly disintegrated; only in places could remains of cristae be seen, near the inclusions (Fig. 4b).

In the cytoplasm of the tumour cells a number of "dense bodies" surrounded by a single membrane were observed. These occurred in large numbers, mainly in the vicinity of the biliary poles (Fig. 7a). Similar formations were also seen in the "fingerprint" patterns (Fig. 3a). In other areas irregularly shaped formations, surrounded by single membranes, reminiscent of lipofuscin, were seen; these are built up from light or dark globuli (Fig. 5a). In some places in the cytoplasm focal necrotic areas of varying extent were observed, sometimes occupying a

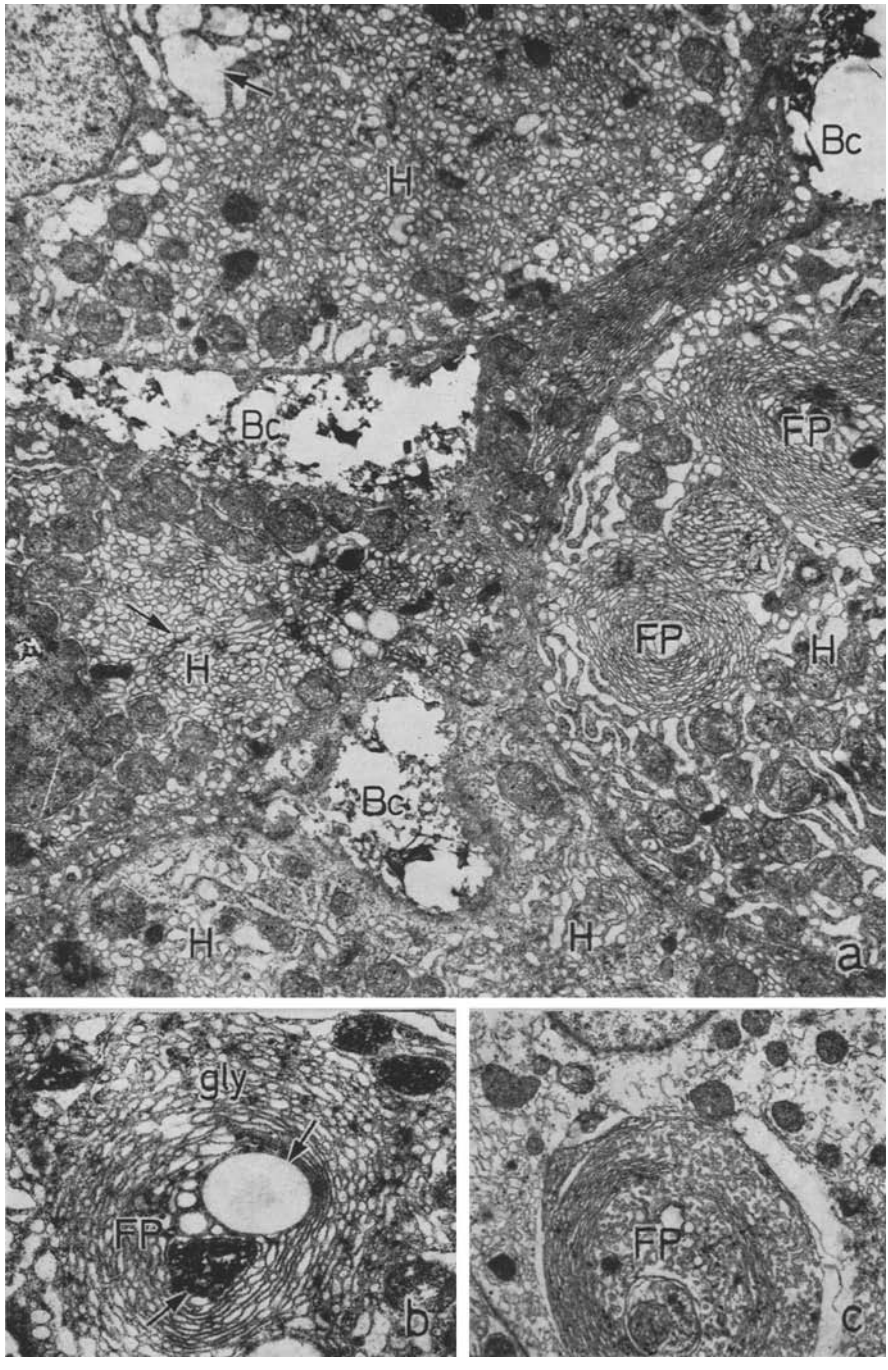

Fig. 3. a Detail of tumour cells *H*. Among them are dilated bile capillaries *BC* containing amorphous material. The endoplasmic reticulum is in some places vesicularized →; elsewhere it displays distended cisterns → or "fingerprint" formations. *FP*. × 9800. b Detail of a hepatoma cell. "Fingerprint" formation of endoplasmic reticulum *FP* with cytoplasmic components → at its centre are seen. Few glycogen granules *gly* are also present. × 12150. c Detail of a hepatoma cell. "Fingerprint" formations *FP* appears to be partly separated from the surrounding cytoplasm. × 5100

