Nodules in the Livers of C3H Mice after Long-Term Carbon Tetrachloride Administration: A Light and Electron Microscopic Study

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Summary

Parenchymal nodules developed in the livers of C3H mice given carbon tetrachloride by rectal administration. These non-encapsulated nodules varied from 4 to 14 mm in diameter and were sharply demarcated from the adjacent parenchyma. Despite a lack of organized lobular structure, the nodules contained occasional biliary ductules and scattered blood vessels resembling capillaries. Histologically, the cells composing the nodules were less bizarre than those of the contiguous parenchyma and were, in fact, comparable, in many ways, to the livers of untreated animals.

Ultrastructurally, when contrasted with control parenchyma, the cells of the nodules exhibited enlarged mitochondria, hypertrophied microbodies, prominent Golgi complexes, and abundant dilated rough-surfaced endoplasmic reticulum with numerous attached ribosomes. Nuclei were frequently invaginated and glycogen was abundant. Lysosomes and autophagic vacuoles were few in number.

The morphologic evidence suggests that the nodules represent areas of hyperplasia rather than neoplasia, and this possibility is discussed. Moreover, it would appear that the dilated rough-surfaced endoplasmic reticulum of the nodule cells reflects metabolic activity rather than cellular injury.

Introduction

Carbon tetrachloride has been shown to produce tumor-like nodules in the livers of several strains of mice (10, 11, 13-15). Special attention has been paid to the C3H mouse which has a high incidence of both CCl₄-induced (10, 11, 14) and spontaneous nodules (2, 7, 12). Whether the nodules induced by CCl₄ are truly neoplastic has been previously considered (11, 12), although they usually have been interpreted as hepatomas (10, 11, 14).

The purpose of the present experiment was to study the histologic and ultrastructural characteristics of such CCl₄-induced nodules in the livers of C3H mice.

Material and Methods

Twenty-five male C3H mice, from the Roscoe B. Jackson Memorial Laboratory (Bar Harbor, Me.) were given twice weekly rectal instillations of 0.1 ml of 40% solution of CCl₄ dissolved in olive oil. Ten control mice were given pure olive oil in a similar manner. All animals were 5 weeks old at the beginning of the experiment. After 40-52 administrations (20-26 weeks), the experimental animals were divided into 2 groups; a group of 14 was sacrificed 9 days after their last treatment, while the remaining 11 experimental animals were sacrificed at periods from 3 to 37 weeks after their last treatment. At least 9 days were allowed to elapse between the last dose and the sacrifice of the animals in order that the liver would have sufficient time to recover from the acute effects of CCl₄.

 Portions of the nodules and of the adjacent parenchyma were fixed, for light microscopy, in Zenker's solution or neutral formalin. Hematoxylin and eosin, periodic acid-Schiff, and reticulum stains were done on this material.

For electron microscopy, slices of the nodules and of adjacent parenchyma were trimmed into small blocks (1-2 cu mm) in 1% OsO₄ buffered to pH 7.25-7.35 with phosphate (17). After being trimmed the blocks were immediately transferred into larger quantities of the fixative, and fixation was continued for 2 hr at 4°C. Subsequently, the tissue was dehydrated in graded acetones and embedded in Araldite epoxy resin (26). Thin sections were prepared with a Porter-Blum MT-1 microtome, stained with lead hydroxide (18), and examined with an RCA EMU-3F or -3G electron microscope.

Results

Of the 2 groups of experimental animals, the group of 14, sacrificed 9 days after their last dose of CCl₄, yielded 5 nodules in 5 livers. The group of 11, killed 3-37 weeks after their last dose, yielded 11 nodules in 8 livers. The nodules from the 2 groups of experimental animals showed identical gross, histologic, and...
Gross Features

Grossly, the nodules (Fig. 1) were pale pink or white, and they varied from 4 to 14 mm in diameter. The nodules were not encapsulated but were sharply demarcated from the contiguous parenchyma. There was no extension, either by direct invasion into adjacent structures or by distant metastasis, in any of the animals studied. Cirrhosis failed to develop in any of the experimental livers.

