Ultrastructure of Spontaneous Hyperplastic Nodules in Mouse Liver

EDWARD ESSNER

Division of Cytology, Sloan-Kettering Institute for Cancer Research, and the Sloan-Kettering Division, Cornell University Medical College, New York, New York

SUMMARY

The fine structure of parenchymatous cells in a series of spontaneous, hyperplastic nodules in aging C3H mice is described. These nodules were circumscribed, frequently lacked central and portal veins, and sometimes exhibited a variety of other changes including hydropic vacuolization, fatty infiltration, and dilatation of sinusoids. The nuclei of the parenchymatous cells were smaller in size, while the cytoplasm was more basophilic and often vacuolated. All the nodules showed varying degrees of alteration in fine structure, particularly in the Golgi apparatus and mitochondria. Most striking, however, were alterations in the rough endoplasmic reticulum in which the normally flattened cisternae were transformed into either a dilated or a tubular form, containing amorphous, granular, or filamentous material. The filaments measured about 33 m in diameter. Of interest is the fact that similar changes in the endoplasmic reticulum were observed in 18-month-old mice of the same age group. The nature of the histologic changes in the hyperplastic nodules are considered. The significance of the various altered forms of endoplasmic reticulum and their unusual contents in parenchymatous cells of both hyperplastic nodules and "normal" liver are discussed. The question whether these changes as well as alterations in other cell organelles are the result of unfavorable environmental conditions in the growing nodule is also considered.

INTRODUCTION

Primary tumors occur at high incidence in the liver of aging mice of the C3H strain (1-3, 6). These tumors are generally regarded as benign although a few instances some features of malignant growth have been observed (6). Recently, Hruban et al. (15) reported on the fine structure of a series of benign hepatomas in this strain. They described among other changes alterations in the rough endoplasmic reticulum and their unusual contents in parenchymatous cells of the liver of a "normal" animal from the same age group. The present investigation illustrates in detail these various changes in the parenchymal cells of hyperplastic nodules including alterations in the endoplasmic reticulum, Golgi apparatus, and mitochondria as well as the appearance of lipid-laden cells from areas of fatty infiltration. In addition, unusual alterations in the endoplasmic reticulum which also occurred in parenchymal cells of the liver of a "normal" animal from the same age group are illustrated. The significance of these changes in endoplasmic reticulum and other organelles and the possible relationship of the altered reticulum in "normal" liver to that in the hyperplastic nodule are discussed.

MATERIALS AND METHODS

The animals used in this study were all C3H/An male mice received in the laboratory at 6-7 weeks of age and maintained until used on Purina chow and tap water ad libitum. The mice were sacrificed by cervical dislocation between 19 and 20 months of age and examined for hepatic lesions. Samples from 11 lesions were initially removed for study. Eight of these, classified histologically as typical, hyperplastic nodules, were prepared for electron microscopy. The remaining 3 hyperplastic nodules were not included in this investigation when histologic examination revealed one to be necrotic and the other to have large areas of necrosis and fibrosis. The third nodule not included in this study was relatively large and contained foci of inflammation and dilated sinusoids. The cell plates were thicker and more irregular, and there was more variation from cell to cell than in the other 8 nodules selected for study. Nuclei and nucleoli varied considerably in size and some were much larger than the nuclei and nucleoli in the other nodules. Although this lesion was classified as a hyperplastic nodule, it was more atypical than the others selected.

Sixteen samples of liver served as controls as follows: (a) 6 samples were obtained from the uninvolved liver lobes of 6 of the 8 mice bearing hyperplastic nodules upon which this report is based; (b) 2 samples were from uninvolved lobes of mice of the same age group as in (a) that had nodules not described here; (c) 5 samples were from livers of normal mice, 22 months of age; and (d) 3 samples were from livers of normal mice, 18 months, 3 weeks of age. One of these livers showed the extensive changes illustrated in this report. Similar although less extensive changes were observed in a sample from an uninvolved liver. The fine structure of a series of benign hepatomas in aging C3H mice were described. Somewhat similar changes to those reported by Hruban et al. (15) occurred in the endoplasmic reticulum of the parenchymal cells. The appearance of "intracisternal protein inclusions" in the endoplasmic reticulum of the tumor cells. In a preliminary report is based; (b) 2 samples were from uninvolved lobes of mice of the same age group as in (a) that had nodules not described here; (c) 5 samples were from livers of normal mice, 22 months of age; and (d) 3 samples were from livers of normal mice, 18 months, 3 weeks of age. One of these livers showed the extensive changes illustrated in this report. Similar although less extensive changes were observed in a sample from an uninvolved liver.
Edwin Essner

lobe of a mouse (20 months old) with a large hepatoma not described here.