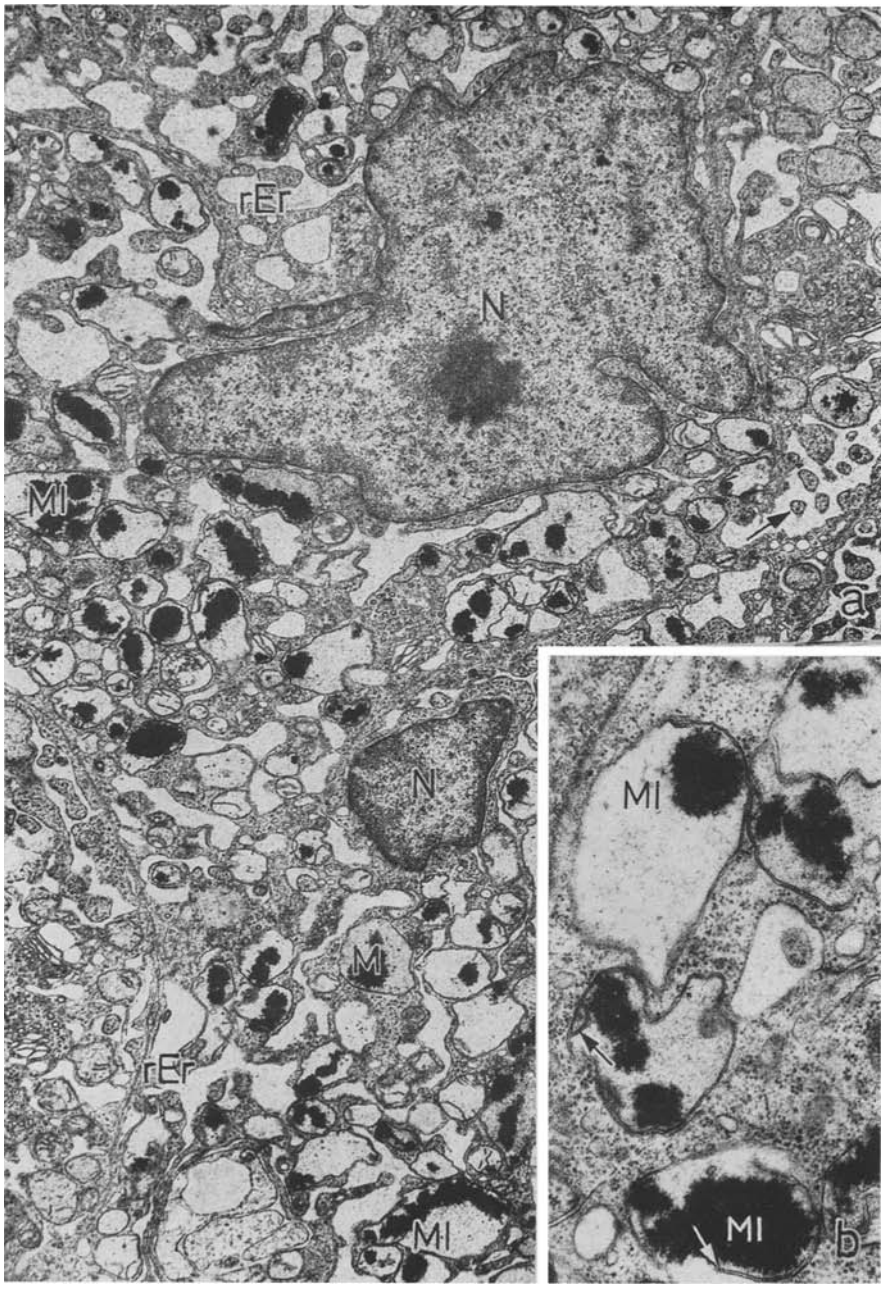

Fig. 4. a Detail of a hepatoma cell. Two nuclei *N* of irregular shape; a considerably dilated endoplasmic reticulum *rEr*, partly papillary →; mitochondria *M* of irregular shape and size, many containing osmophilic, irregularly bordered electron-dense inclusions *MI*. × 5500. b A group of mitochondria *M* containing electron-dense inclusions *MI* and remnants of cristae →. × 18000

considerable part of the cell. In the cytoplasm of some of the cells myelin figures were seen.

A considerable number of lipid droplets were also found in the cytoplasm of the tumour cells. Cell disintegration presented unusual forms in places. The cytoplasm appeared as if pealing off the nucleus and the cytoplasmic organelles disappeared while the nuclear membrane and the nucleus itself remained almost intact. Cells damaged in this way enter the sinusoids, in which apparently intact but "naked" nuclei without surrounding cytoplasm could thus be seen (Fig. 8a).

The amount of glycogen granules was diminished in almost all the cells. In some foci, however, large amounts of glycogen granules were found, while other areas were altogether without such granules. At times glycogen granules were seen between the lamellae of the "fingerprint" formations of endoplasmic reticulum, or even in the centre of these formations (Fig. 3b).

The relations of most of the tumour cells to the sinusoids were essentially similar to those observed in normal liver, *i.e.* the vascular pole was usually bordered by the endothelial cells of the sinusoids and the Disse spaces were recognizable (Fig. 6). In the latter, however, fibrin and cell debris were often seen (Fig. 7b). In places the endothelial cells were found to be arranged in multiple layers (Fig. 6): among them there was amorphous osmophilic substance that occasionally displayed agglomeration reminiscent of basement membrane (Fig. 6). A basement-membrane-like osmophilic layer was often found not only on the surface of the endothelial cell facing a liver cell but also along the vascular pole of the latter (Fig. 6). The basement membrane along the vascular pole was usually irregular and discontinuous. In some places there was no endothelial wall to the sinusoids and the tumour cells, mostly showing necrobiotic changes, then abutted directly on the lumens of the sinusoid (Fig. 8a). Tumour cells, loosened from their bonds, were frequently observed in the lumen of the sinusoids.

