AN ELECTRON MICROSCOPIC STUDY OF ALCOHOLIC HYALIN

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As described and defined originally by F. B. Mallory,[1] alcoholic hyaline bodies occur characteristically in abundance only in the diseased livers of alcoholic patients. Similar eosinophilic cytoplasmic masses have been observed, however, in non-alcoholic human cirrhosis,[2–4] in non-cirrhotic human liver [4] and in certain types of experimental damage to the animal liver.[5–7] It is thus not clear from light microscopic studies whether or not the "classic" alcoholic hyaline bodies are related specifically to the metabolic effects of ethanol or, of equal importance, if the other various types of cytoplasmic masses are truly comparable in their structure, evolution and implications.

The purpose of the present study was to determine the ultrastructure and morphogenesis of alcoholic hyalin in biopsy material obtained from patients with active alcoholic liver disease. It was hoped to gain further insight into the pathogenesis of Laennec's cirrhosis.

MATERIAL AND METHODS

Percutaneous needle (Vim-Silverman) liver biopsy was carried out in 14 adult patients shortly after their admission to the Massachusetts General Hospital. All patients gave histories of moderate to heavy alcoholic consumption and concomitant poor food intake for periods of months to years; all had signs and symptoms of hepatic dysfunction ranging in duration from 2 weeks to 3 years. On initial study, all had some clinical and laboratory evidence of liver dysfunction but appeared free of hepatobiliary disease unrelated to alcoholism.

Portions of each biopsy specimen were placed in Technicon fixative, embedded, sectioned and examined by conventional microscopy using the hematoxylin and eosin and Masson stains. Other portions of the tissue were fixed immediately in cold buffered 1 per cent osmium tetroxide (with 45 mg. sucrose per ml.), dehydrated in a graded series of ethanol and embedded in n-butyl methacrylate. Ultrathin sections were cut with a Porter-Blum microtome and examined with an RCA EMU-3B electron microscope.

RESULTS

Light Microscopic Findings

By light microscopy the following diagnoses were established in the 14 patients: fatty liver, 2 cases; periportal fibrosis, 2 cases; Laennec's

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(portal) cirrhosis, slight to advanced in degree, 9 cases; and finely nodular cirrhosis of uncertain type, 1 case.

Five of the 9 specimens showing Laennec's cirrhosis contained liver cells with clustered eosinophilic perinuclear masses characteristic of alcoholic hyalin (Figs. 1 and 2). As noted by previous workers,[8] these cytoplasmic structures varied considerably in size and number, and the liver cells involved were occasionally surrounded by neutrophils (so-called satellitosis). Parenchymal cells containing alcoholic hyalin rarely showed significant fat vacuolation.

Electron Microscopic Findings

In 5 biopsy specimens, the liver cells contained abnormal cytoplasmic masses that corresponded closely in size, number and distribution to the hyaline bodies found in the corresponding paraffin sections. Although the sequential cytoplasmic changes that resulted ultimately in the appearance of "typical" alcoholic hyalin were not evident in each specimen, in one a variety of additional cytoplasmic changes were observed which seemed to constitute the intermediary stages.

Typical hyaline bodies, as seen in all 5 biopsy specimens, consisted of large, irregular, amorphous perinuclear masses within hepatic cells (Fig. 3). These aggregates were without a limiting membrane, and consisted primarily of irregularly disposed, fine granular and filamentous structures (Fig. 4). Occasionally an inner, densely osmiophilic core was seen in the midst of these aggregates (Fig. 5). The masses were usually clearly delimited from the adjacent cytoplasm, and normal cytoplasmic organelles were generally excluded. Occasionally, concentric membranous structures (myelin forms) were found at the margins of the hyalin (Fig. 6). It is significant that cells containing hyalin were generally otherwise unaltered and exhibited normal-appearing cytoplasmic organelles; fat inclusions were uncommon, and nuclear alterations were not seen. Since there was no appreciable active cellular degeneration in neighboring parenchymal cells in 4 of the specimens, it seemed likely that the hyaline bodies constituted "late" or "mature" forms. Nearby cells occasionally showed mitochondrial abnormalities, including giant forms with alteration in number, structure and distribution of cristae (Fig. 7).