For histologic study, tissues were fixed in formaldehyde-calcium and stained with hematoxylin and eosin. For electron microscopy the tissues were diced into small cubes, fixed for 1 hour in cold 1% osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in Epon 812. Thin sections were cut on diamond knives, mounted on uncoated copper grids, and stained with lead citrate. A light layer of carbon was evaporated over them. The sections were examined with an RCA-EMU-3G electron microscope and photographed at magnifications of from 2,900 to 18,000. These were enlarged photographically.

RESULTS

Gross Appearance of Hyperplastic Nodules

The hyperplastic nodules usually occurred on the diaphragmatic surface or at the edge of the liver. They were small (2-5 mm in diameter), light brown, somewhat paler than the remainder of the liver, and well demarcated from the surrounding liver tissue. Usually, only a single nodule occurred in the liver. No other gross changes were observed in these animals.

Histologic Appearance of Hyperplastic Nodules

All the lesions were classified as hyperplastic nodules. They possessed several histologic features in common. All were circumscribed and clearly demarcated from the adjacent normal liver parenchyma (Figs. 1, 2). The surrounding parenchyma was sometimes compressed as a result of the growth of the nodule. Central and portal veins were fewer in number or were entirely absent. At higher magnifications, nodule cells immediately adjacent to normal liver cells were easily distinguished (Fig. 3). The cell plates in the nodules were 1, 2, or 3 cells wide and were more irregular in arrangement. The cytoplasm of the parenchymatous cells was more basophilic (Fig. 3) than in normal liver cells and frequently more vacuolated. In some of the nodules, a few mitotic figures were evident. They also sometimes occurred at the periphery of the nodule where it was difficult to determine whether the dividing cells were in the nodule or in the normal tissue (Fig. 3). Additional changes were observed in parenchymatous cells of 1 or more of the nodules. These included: hydropic vacuolization (Fig. 5), the most common change; extensive dilution of sinusoids (Fig. 2); and accumulation of lipid (Fig. 6). In a few cells of some nodules, large eosinophilic inclusion bodies were observed. Bile duct proliferation was not present, but in almost all nodules clusters of unidentifiable cells were evident (Fig. 4). The nuclei of these cells were elongated and more lightly stained than those of normal liver cells. The nature of these cells is uncertain, but they may represent early ductular proliferation or, possibly, proliferated endothelial cells. It is of interest that "islands of atypical cells" similar to those observed in ethionine-induced hyperplastic nodules (Refs. 11, 12; see also Ref. 25) of rat liver where they are thought to represent early malignancies were not observed in any of the spontaneous mouse nodules.

Fine Structure of Parenchymal Cells in Hyperplastic Nodules

The samples of livers of the 2 groups of normal mice of different ages as well as the uninvolved portions of livers of nodule-bearing mice (see Materials and Methods) were essentially normal in fine structure. None showed the extensive changes observed in one "normal" aged mouse as described in the second part of this report. However, it was noted that in the livers of most aged mice the rough endoplasmic reticulum was more disorganized. Parallel arrays of cisternae were much less evident and there was an increased tendency for individual cisternae to surround individual mitochondria partially or entirely. Occasionally, a cell was observed with mitochondria that were more irregular in size and shape. In virtually all livers, Kupffer cells contained increased numbers of large, electron-opaque bodies, presumably acid phosphatase-rich phagosomes (18).

In the cells of the hyperplastic nodules, the endoplasmic reticulum also showed increased disorganization, and the association of endoplasmic reticulum with mitochondria was even more evident. Occasionally, the cisterna surrounding a mitochondrion was dilated and contained material of low electron opacity (Fig. 17). The membrane of the reticulum adjacent to the mitochondrion usually possessed attached ribosomes and remained separated from the outer mitochondrial membrane by a space of uniform width (Fig. 17). In other cells the endoplasmic reticulum was disorganized and considerably more dilated containing amorphous material of medium opacity (Fig. 9). Some of these cisternae also contained globules of material, possibly lipid, having greater opacity than the surrounding contents. Images of some of these globules that were close to the reticulum membrane gave the impression that this material might be conveyed into or out of the cavity of the cisterna (Fig. 9).