The cell membrane usually showed a diminished number of microvilli at the vascular pole and the cell surface was unusually smooth (Fig. 7b). Microvilli of abnormal shape were also frequently seen.

The intercellular gaps among the tumour cells were often dilated. This was particularly conspicuous in the undifferentiated areas of the tumours. Microvilli were to be seen on the surface of the tumour cells adjoining the dilated intercellular space.

In the hepatocellular carcinomas we observed bile canaliculi frequently (Fig. 3a). In most instances more than two and often as many as 5 or 6 cells contributed to the formation of these bile canaliculi (Fig. 7a). Some tumour cells possessed several biliary poles. The number of microvilli at the biliary pole was at times very much increased (Fig. 8b), at times diminished (Fig. 3a). The villous structure was often abnormal, and microvilli of varied length and thickness, sometimes considerably enlarged, were frequent. Desmosomes, closing the bile canaliculi from the rest of the intercellular space, was also observed; sometimes they were more extensive and more numerous than in normal liver (Fig. 8b). In the lumen of the bile canaliculi an amorphous precipitate of varying electron density was often seen (Fig. 3a, 7a). The bile canaliculi, however, might appear empty optically (Fig. 5b).

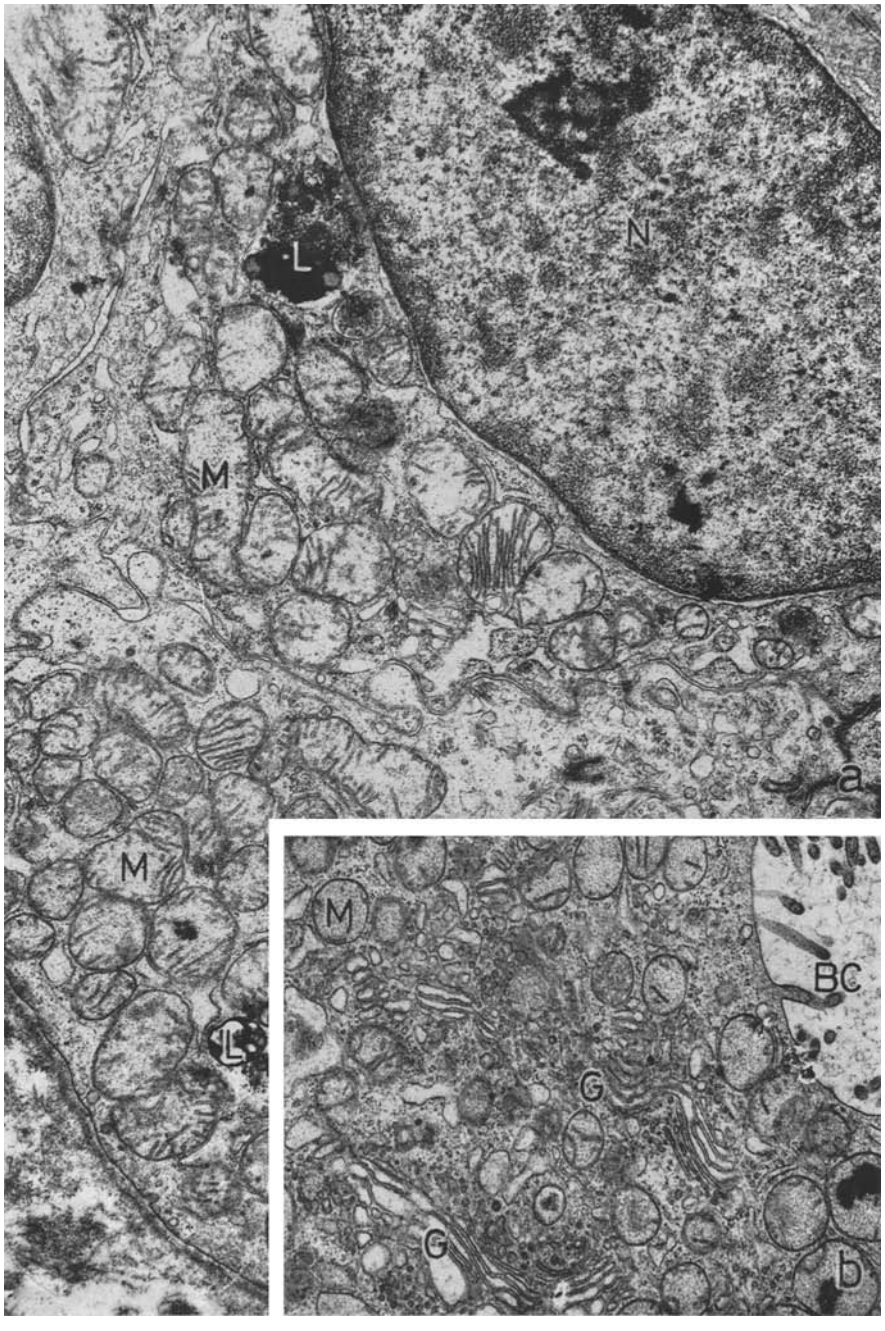

Fig. 5. a Detail of a hepatoma cell. Oval nucleus *N*; mitochondria *M* closely attached to each other and varying in shape and size; organelles, suggesting lipofuscin *L*. × 12000. b Detail of a hepatoma cell. In the rounded and oval mitochondria *M* most of the cristae have degenerated. In the vacinity of a bile canaliculus *BC* a Golgi apparatus *G* is seen. × 18000