Light Microscopy

In hematoxylin and eosin-stained sections the nodules were seen to consist of uniform parenchymal cells with relatively small nuclei (Fig. 2). Although no organized lobular structure was recognizable (Figs. 2-4), scattered, well-formed bile ductules appeared within the substance of the nodules, as well as at their periphery (Fig. 3).

The nodules abutted directly upon the adjacent parenchyma (Fig. 2), and the contrast between the cells of the nodules and those comprising the remainder of the parenchyma was striking, especially in respect to nuclear features. Nuclei in the nodules were small and uniform, with 1-2 nucleoli; those in the surrounding parenchyma were large and irregular, and often contained multiple nucleoli.

Interposed between groups of parenchymal cells were occasional regular, cylindrical blood vessels, resembling capillaries (Fig. 4).

Electron Microscopy

Controls. The normal ultrastructure of the mouse liver has been amply discussed elsewhere (29); the controls of the present experiment differed in no respect from this. Parenchymal cell nuclei (Fig. 5) were regular in shape, with evenly dispersed chromatin and 1-2 nucleoli, often partially applied to the nuclear membrane (Fig. 5). Mitochondria were small or medium-sized and dispersed at random through the cytoplasm. Several cristae could be seen in each mitochondrion, usually near the periphery of the organelle (Fig. 5). The rough-surfaced endoplasmic reticulum was rather sparse (Fig. 5) and cisternae were surrounded by numerous apparently free ribosomes; the latter, however, might represent attached ribosomes where the cisternae were tangentially cut. Microbodies were present as small, dense, single-membrane-limited particles with denser, laminated or serpentine nucleoids in their centers (Fig. 5). The Golgi complexes were small, with many dilated vesicles (Fig. 5). Occasional lysosomes and autophagic vacuoles were seen, most often near Golgi zones (Fig. 5). Glycogen was plentiful and an occasional lipid droplet was encountered (Fig. 5).

Nodules. Many nuclei of the nodule cells exhibited strikingly irregular profiles (Figs. 6, 8) with tongues of cytoplasm filling clefts formed by invaginations of the nuclear membrane. Such invaginations, in cross sections, appeared as nuclear inclusions filled with cytoplasmic components (Fig. 7).

The mitochondria in many cells were enlarged and contained increased numbers of cristae which, on occasion, seemed to form a central sheaf (Figs. 9, 10). The rough-surfaced endoplasmic reticulum in many instances was moderately dilated. The vesicles and cisternae were usually filled with a fine, flocculent substance (Figs. 9, 11) but rarely with lipid-like granules. Numerous ribosomes were attached to the dilated cisternae (Figs. 9, 11). Polyribosomes were occasionally prominent (Figs. 9, 11).

One of the most unusual features was an enlargement of microbodies in many of the cells. In addition, microbody nucleoids underwent marked elaboration and appeared as greatly enlarged, laminated profiles or as a jumble of threadlike densities (Figs. 12, 13).

The Golgi zones were often quite increased in size and contained many layers of flattened sacs and vesicles, frequently filled with electron-dense material (Figs. 13, 14). Glycogen was abundant (Figs. 9, 10, 13, 14, 15); lipid droplets appeared in small numbers (Figs. 7, 9, 11-14). Both autophagic vacuoles and lysosomes were infrequently seen.

Within the substance of the nodules occasional bile ductules were encountered (Fig. 15). These were lined by cuboidal cells with relatively large nuclei. The cell membranes of adjacent cells interdigitated. Desmosomes were evident on the lumen side of the cells at their junctions with one another (Fig. 15). The entire ductule was separated from surrounding parenchymal cells and adjacent sinusoids by an almost continuous, amorphous basement membrane (Fig. 15).

Now and then blood vessels intervened between adjacent groups of hepatic cells (Fig. 16). The perivascular space contained a basement membrane, interposed between the endothelium and the parenchyma. The endothelial cells were moderately thick. Occasional pseudopodia projected from their external surfaces, and tight intercellular junctions were manifest between neighboring cells. Such structures were thought to be the ultrastructural counterparts of the capillary-like vascular channels viewed by conventional microscopy (Fig. 4).