In addition to these variations, striking alterations of the rough endoplasmic reticulum were observed in 4 of the 8 nodules studied. These alterations assumed 2 morphologically different forms. The first is illustrated in Fig. 7.

In this form of endoplasmic reticulum, the characteristic pattern of parallel, flattened cisternae was almost entirely replaced by individual randomly disposed cisternae that were dilated in varying degrees. Continuities between dilated cisternae and remnants of normal reticulum were evident (Fig. 7). In the cells of some nodules these changes in the endoplasmic reticulum were limited to a few cisternae while in other cells they appeared to involve entire clumps that became altered en masse. The diluted cisternae contained contents of 2 types, an amorphous material of low electron opacity and, embedded in it, a series of filamentous, rodlike structures approximately 30 m in diameter. As extensive as these changes were in some areas they did not seem to involve an actual increase in the amount of endoplasmic reticulum. Rather, it appeared that each pre-existing cisterna became altered in situ. The second type of altered endoplasmic reticulum, observed less frequently, appeared to involve an increase in the amount of reticulum (Fig. 8). In this form of reticulum, large areas of the cytoplasm were occupied by long tubular arrays of endoplasmic reticulum tightly packed together and oriented in somewhat parallel
Ultrastructure of Hyperplastic Nodules

In this study, we observed the fine structure of parenchymatous cells in an 18-month-old mouse. As in the 3-week-old mouse, nodules were present in liver from an uninvolved lobe of an animal (20 months old) containing altered rough endoplasmic reticulum. The Golgi apparatus (Fig. 12). Although in general there appeared to be fewer of these dense bodies in the parenchymatous cells of the nodules, this would require confirmation from studies of light microscopy of preparations such as those incubated for acid phosphatase activity (18).

Alterations in mitochondrial fine structure were evident in the parenchymal cells of the nodules. An example of these changes is shown in Fig. 18. The matrix of these mitochondria was less opaque than that of normal mitochondria, and the cristae were diminished in amount or grouped to the sides forming compacted structures. Other types of changes in mitochondria (Figs. 19, 20, 25, 26) occurring in the liver cells of a "normal" mouse are discussed below. Microbodies were present in parenchymatous cells of the nodules. Although most of them possessed normal morphology, a few contained a somewhat altered nucleoid (Fig. 14). The Kupffer cells of the nodules are not illustrated in this report, since they were similar to those in livers of normal, control mice. However, these cells were usually large, occupying much of the sinusoid and generally contained many more of the characteristic large, pleomorphic bodies commonly observed in Kupffer cells of livers of younger mice. These bodies resembled the lipofuscin pigment of human liver (10) and were composed of 2 or 3 components distinguishable by their differing opacities. Some bodies were elongated and contained crystalline-like material not observed in the Kupffer cells of livers of younger mice.

Fine Structure of Parenchymal Cells in an 18-Month, 3-Week-Old "Normal" Mouse

The fine structure of parenchymatous cells of the liver in normal, aged mice without grossly visible nodules or other lesions was similar to that of younger mice except for the occasional reticulum-mitochondrial associations of the kind seen in the nodule cells (Fig. 17) and referred to earlier. However, in one of these "normal" mice that grossly showed no lesions, the fine structure of the liver cells showed extensive changes particularly in the rough endoplasmic reticulum (Figs. 21–24). Similar changes involving smaller regions of endoplasmic reticulum were observed in liver from an uninvolved lobe of an animal (20 months old) bearing a large hepatoma (see Materials and Methods). As in the parenchymal cells of the hyperplastic nodules, these changes in the endoplasmic reticulum of liver cells of a "normal" mouse occurred in two morphologic forms. In one form the cisternae

NOVEMBER 1967
of the endoplasmic reticulum were transformed into elongated, tubular structures (Fig. 21) that retained some parallel organization and resembled one of the types of altered reticulum that occurred in the nodules cells (Fig. 8).