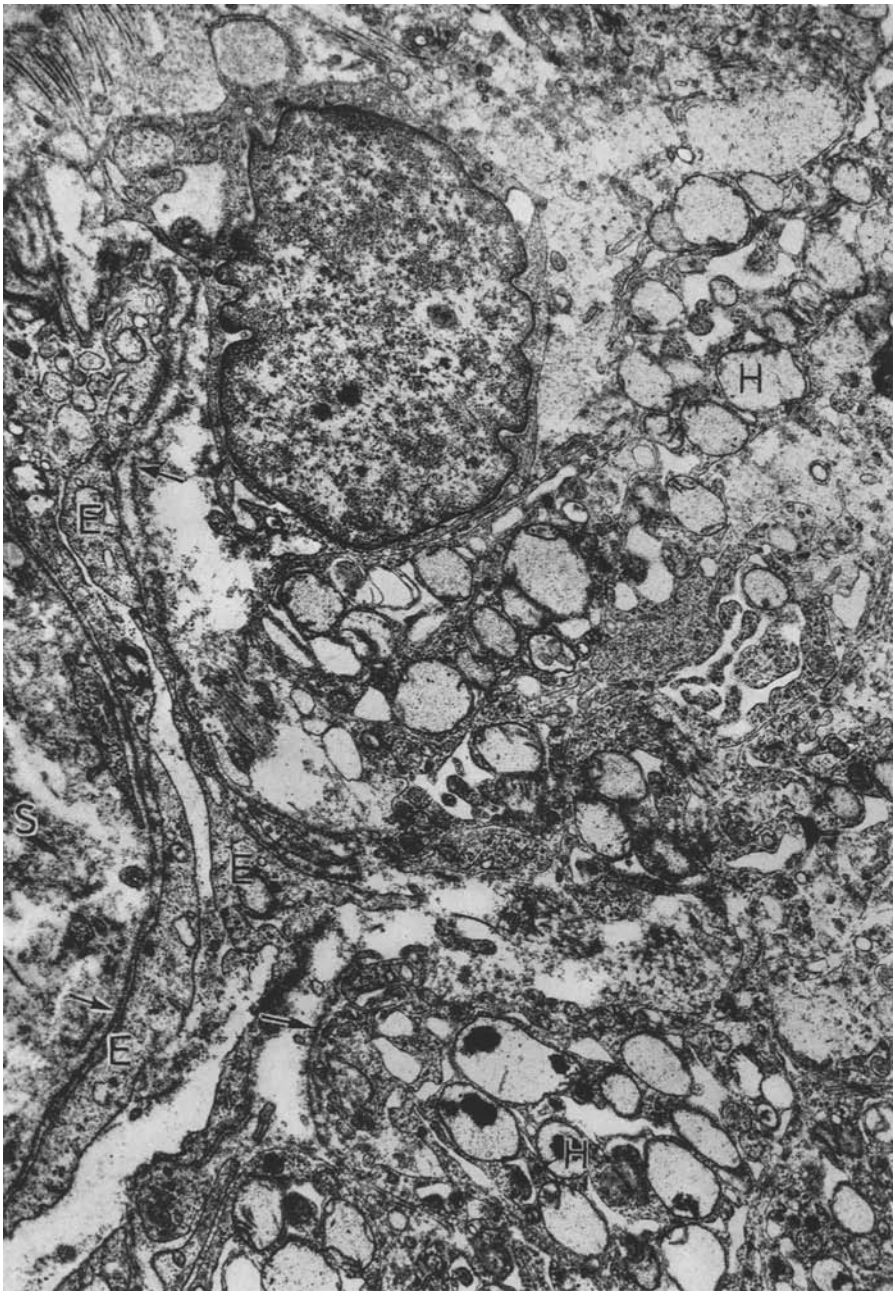

Fig. 6. Vascular pole of hepatoma cells *H*. Endothelial cells *E* line the sinusoid *S* in several rows; among them a formation similar to a basement membrane is observed ↗.
× 7500