The ultrastructural features of the nodule cells also differed from those of the adjacent parenchyma. The latter, however, were quite extensive and will constitute the basis of a separate report.

Discussion

The hepatic parenchymal nodules reported here, despite their size and multiplicity, appear to represent foci of regenerative hyperplasia rather than true neoplasia. There was no frank invasion of the adjacent parenchyma or metastasis beyond the confines of the liver. Moreover, the cells of the nodules had histologic features which were less atypical than those of the contiguous parenchyma. This was especially true of nuclear characteristics. Nuclei of the nodule cells were small and uniform in size with only 1-2 nucleoli, whereas many of those in the adjacent parenchyma were large and hyperchromatic, with multiple nucleoli. There was no consistent increase in mitotic activity in the nodules.

These benign cytologic characteristics were also manifest at the ultrastructural level. Thus, it has been reported that neoplastic cells often show marked degrees of cytoplasmic dedifferentiation, with a decrease in the size and number of mitochondria as well as an increase in the amount of free ribosomes (5, 9, 20). These features were coupled with a sparsity of other cytoplasmic com-
components (5, 9, 20). In contrast, the cells of the nodules reported here contained many mitochondria (Figs. 9, 10, 13, 14) and exhibited hypertrophied Golgi complexes (Figs. 13 14), prominent rough-surfaced endoplasmic reticulum (Figs. 9, 11, 14), and rich glycogen stores (Figs. 9, 10, 13-15). The generalized increase of interchromatin granules, reported in the nuclei of many neoplastic cells (6), was also lacking in the CCl4-induced nodules reported here. Finally, as has also been suggested in previous reports (23, 30), the numerous enlarged microbodies (Figs. 12, 13) might constitute an added indication of regenerative activity.

The cytologic characteristics of the nodules reported here stand in contrast to those of hepatomas studied by electron microscopy. Thus, the ultrastructural features of hepatomas induced by p-dimethylnitrosobenzene (1, 27), as well as those described in several transplantable hepatomas of various origins (28), differed significantly from the lesions described here. In a spontaneous transplantable hepatoma from a strain of C3H mice (9), for example, the neoplastic cells exhibited small mitochondria, many free ribosomes, and Golgi areas filled with empty vesicles, whereas the nodules induced in the present experiment showed increased numbers of large mitochondria with abundant cristae (Figs. 9, 10), few free ribosomes (Figs. 9, 11), and Golgi areas containing vesicles filled with an electron-dense material (Figs. 13, 14).

CCl4-induced hepatic nodules have often been interpreted as hepatomas (10, 11, 14); however, such tumors have proved to be notably resistant to transplantation (3, 11). Successful transplantation, reported in only one series of experiments, has been accomplished with the line of C3H mice utilized by Andervont (4). Such tumors appeared as cords of cells, 2 cells thick, flanked on either side by sinusoids. Bile ductules were encountered, but were most often at the periphery of the lesions (10, 11). The nodules in the present experiment were produced in the substrain of C3H mice from the Roscoe B. Jackson Memorial Laboratory. Andervont (2) has stated that these 2 substrains apparently had significantly different characteristics, so that the results obtained in one could not be equated with results in the other. In actuality, the nodules reported here were not characterized by obvious cell cords (Figs. 2-4), and bile ductules were found with regularity within their substance (Fig. 3). This position of the ductules would seem to eliminate the possibility that they were simply preexisting structures incorporated into an expanding neoplasm.

Studies of acute CCl4 poisoning have shown that dilated rough-surfaced endoplasmic reticulum might reflect cell injury (22, 24, 25). In contrast, this did not seem to be the case with the dilated endoplasmic reticulum of the nodules studied here (Fig. 11). This dilution could not reasonably have been a direct toxic effect, since it was present as long as 37 weeks after the last administration of CCl4. It also could not have been related to the structural disarray of cirrhosis, since none of the livers were cirrhotic. Moreover, the hyperplastic nodules exhibited none of the stigmata of injury, such as increased numbers of autophagic vacuoles (8) or dissociation of ribosomes from the endoplasmic reticulum (25). Instead, the cells of the nodules exhibited areas replete with glycogen (Figs. 9, 10, 13-15), many mitochondria with increased numbers of cristae (Figs. 9, 10), and abundant cisternae of rough-surfaced endoplasmic reticulum with numerous, closely applied ribosomes (Figs. 9, 11, 14). In consideration of all these points, it seems likely that the dilated rough-surfaced endoplasmic reticulum reflected metabolic activity rather than cellular injury, and might in this respect be analogous to the dilated rough-surfaced endoplasmic reticulum of active fibroblasts (19, 21) or plasma cells (16).