This tubular form of endoplasmic reticulum contained amorphous material, longitudinally oriented filaments and small, opaque granules (Figs. 22, 23) very similar to the materials in both types of altered reticulum of the nodules cells (Figs. 7, 8). In longitudinal sections these tubules measured approximately 100 μm wide. The membranes had few attached ribosomes, but free ribosomes and clusters of ribosomes were apparent in the matrix between the tubules of endoplasmic reticulum (Figs. 22, 23). In addition to the tubular type of endoplasmic reticulum some areas of the same cell or other cells also contained a more dilated form of cisternae (Fig. 22). This dilated form also contained amorphous material, filaments, and opaque granules. As in the nodule cells (Fig. 8), both altered forms of endoplasmic reticulum were frequently oriented toward the Golgi apparatus (Fig. 22). Although more numerous, the small opaque granules within the cisternae were similar to those commonly observed in the endoplasmic reticulum of normal liver cells or of liver cells under various experimental conditions (4, 20). They also appear to increase in number within the fragmented endoplasmic reticulum produced by administration of certain carcinogens (8). As in normal liver, these granules resembled those present in the Golgi sacules.

In glycojen areas the altered endoplasmic reticulum assumed a characteristic appearance (Fig. 24) and contained fibrous material and opaque granules similar to those present in altered reticulum in other regions of the cell.

In addition to the changes in the endoplasmic reticulum many of the cells contained abnormal mitochondria. In one form several mitochondria were compacted together (Fig. 19) and showed changes in the form and orientation of the cristae. In others, the cristae formed loops (Fig. 20). In a few cells, virtually all the mitochondria were markedly elongated (Fig. 25) or distorted into a variety of forms (Fig. 26). The cristae of these mitochondria were oriented in unusual patterns, and the matrix contained finely fibrillar material which, in elongated mitochondria (Fig. 20), was oriented longitudinally (Fig. 26, inset).

DISCUSSION

Histologic Considerations

Histologically, the spontaneous hyperplastic nodules of the C3H mouse were similar to the hyperplastic nodules (14) induced in rat liver after administration of N-2-fluorenylidacetamide (25, 26), xanthine-7-N-oxide (5), ethionine (11, 12), and the miticide, Aramite (23), and in mouse liver after administration of N-nitrosodimethylamine or N-nitrosodiethylamine (29). These lesions are circumscribed, resemble normal liver, and frequently cause compression of the surrounding parenchyma. In both types of nodules, alteration or decrease of central and portal veins are evident, the cell plates are wider and more irregular and the cell cytoplasm more basophilic. The several other changes observed in the mouse spontaneous nodules, dilution of sinusoids, fatty changes, and hydroptic vacuolization, are interpreted as secondary effects resulting from growth of the nodule, crowding of the cells, and perhaps most important, alterations of the blood supply. In cell plates comprised of 2 cells, a portion of the cell surfaces evidently does not border on the space of Disse and hence is deprived of access to the blood sinusoid. In plates 3 cells wide, the middle cells may have considerably less access to blood supply. It will be of interest to study the relationship of parenchymatous cell membranes to each other and to the sinusoids in these widened cell plates. Similar considerations regarding altered distribution of oxygen, carbon dioxide, and nutrients between the liver cells and the blood which might lead to a "new cellular environment" have been discussed by Farber (11) in ethionine-induced nodular hyperplasia. In addition, Reeves and Farber (cited by Farber (11)), noted that many nodules appear to have greatly diminished or absent portal blood supply and a correspondingly increased hepatic arterial supply. Although in these studies it is suggested that such changes may lead to an "irreversibly altered neoplastic cell" (11), it is possible that such conditions are also responsible for some or all of the fine structural changes described in the present investigation.

Fine Structure

The most striking changes in the parenchymatous cells of the hyperplastic nodules were the alterations in the endoplasmic reticulum and their unusual contents of homogeneous, filamentous, and granular materials. The altered reticulum appeared in 2 forms, one in which each cisterna was dilated but retained attached ribosomes, and the other, observed less frequently, characterized by apparent proliferation of smooth endoplasmic reticulum and extensive loss of ribosomes. Despite these morphologic differences, both forms contained the same kind of materials suggesting that these changes reflected modulations of essentially the same basic alteration. It is interesting to note that proliferation of smooth endoplasmic reticulum was the most prominent effect observed by Porter and Bruni (24) in their study on the early changes in rat liver following administration of 3'-methyl-4-dimethylamionozobenzene. It is too early, however, to speculate whether such changes are related to those observed in the endoplasmic reticulum of cells of the spontaneous hyperplastic nodules.