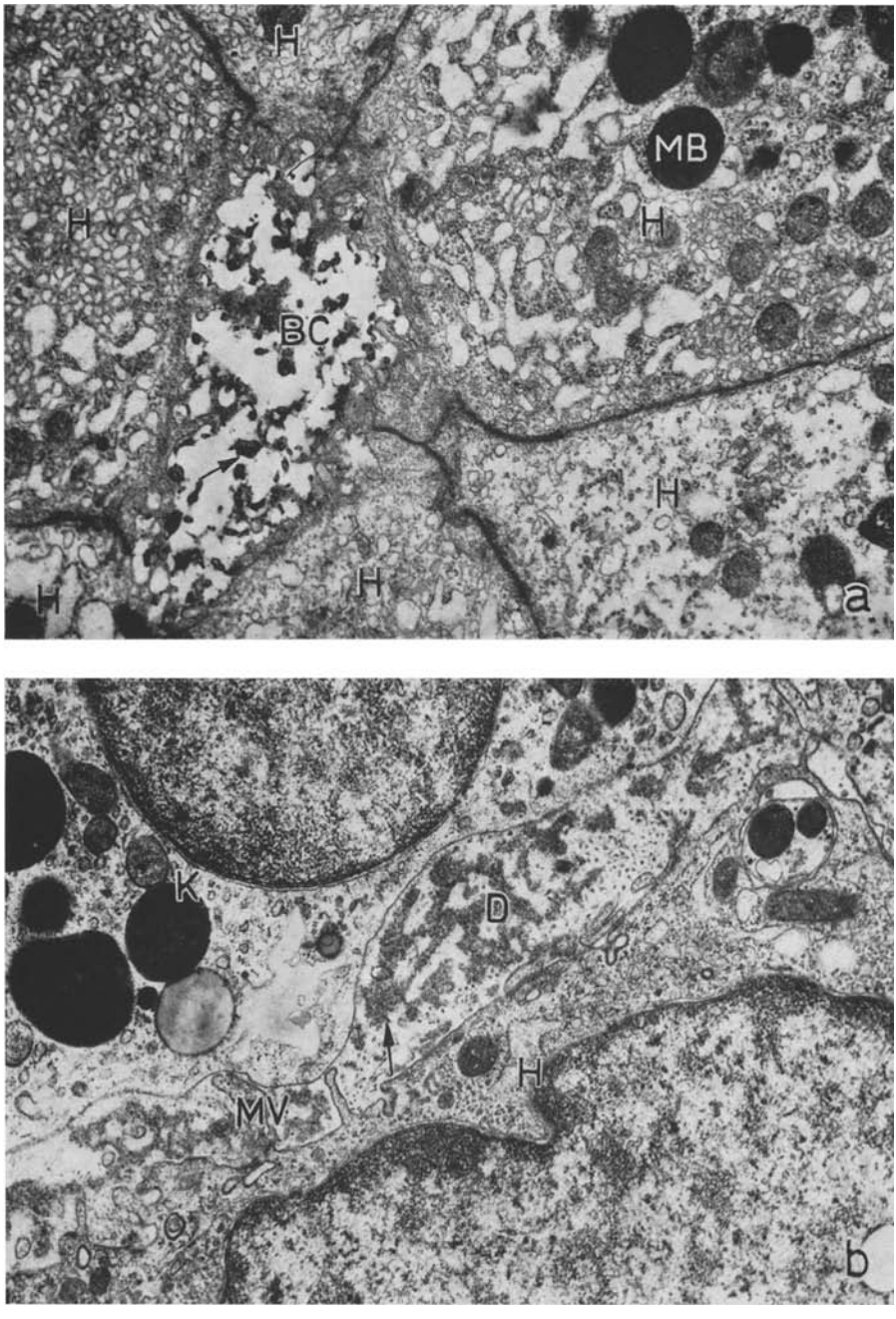

Fig. 7. a Bile canaliculus *BC* enclosed by six hepatoma cells *H*. Its lumen contains amorphous material →. On the biliary pole of a tumour cell microbody *MB* are seen. × 5200. b Detail of a hepatoma cell *H* and of a Kupffer cell *K*. On the vascular pole of the tumour cell the microvilli *MV* are irregularly shaped, and their number is diminished. In the Disse space *D* there is amorphous osmophilic material →. × 10800