In one specimen there was, in addition, a great variety of parenchymal degenerative changes from focal cytoplasmic lesions to involvement of the entire cell. The smallest lesions consisted of dense amorphous bodies and irregular osmiophilic masses intermingled with fragmented membranous structures (Fig. 8), the latter sometimes arranged as myelin forms. The dense bodies resembled lysosomes, said to be associated

with certain autolytic processes.[9] Some of the focal alterations appeared initially to be membrane-limited. The origin of these abnormal structures was uncertain, but morphologic evidence suggested that they were derived in part from altered cytoplasmic organelles. For example, mitochondria were observed undergoing partial or total fragmentation, becoming vacuolated structures containing fine and coarse electron-dense debris, apparently derived from the cristae (Fig. 9). Denser amorphous bodies suggestive of lysosomes were also seen within foci of degeneration (Fig. 9). In more extensive lesions the focal alterations appeared to coalesce and to form large pleomorphic aggregates which were no longer membrane-limited (Figs. 10 and 11). In their midst, the characteristic fibrillar and granular hyaline masses appeared (Figs. 11 and 12). In some instances, altered mitochondria were enmeshed in these large amorphous masses (Fig. 10). At this stage, residual normal cytoplasmic organelles remained in adjacent areas of the cell, emphasizing the "piecemeal" nature of the degenerative process. Some cells, in which the process was most advanced and involved the entire cytoplasm, were surrounded by neutrophils (satellitosis).

Discussion

These electron microscopic studies of the liver in alcoholic patients with Laennec's cirrhosis have shown the underlying alteration to be a focal cytoplasmic degeneration involving mitochondria and other cytoplasmic structures such as lysosomes. The masses recognizable by conventional microscopy as alcoholic hyalin or Mallory bodies arose within these areas of acute degeneration. The observations confirm and extend those of Schaffner, Loebel and Weiner,[10] who reported that mitochondrial enlargement and degeneration were frequent ultramicroscopic features of biopsy specimens in "acute alcoholic hepatitis." Similar mitochondrial degeneration and clumping have been described in human infantile cirrhosis [2] and in the livers of choline-deficient rats,[11] indicating that mitochondrial alterations are not unique to alcoholic liver disease. Parenthetically, the lack of specificity might well justify the use of the less limited term "Mallory bodies." None of these morphologic studies have provided direct insight into the mechanisms by which alcohol, undernutrition or specific nutritional deficiencies induce mitochondrial damage and dysfunction. Swelling and disruption of hepatic mitochondria may be produced by many agents that alter membrane permeability or uncouple oxidative phosphorylation, the critical process that generates utilizable chemical energy within the cell. It is known experimentally that alcohol depresses certain mitochondrial oxidative reactions,[12] perhaps by "fragmenting" the electron transport chain, but

there are no studies that correlate the metabolic impact of ethanol with its possible role in progressive structural damage to the mitochondrion. Since mitochondrial changes induced by factors such as anoxia, surface-acting agents [13] and deficiency of essential fatty acids [14] do not proceed to the final distinctive pattern of alcoholic hyalin, additional significant factors must be involved.

The form and variable electron density of certain portions of the evolving hyaline bodies suggested that lysosomes and other cytoplasmic structures were also involved in the acute degenerative stages. The "myelin forms" indicated the presence of phospholipid-containing material. This phenomenon is frequently associated with damaged membranes. Frequently, the earliest foci were membrane-limited structures which contained a variety of dense osmiophilic material, dense bodies and membranous structures. The morphologic resemblance of some of the dense bodies to lysosomes was in keeping with the role of the latter in autolytic phenomena.[15] Coalescence of cytoplasmic degenerative foci led to the formation of large areas which were no longer membrane-limited. The characteristic hyaline masses arose within these areas in an undetermined fashion. The observation that alcoholic hyalin was present in many hepatic cells otherwise morphologically intact was of considerable interest. The lack of associated degenerative changes in these cells suggested strongly that some of the cytoplasmic alterations were reversible and did not necessarily eventuate in complete cell destruction. The alternative possibility that the cells with only hyaline masses represented early stages of the process seems unlikely.