The nature of the substances in the altered endoplasmic reticulum is unknown. Hruban et al. (15) recently reported similar changes in the endoplasmic reticulum in a series of "benign hepatic tumors" of C3H mice. In addition to amorphous and filamentous materials they observed larger bodies that appeared to correspond to eosinophilic inclusions observed by light microscopy. Hruban et al. (15) believed these substances to be protein or lipoprotein stored in the endoplasmic reticulum in a fashion similar to the intracisternal granules in pancreatic exocrine cells (22). The filamentous material, which they observed less frequently, resembled the "crystalloid" of abnormal microbodies in the same material and was thought to contain enzymes and possibly to represent a stage in the formation of microbodies. In the present study, a few eosinophilic inclusion bodies were observed histologically but none was encountered in thin sections. The small opaque granules observed within the cisternae of the endoplasmic reticulum were more numerous but similar in appearance to those commonly observed in the endoplasmic reticulum of normal liver cells. These particles resembled those in the Golgi sacules and, may be related to similar bodies now generally referred to as liposomes (Refs. 4, 20; see also discussion.
of lipid-laden cells below). Moreover, in favorable planes of section, the endoplasmic reticulum of both nodule cells and cells of the "normal" liver was oriented toward the Golgi apparatus (Figs. 21, 22). Some of the small particles present within the cisternae appeared close to Golgi saccules (Fig. 22). Although additional evidence is required, these orientations suggest the possibility that the small particles are conveyed from the endoplasmic reticulum to the Golgi saccules. Novikoff et al. (20) have also suggested this pathway for movement of liposomes in orotic-acid-fed rat liver via either individual sacs or intermittent continuities between endoplasmic reticulum and Golgi saccules.

It is of interest that altered endoplasmic reticulum was also observed in the liver cells of a "normal" animal of approximately the same age. Moreover, this alteration assumed 2 morphologically different forms, tubular and dilated, resembling the 2 altered forms in the nodule cells. These observations strengthen the suggestion that the various forms of endoplasmic reticulum in both the nodule cells and the cells of the "normal" animal are variations of the same basic change.

Although no information was available on the histology of the liver of the "normal" animal there was no gross evidence of lesions. Several interpretations may be proposed to explain these observations. Although thus far detected only in 1 normal, aged mouse (described in this report) and in the uninvolved lobe of a hepatoma-bearing mouse, these changes may occur generally in the livers of aging mice, and perhaps remain latent within those cells that later proliferate to form hyperplastic nodules. It is also possible that the area taken for electron microscopy and illustrated in this report represented a very small nodule not grossly visible or perhaps an area of "diffuse hyperplasia" similar to those demonstrated in rat liver by Reuber (25) after feeding N-2-fluorenyldiacetamide. However, the fine structure of the cells in such areas of diffuse hyperplasia in rat liver has not been described to our knowledge nor is it certain whether similar areas of diffuse hyperplasia actually develop in the C3H mouse prior to the onset of nodular hyperplasia.

Two types of lipid-laden cells were observed in the hyperplastic nodules. In the first, the droplets were interpreted as lipid on the basis of their similarity to the lipid of normal liver cells as commonly observed after osmium tetroxide fixation. All these lipid droplets were closely associated with rough endoplasmic reticulum but only some were clearly within the cisternae. They resembled, somewhat, the lipid accumulations in endoplasmic reticulum reported by Novikoff et al. (20) in the liver cells of rats fed orotic acid. The second type of cell contained numerous, opaque particles somewhat larger than those in the altered endoplasmic reticulum (Figs. 8, 23). Except for their less opaque centers, they resembled the "osmiophilic bodies" (liposomes) described by Baglio and Farber (4) that increase in number in the endoplasmic reticulum of rat liver cells after administration of ethionine [see also Emmelot and Benedetti (8)]. Although their chemical nature has not been established unequivocally, Baglio and Farber (4) offered several lines of evidence, suggesting that these bodies were lipid, and their accumulation in the endoplasmic reticulum was interpreted as the result of interference with lipoprotein metabolism [see also Novikoff et al. (20) for discussion of this type of particle]. In orotic-acid-fed rat liver, Novikoff et al. (20) demonstrated that lipid (triglyceride) accumulates in the vesiculated rough endoplasmic reticulum, a condition that can be reversed by addition of adenine to the diet. Apparently, this accumulation of triglyceride is due to specific inhibition of $\beta$-lipoprotein synthesis. Although the mechanisms involved in deposition of lipid and lipid-like bodies in the cells of the hyperplastic nodule are unknown it is of interest that, here too, similar droplets and granules are associated with and accumulate within the vesiculated endoplasmic reticulum in the absence of treatment with exogenous agents.