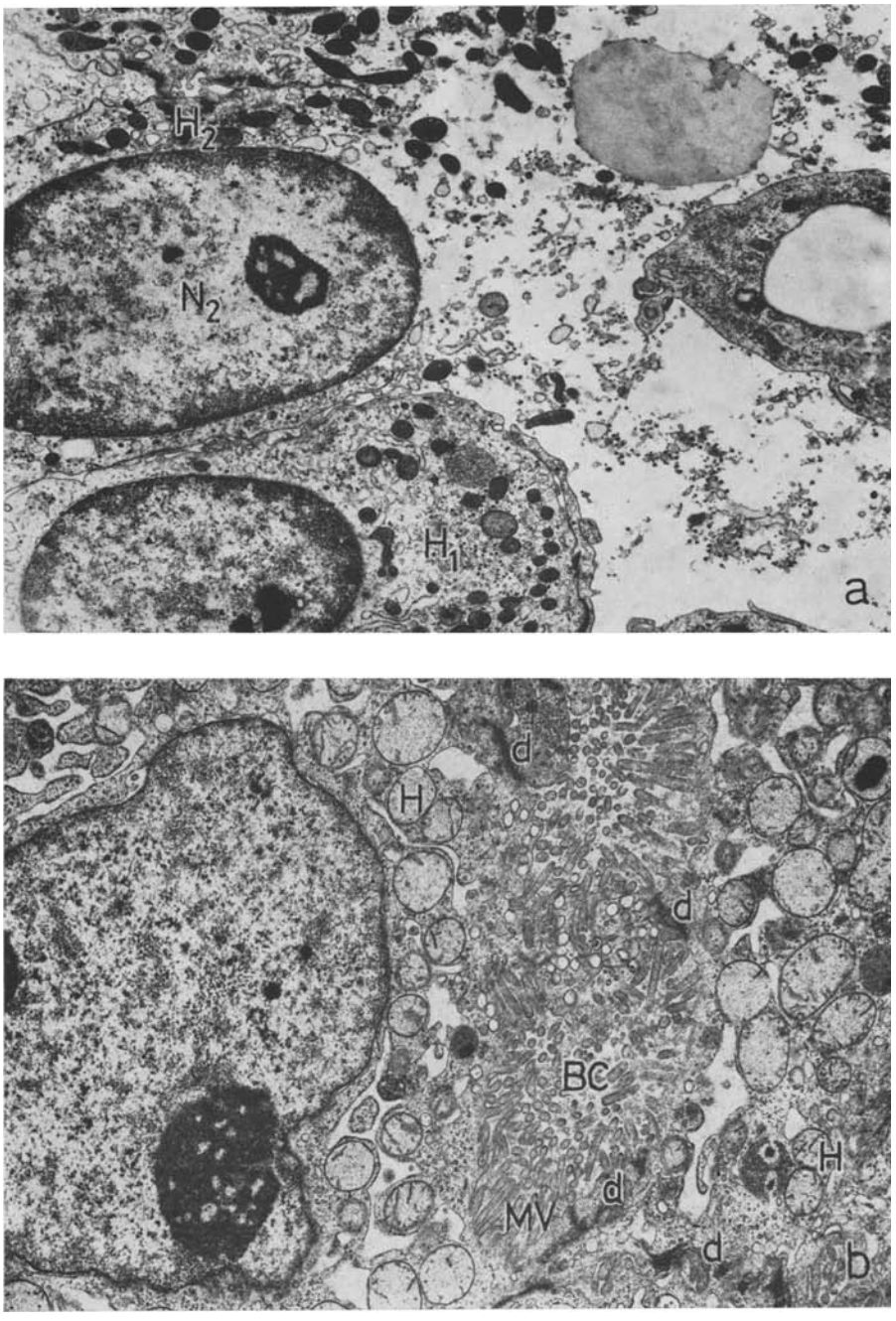

Fig. 8. a Vascular pole of hepatoma cells H_1, H_2. One of the tumour cells seems to be almost entirely intact H_1; the cytoplasm of the other shows advanced degeneration H_2 although its nucleus N_2 appears intact morphologically. × 6300. b Bile canaliculus *BC* formed by adjoining tumour cells *H*, with numerous microvilli *MV*. The desmosomes *d* appear to be more numerous than is normal. × 5700

Discussion

The nuclear pleomorphism of hepatomas is well known (Creemers and Jadin, 1968; Ghadially and Parry, 1966; Ruebner *et al.*, 1967; Tanikawa, 1968; Theron and Mekel, 1964; Toker and Trevino, 1966). Light microscopical (Graffi and Bielka, 1959; Kendrey, 1965) and electron microscopical (Thoenes and Bannasch, 1962; Kendrey, 1965) investigations of this extreme variety in shape and size of the nuclei have also been reported in relation to liver tumours induced experimentally by various carcinogens. Cells with two or more nuclei are quite frequent in human hepatomas. Binucleate cells are also quite frequent in normal liver (Kettler, 1958; Pfuhl, 1932), but more than two nuclei have not yet been reported in normal liver cells (Cossel, 1964).

We have found a considerable variety of inclusions in the nucleus of hepatoma cells. Our electron microscopical studies enable us to state unequivocally that these inclusions are cytoplasmic invaginations containing various organelles. Similar inclusions have been observed in several other pathological conditions of the liver: in transplantable hepatomas of mice (Leduc and Wilson, 1959), in colchicin poisoning (Wessel, 1958), following damage by thioacetamid (Rouiller and Simon, 1962; Kendrey, 1965) and in cholestasis (Izard, 1960). Increase in the area of the nuclear surface caused by invagination of the nuclear membrane occurs when there are enhanced functional requirements. Judging from observations on tumours (Oberling and Bernhard, 1961; Lapis, 1967), this is a frequent but by no means specific characteristic of hepatoma cells. According to David (1965), particularly intensive exchange of substances may occur between the nucleus and the cytoplasm through these cytoplasmic invaginations. Rouiller and Jézéquel (1963) suggested that these nuclear inclusions have a functional role comparable with the phenomena of pinocytosis and phagocytosis.

Nuclear inclusions of cytoplasmic origin include swelling of the cytoplasm (Sobel *et al.*, 1969), nucleocytoplasmic exchange of substances in conditions associated with cell proliferation (Cossel, 1962; David, 1965; Lapis, 1967) and shifting of nucleocytoplasmic ratio in favour of the nucleus (Luse, 1961). Through their continuous contact with the nuclear membrane the invaginated membranes serve as pathways for the exchange of substances between cytoplasm and karyoplasm and between the nucleolus and the cytoplasm. According to Yasuzumi and Sugihara (1965) the intranuclear membranes provide the nucleus with a relatively large inner surface at which the various metabolic processes can take place. Our observations have shown that following treatment with cytostatics the nuclear membrane and the cytoplasmic invaginations become especially marked and frequent in the tumour cells.

Invaginations not separated by membranes from the karyoplasm were also seen. Usually they contained degenerated cytoplasmic organelles. Such inclusions possibly formed by complete separation of the invaginated material with subsequent loss of its limiting membrane (Leduc and Wilson, 1959; Rouiller and Simon, 1962; Simon and Rouiller, 1962; Sobel *et al.*, 1969).