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References

CCl₄-induced Hepatic Nodules

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Figs. 1–16.—Sections for electron microscopy were stained with lead hydroxide.

Fig. 1.—Nodules in the liver of a CCl₄-treated C3H mouse. The liver contains discrete, sharply demarcated, white nodules. × 2.7.

Fig. 2.—Junction between a nodule and the adjacent parenchyma. The cells of the nodule, on the upper right, are seen, abutting directly upon those of the contiguous parenchyma. Note the difference in size and appearance of the nuclei in the 2 areas. × 144.

Fig. 3.—Portion of a nodule. The cuboidal epithelium of the dilated bile ductules contrasts with the flattened epithelium of the large blood vessels. × 240.

Fig. 4.—Portion of a nodule. A capillary-like blood vessel, cut in longitudinal section, is shown. × 240.

Fig. 5.—Control liver. This portion of a control liver cell exhibits many normal features including a nucleus (N) with a nucleolus (Nu) partially applied to the nuclear membrane, many mitochondria (M), small microbodies (Mb), sparse rough-surfaced endoplasmic reticulum (RER), areas replete with glycogen (Gly) and a few small lipid bodies (L). R, Free ribosome; G, Golgi zone; Ly, lysosome. × 24,400.

Fig. 6.—Portion of a nodule. The nucleus (N) presents a very irregular profile. A myelin figure (arrow) is seen in the cytoplasm. × 16,800.

Fig. 7.—Portion of a nodule: The nucleus (N) contains a large inclusion filled primarily by lipid droplets (L). The double nuclear membrane (arrows) can be seen surrounding the inclusion. × 23,100.

Fig. 8.—Portion of a nodule. An irregular, horseshoe-shaped nucleus is shown here. × 23,500.

Fig. 9.—Portion of a nodule. The mitochondria (M) in this picture are enlarged and one shows a central sheaf of cristae. Small segments of dilated rough-surfaced endoplasmic reticulum (RER) are also present. × 24,400.

Fig. 10.—Portion of a nodule. Hypertrophied mitochondria (M) are shown with increased numbers of cristae. × 28,600.

Fig. 11.—Portion of a nodule. Many dilated cisternae of rough-surfaced endoplasmic reticulum (RER), filled with a flocculent material, may be seen here. Note small ribosomes studding the membranes. L, Lipid. × 24,400.

Fig. 12.—Portion of a nodule. This picture contains 3 hypertrophied microbodies (Mb) with elaborate, dense nucleoids. L, Lipid. × 44,700.

Fig. 13.—Portion of a nodule. Several large microbodies (Mb) can be seen in this picture. A prominent Golgi zone (G) is also shown. × 23,500.

Fig. 14.—Portion of a nodule. An enlarged Golgi zone (G) fills a large portion of this picture. Note the electron-dense material in many of the Golgi vesicles. L, Lipid. × 22,700.

Fig. 15.—Portion of a nodule containing a bile ductule: The cuboidal cells of a bile ductule (BD) are illustrated here. The cells may be seen to interdigitate along their borders, and desmosomes (D) are prominent. A thin, amorphous basement membrane (arrows) separates the bile ductule from adjacent cells. × 11,500.

Fig. 16.—Portion of a nodule. This micrograph shows a cross-sectioned blood vessel whose lining cells exhibit tight, intercellular junctions (J), as well as an occasional pseudopodium (P). Notice the duplication of the basement membrane (arrows) in the perivascular space. × 23,500.
CCL_4-induced Hepatic Nodules
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