The changes that occurred in the mitochondria and Golgi apparatus of parenchymal cells in both the hyperplastic nodules and "normal" liver are difficult to interpret. The sensitivity of organelles like the mitochondria to hepatotoxic agents or to various forms of stress is widely appreciated (28), and it is thus entirely possible that such changes reflect reactions to unfavorable conditions and are not related to proliferation of the cells per se. The occurrence of fibrillar material in the matrix of some of the abnormal mitochondria raises the question whether some of these fibers might consist of or contain DNA. Although the DNA fibers of mitochondria that have recently been described usually appeared as rod-shaped clumps after OsO$_4$ fixation (17), it is possible that in this type of altered mitochondrion the fibers remained as discrete units. However, cytochemical studies are obviously required to determine whether such fibers actually contain DNA. On the other hand, changes like those in the Golgi apparatus could, conceivably, reflect interference or suppression of normal secretory activity as a consequence of proliferative activity. Hruban et al. (15) also noted changes in the Golgi apparatus as well as somewhat similar alterations in mitochondria. In addition, they observed abnormal microbodies, fewer dense bodies, and large areas of "focal degradation." We observed only an occasional microbody that may have had an abnormal nucleoid (Fig. 14) and few autophagic vacuoles. Although clusters of dense bodies were noted, it was not possible in the absence of cytochemical studies to estimate their number or distribution in thin sections.

REFERENCES

8. Emmelot, P., and Benedetti, E. L. Changes in the Fine Struc-

Figs. 1–6. Photomicrographs of paraffin sections stained with H & E.
Fig. 1. Hyperplastic nodule (HN) located at edge of liver. Arrows indicate line of demarcation between the nodule and normal liver parenchyma (LI). Note virtual absence of central and portal veins in the nodule. × 35.
Fig. 2. Portion of hyperplastic nodule (HN) illustrating dilation of sinusoids (S). Arrows indicate edge of nodule. Adjacent normal liver parenchyma is slightly compressed. × 40.
Fig. 3. High magnification of border line between hyperplastic nodule (HN) and normal liver parenchyma (LI). Arrows point to outermost nodule cells that lie adjacent to normal liver cells. Many of the cell plates in the nodule are more than 1 cell wide and are more irregular than in the normal parenchyma. The nodule cells have smaller nuclei that are somewhat more uniform in size and a more basophilic, vacuolated cytoplasm. Note mitotic figure (M). × 700.
Fig. 4. Cluster of unidentified cells in a hyperplastic nodule. Their nuclei are elongated and more lightly stained that those of the other parenchymal cells. They may represent early proliferation of bile duct cells or possibly proliferated endothelial cells. × 700.
Fig. 5. High magnification of region of hyperplastic nodule illustrating extensive cytoplasmic vacuolization (hydropic vacuolization). × 900.
Fig. 6. High magnification of region of hyperplastic nodule showing extensive cytoplasmic vacuolization, probably lipid, in the cells. Note pyknotic nuclei in some of these cells. × 850.
Ultrastructure of Hyperplastic Nodules

[Images of tissue sections showing various details of hyperplastic nodules.]
Edward Essner

Figs. 7-18. Electron micrographs of hyperplastic nodule cells.

Fig. 7. Low-magnification micrograph of a portion of a parenchymatous cell between nucleus (N) and bile canaliculus (BC) from a hyperplastic nodule. The characteristic parallel arrays of rough endoplasmic reticulum are replaced by randomly oriented individual cisternae (ER). These cisternae retain attached ribosomes but are greatly diluted and contain a series of rodlike elements (seen in various planes of section) embedded in a matrix of low opacity. Note smaller Golgi apparatus (G) with undilated, somewhat abnormal saccules, lipid droplets (L), microbodies (MI), dense body (DB), and a few slender, tubule-like profiles of smooth endoplasmic reticulum (SER) located predominantly in the glycojen areas (see Fig. 13). × 17,000. Inset A, enlargement of an altered cistera showing rodlike elements in cross-section. × 61,000. Inset B, enlargement of two altered cisternae. One of the cisternae contains a few of the rodlike elements in cross-section, and its membrane is continuous with an undilated segment of endoplasmic reticulum (arrow). The other cisterna shows rodlike elements in oblique and longitudinal section. × 37,000.