In agreement with our electron microscopic findings, most authors using the light microscope to study hepatomas in man and experimentally induced hepatomas in animals have also reported an increase in the number and size of the nucleoli in the tumour cells (Graffi and Bielka, 1959; Kendrey, 1965). Besides

variations in size and number of nucleoli a very frequent phenomenon was the penetration of the nucleolar substance by the karyoplasm, forming a network in its substance. Occasionally peculiar "plait" and "target" nucleoli were also observed. Formations similar to the latter have been observed by Luse (1961) in oligodendroglioma; he doubted, however, that it was characteristic of the neoplastic process.

We observed degranulation, vesiculation and cistern-like dilatation of the lamellae of the rough-surfaced endoplasmic reticulum. Other authors have described similar observations (Creemers and Jadin, 1968; Ghadially and Parry, 1966; Toker and Trevino, 1966). Theron and Mekel (1964) observed finely granular protein-like deposits of low electron density inside the cisternae. Such deposits were not observed in our material. According to these authors, the endoplasmic reticulum is the first among the cytoplasmic organelles to get damaged during carcinogenesis, thus implying that in human hepatoma cells the alterations of the endoplasmic reticulum may be considered primary. Similar alterations in the papillary pattern of the endoplasmic reticulum were reported by Lapis (1967) in experimentally induced thyroid tumours.

The "fingerprint" or Nebenkern-like endoplasmic reticulum was a peculiar finding in our material. Similar formations were described by Rouiller (1957), Bernhard (1958), and Ghadially and Parry (1966) in human hepatomas and by Fawcett and Wilson (1955) in the spontaneous hepatomas of inbred C3H mice. We have not seen such formations of the endoplasmatic reticulum either in normal human liver or in the pathological liver of Gilbert's syndrome (Schaff *et al.*, 1969), Dubin-Johnson syndrome (Sáfrány and Lapis, 1967), Rotor-syndrome (Schaff *et al.*) or intrahepatic and extrahepatic cholestasis (Balázs *et al.*, 1970).

In experimental material, "fingerprint" formations were seen in the liver by Driessens and his collaborators (1959) after administration of azo dyes and by Verbin and his collaborators (1969) after administration of cycloheximide. The latter authors observed a decrease of protein synthesis in the liver parallel to the particular morphological alterations described. The appearance of "fingerprint" formations was reported also as an effect of other drugs inhibiting protein synthesis—dimethylnitrosamine (Benedetti and Emmelot, 1961; Emmelot and Benedetti 1960) and thioacetamide (Rouiller and Simon, 1962; Salomon, 1962; Thoenes and Bannasch, 1962; Kendrey, 1968). As a result of these observations we feel justified in regarding the "fingerprint" formations in human liver tumours as evidence of a disorder in protein synthesis.

As for the *Golgi-apparatus*, some authors (Creemers and Jadin, 1968; Ghadially and Parry, 1966; Tanikawa, 1968) failed to find any alteration in most of the tumour cells, in agreement with our own observations. Some others, however, found multiple Golgi-complexes in some cells (Ghadially and Parry, 1966) and damaged complexes have also been seen (Toker and Trevino, 1966).

Published data on the alterations in the mitochondria of the tumour cells vary. Some authors found the number of mitochondria diminished (Toker and Trevino, 1966), others found them increased (Creemers and Jadin, 1968; Toker and Trevino, 1966) or unchanged (Tanikawa, 1968). Theron and Mekel (1964) observed the grouping of membranes of endoplasmic reticulum around the altered mitochondria. In their opinion this morphological feature indicates that the

mitochondrial damage was secondary and due to alterations of the endoplasmic reticulum. In experimental material the damage to the cristae of the mitochondria were observed frequently as an effect of hepatoxic and carcinogenic agents (Rouiller, 1964). There are, however, opposite views of the significance of the mitochondrial alterations induced by carcinogenic agents (Rouiller and Jézéquel, 1963). Bernhard's opinion (1963) sounds to us the best justified: according to him the mitochondria are the organelles most frequently altered in the tumour cells. The alterations are, however, not specific, and they are possibly related to lack of oxygen and of other cell requirements.

In one of our cases we saw peculiar inclusions in the mitochondria. Their nature is speculative. In the mitochondria of human hepatomas Ghadially and Parry (1966) found a similar substance, though of lower electron density: they thought it to be lipid. Similar inclusions thought to be of lipid nature were seen by Oberling and Rouiller (1956) in mitochondria after chloroform intoxication. Roux (1960) reported an increased phospholipid and glyceride content in the mitochondrial fraction of the liver in a case characterized by similar intramitochondrial inclusions of lipid nature. On the basis of their morphology these inclusions might be a heavy metal chelate, but we obtained a negative result on applying electron diffraction. They do not seem to be specifically related to the tumour as such, for they were found in only one of our 14 tumours, and they have been seen as an effect of other damaging agents (Oberling and Rouiller, 1956).

In our material we also found large numbers of microbodies, as well as lipofuscin granules. The presence of such organelles is mentioned by other investigators (Creemers and Jadin, 1968; Toker and Trevino, 1966) but in other reports they have not been described (Ghadially and Parry, 1966; Tanikawa, 1968).

It has been proved by light microscopy that cytoplasmic inclusions are frequent in human and experimental hepatomas. Kendrey (1965) distinguished seven types of these on the basis of their morphology. Our electron microscopical findings as well as the opinions of other authors (Anderson *et al.*, 1961; Biava *et al.*, 1965) lead us to conclude that the inclusions consist of various organelles showing regressive alterations. Lipid inclusions and myelin figures are especially frequent in tumour cells. Focal necrotic areas as well as the sequestration of "fingerprint" formations might account for the picture of such cytoplasmic inclusions under the light microscope. These alterations are related to necrobiotic phenomena. The loss of cytoplasmic substance of cells with relatively well preserved nuclei, the so-called "naked" nuclei may also be considered as evidence of necrotic processes occurring in the tumour.

The glycogen content of liver cells differs widely in the various pathological conditions of the liver. Carcinogenic agents usually cause the glycogen content of liver cells to fall rapidly (Rouiller, 1964). Decreased amounts of glycogen might be found also in human hepatomas. Sometimes the presence of glycogen, even in such a decreased amount might have diagnostic importance in distinguishing between hepatocellular and cholangiocellular carcinomas.

We have not seen particles which might have been considered as viruses in the tumours studied. Some authors reported the occurrence in hepatoma cells of particles, 80–100 Å in diameter, bounded by a single membrane and considered

them to be viruses (Blackburn, 1966; Theron *et al.*, 1962). Others have suggested that these particles may be secretion granules (Creemers and Jadin, 1968).

We have regularly observed bile canaliculi and desmosomes in the hepatoma cells, except in anaplastic or necrotic areas. Most other authors have reported similar observations, but Ghadially and Parry (1966) noted the absence of desmosomes. Only a few authors (Tanikawa, 1968) have noted that the bile canaliculi in the tumours were usually bordered by 4 to 5 cells instead of 2, as is usually seen in the normal liver. In addition, the same tumour cells may take part in the formation of several bile canaliculi. This phenomenon may serve as a good example that a feature characteristic of a normal tissue (in this instance the formation of bile canaliculi) may also be present, but in a distorted form, in tumours derived from the tissue in question. We have not seen a similar appearance of the bile canaliculi in other liver diescases. In contrast, alterations of microvilli of bile canaliculi (swelling, increased number, etc.) seen in the tumours is also observed in other liver diseases (cholestasis, toxic damage, etc.).

Various alterations of the vascular pole of hepatic cells were observed in our material, sometimes within one and the same tumour. A decreased number of microvilli on the vascular pole has been seen in several liver diseases (acute and chronic hepatitis, cirrhosis) (Cossel, 1964). However, the microvilli are rather inconstant structures, depending on the metabolic state of the cells (Cossel, 1964). Some authors found the microvilli of vascular poles in hepatomas to be normal (Creemers and Jadin, 1968; Toker and Trevino, 1966); others found them rather underdeveloped and irregular (Tanikawa, 1968).

According to our observations the arrangement of the cells and their relation to the sinusoids is in many respects similar to the organization seen in the normal liver. Similar observations have been reported by Ghadially and Parry (1966), Tanikawa (1968), Toker and Trevino (1966). According to Tanikawa (1968) the liver cell trabeculae in hepatomas can often be regarded as multilayerd plates. Some tumour cells have no direct contact with sinusoids. The intercellular gaps around these "closed in" or confined cells are mostly dilated and the formation of microvilli may be observed on the surface of the cells abutting on the intercellular spaces. The phenomenon is presumably related to the adaptation of the cells to circumstances prevailing in anoxia, i.e. the lack of direct contact with the sinusoids is compensated by an increase of the cell surface. If its nutrition cannot be assured in this way, the cell will die. We have often noted the isolated degeneration of such "closed in" cells.

Ghadially and Parry (1966) failed to find any endothelial sinusoids in cancers of the liver in man. In contrast to this finding we regularly observed the presence of sinusoids in these tumours. In some we also noted the formation of a structure similar to basement membrane around the sinusoids in the tumours. The presence of basement membranes has been found in numerous other pathological conditions of the liver, e.g. in cases of experimentally induced extrahepatic cholestasis in rats (Carruthers *et al.*, 1962), following allylformate intoxication (Rouiller and Haenni, 1963), and in hepatomegaly of unknown origin (Rouiller and Camain, 1961). According to observations in the literature, "capillarisation" of the sinusoids (i.e. the formation of a basement membrane) in cirrhosis may be considered as a regularly encountered alteration (Schaffner and Popper, 1963;

Tanikawa, 1968). With the exception of two cases, all the carcinomas in our series developed on the basis of cirrhosis. In these two exceptional cases we noted development of the basement membrane in the absence of cirrhosis. Hence it follows that the development of a basement membrane around sinusoids—their "capillarisation"—may occur not only in cirrhosis but also in primary hepatocellular carcinoma arising in the absence of cirrhosis. These observations are in agreement with those of other workers (Tanikawa, 1968).

The nature of the amorphous, osmophilic substance found by us in the Disse space, has not yet been demonstrated. Its morphological picture suggests that it is fibrin.

Conclusion. The observations that we have recorded here—multilayering of the cell plates, "capillarisation" of the sinusoids, stratification of the endothelium, filling of the Disse spaces by fibrin-like material—are relevant to the development of hypoxia and nutritional disturbances. Deficiency of oxygen and of nutrition must increase the sensitivity of the tumour cells to toxic factors, among which products of metabolism released by disintegrated cells must be specially mentioned. In this way a vicious circle is initiated in which necrobiotic processes in the tumour, i.e. cell necrosis, are enhanced. These phenomena are conspicuous in hepatocellular carcinomas.

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