Fig. 8. Low-magnification electron micrograph of a parenchymatous cell from a hyperplastic nodule. Masses of smooth endoplasmic reticulum (SER) consisting of tubules of various lengths are oriented in roughly parallel fashion. Depending upon the plane of section, the tubules are seen in longitudinal (middle), oblique, or cross-section (lower left). Individual glycojen rosettes are scattered throughout the area (unmarked arrows). Note a portion of the nucleus (N), the Golgi apparatus (G), a dense body (DB), lipid droplets (L), and autophagic vacuoles (AV). × 15,000. Inset, enlargement showing opaque granules (liposomes 4, 20) in altered cisternae. × 30,000.

Fig. 9. Portion of parenchymatous cell in hyperplastic nodule. Rough endoplasmic reticulum (ER) is dispersed in the form of enlarged, individual cisternae containing an amorphous material of moderate opacity. One very dilated cisterna also contains large globules (IC) of a more opaque material. Part of a nucleus and a small Golgi apparatus (G) are indicated. × 17,000. Inset, enlargement of portion of the dilated endoplasmic reticulum shown in Fig. 9. It suggests the possibility that the opaque material may become engulfed by the membrane of the endoplasmic reticulum and thus gain entry into the cavity of the cisterna or that the material is being extruded from the cisterna. × 42,000.

Fig. 10. A lipid-laden parenchymatous cell from an area of a hyperplastic nodule probably similar to that shown in Fig. 6. Many of the lipid droplets (L) appear to lie within cisternae of rough endoplasmic reticulum (arrows). Other profiles are more difficult to interpret. Some lipid droplets are not completely surrounded by a membrane or are associated with a "double" membrane of rough endoplasmic reticulum and may not be within the cisternae. Note nucleus (N), Golgi saccules (G) reduced in size and number, and microbodies (MI). × 13,000. Inset, enlargement of lipid droplet "within" cavity of rough endoplasmic reticulum. Arrow points to a region of the reticulum membrane that appears "double" making interpretation difficult. × 26,000.

Fig. 11. A cell from a hyperplastic nodule containing numerous small, electron-opaque granules. The rough endoplasmic reticulum is reduced in amount, completely fragmented and dispersed into small, randomly oriented elements. Virtually all the opaque granules are within cavities of these small fragments of the reticulum. Nucleus (N) is indicated. × 11,000. Insets A and B, enlargements showing small, opaque granules within fragments of the endoplasmic reticulum. In Inset A, the reticulum membrane still has a few attached ribosomes, while in Inset B, the reticulum membrane is virtually devoid of ribosomes. Inset A, × 44,000. Inset B, × 44,000.

Fig. 12. Area of a parenchymatous cell near the bile canaliculus (BC) showing Golgi apparatus (G) and a cluster of peribiliary dense bodies (DB). Some of the Golgi saccules (unmarked arrows) remain undilated and appear in atypical angular configurations (arrows). A lipid droplet (LI) is indicated. × 18,000.

Fig. 13. Parallel, tubule-like arrays of smooth endoplasmic reticulum are seen in glycojen area of cell. × 23,000.

Fig. 14. Area showing atypical, circular profiles of undilated Golgi saccules (G). A microbody (MI) (7), possibly containing an abnormal nucleoid, is indicated. × 17,000.

Fig. 15. Circular configuration of undilated Golgi saccule (G) enclosing a number of small vesicles. × 17,000.

Fig. 16. Golgi apparatus (G) composed of unusually long, undilated saccules. One saccule is undilated except for a small area containing opaque material at one extremity. (arrow) × 24,000.

Fig. 17. A group of mitochondria each of which is encircled by an individual cisterna of rough endoplasmic reticulum. Note relatively constant distance between outer mitochondrial membrane and innermost membrane of the endoplasmic reticulum. × 14,000.

Fig. 18. Group of atypical mitochondria (M). Opacity of the mitochondrial matrix is different from normal mitochondria. Cristae are diminished in number and grouped to one side, sometimes appearing as compacted membranes (arrows). × 17,000.

Figs. 19-26. Electron micrographs from "normal" liver of an 18-month, 3-week-old mouse without grossly visible lesions.

Fig. 19. Three mitochondria (M) in compacted form. Note atypical appearance of cristae extending lengthwise in the mitochondria. Due to tangential sectioning, parts of the outer mitochondrial membranes are not visible. At several places small fragments of rough endoplasmic reticulum (arrows) are closely applied to the mitochondria. Note resemblance to similar mitochondria in Novikoff et al. (19) and in Oudea (21). × 22,000.

Fig. 20. Group of mitochondria (M) showing atypical configurations of cristae. Note the vesciculated endoplasmic reticulum. A microbody (MI) is indicated. × 16,000.

Fig. 21. Portion of cytoplasm of a parenchymatous cell in an area bounded by the nucleus (N), sinusoid (S), and bile canaliculus (BC). Masses of altered rough endoplasmic reticulum (ER) occupy center of micrograph. The altered reticulum consists of individual, elongated, tubule-like elements arranged in roughly parallel fashion. These tubule-like elements measure about 100 μm wide and contain longitudinally oriented filamentous material (see Figs. 22, 23). Many small, opaque bodies (liposomes?) lie within (see Figs. 22, 23) and between the altered tubules. Some (LI) approach the size of small lipid droplets. Similar particles can be seen within the space of Disse (S) at the right hand margin of the micrograph. Some lie within pinocytosis vesicles (arrow) forming at the parenchymal cell surface. In this micrograph, the tubule-like elements of endoplasmic reticulum appear to be oriented around a large Golgi area. In addition to the elements of the Golgi apparatus (G), the region also contains several dense bodies (DB) and microbodies (MI). Normal rough endoplasmic reticulum is evident in the upper left portion of the micrograph. A glycogen area (GL) containing smooth endoplasmic reticulum and a microbody (MI) are seen in an adjoining cell at lower right. × 10,000.
Ultrastructure of Hyperplastic Nodules

[Image of an electron micrograph showing cellular structures with labels such as ER, SER, G, DB, N, and MI.]

Downloaded from cancerres.aacrjournals.org on July 20, 2017. © 1967 American Association for Cancer Research.
Edward Essner

Fig. 22. Area of parenchymatous cell illustrating altered endoplasmic reticulum oriented around a Golgi apparatus (G). Although these altered cisternae are shorter and more dilated as compared with those in Fig. 21, they also contain similar small, electron-opaque granules (arrows) and filamentous material, suggesting that both forms are related to the same type of alteration. × 22,000.

Fig. 23. Enlargement of an area containing altered rough endoplasmic reticulum seen in longitudinal section. These elements are similar to those shown in Figs. 21 and 22. They also contain small opaque granules and filamentous material. Only a few ribosomes remain attached to the reticulum membranes. Note ribosomes and ribosomal clusters in matrix between tubules. × 57,000.

Fig. 24. In glycogen areas the altered endoplasmic reticulum is smooth-surfaced and forms a meshwork of smaller elements. Note small opaque granules (arrows) and fibrous material within these altered cisternae. × 60,000.

Fig. 25. A greatly elongated, abnormal mitochondrion is illustrated. The cristae appear reduced in size and are arranged in parallel fashion along the length of the mitochondrion. In the center, oriented longitudinally, is a central core of fine, fibrillar material. Note that the other mitochondria (M) in the cell are smaller and have fewer cristae than those in normal cells. The cytoplasm contains fragmented elements of both smooth and rough endoplasmic reticulum, some of which contain small opaque granules (arrows). A portion of the nucleus (N), microbodies (MI), and a small Golgi apparatus (G) are indicated. × 17,000.

Fig. 26. Parenchymal cell containing altered mitochondria. These mitochondria resemble the one in Fig. 25. Note their irregular shape and the delicate, parallel cristae (arrows). It is not clear whether the cristae are reduced in size or “masked” in some manner by the material comprising the mitochondrial matrix. × 17,000. Inset A, enlargement of a portion of an altered mitochondrion showing longitudinally oriented, fibrillar material of the matrix which appears to pass “through” the cristae. × 68,000.
Ultrastructure of Spontaneous Hyperplastic Nodules in Mouse Liver

Edward Essner


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/27/11_Part_1/2137

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.