Another form of hepatic hyaline degeneration, similar at the light microscope level, has been described following treatment of rats with the carcinogen, 3′-Me-DAB.[16] Electron microscopy revealed that these areas consisted almost exclusively of closely packed smooth-surfaced endoplasmic reticulum.[16] The fine structural difference between this form of hyalin, indistinguishable by conventional microscopy from alcoholic hyalin, points up the need for further analysis of hyaline degeneration induced by various agents.

In summary, the current study has demonstrated the structure and probable development of alcoholic hyalin so characteristic of human liver disease in alcoholism. To paraphrase the comment of F. B. Mallory,[17] we may have the fingerprint of the criminal but we still do not know how the crime was committed.

Summary

Correlative light and electron microscopic studies of liver biopsy specimens obtained from alcoholic patients with various stages of Laen-

nec's cirrhosis have shown "mature" alcoholic hyalin to consist of amorphous granular and filamentous perinuclear cytoplasmic masses that are not membrane-limited. These observations suggest also that swollen and degenerated mitochondria and altered lysosomes contribute to the formation of alcoholic hyalin, often with preservation of other subcellular structures. Critical investigations are needed to correlate the biochemical and morphologic events induced within the hepatic cell by chronic ethanol ingestion.

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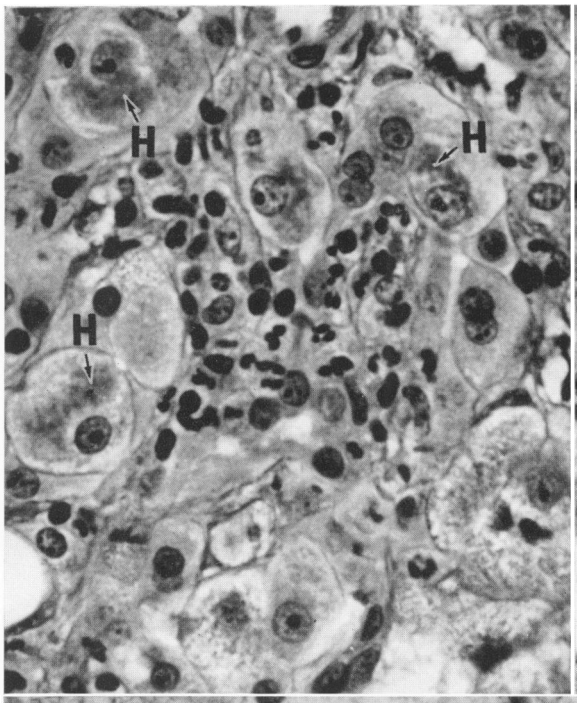
Legends for Figures

FIG. 1. Active and moderately advanced Laennec's cirrhosis with abundant alcoholic hyalin (H) clumped around cell nuclei. Hematoxylin and eosin stain. × 400.

FIG. 2. Coarse aggregates of alcoholic hyalin (H) in a degenerating hepatic cell, with surrounding satellitosis by neutrophils. Hematoxylin and eosin stain. × 400.

FIG. 3. Liver parenchymal cell showing several irregular masses of hyalin (H) adjacent to the nucleus (N). Although there is no limiting membrane, the mass is well demarcated. The adjacent mitochondria (M) and endoplasmic reticulum (ER) are normal. × 19,000.



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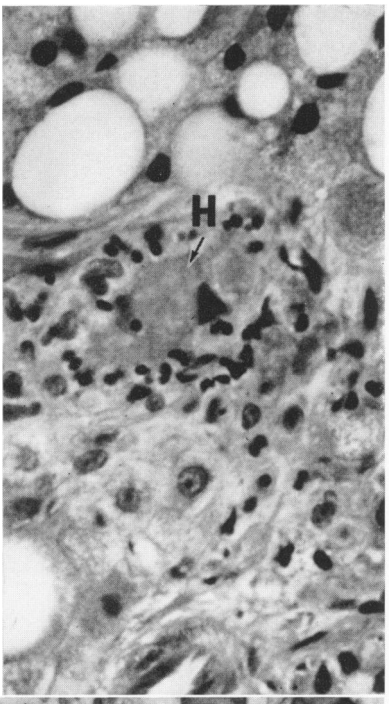

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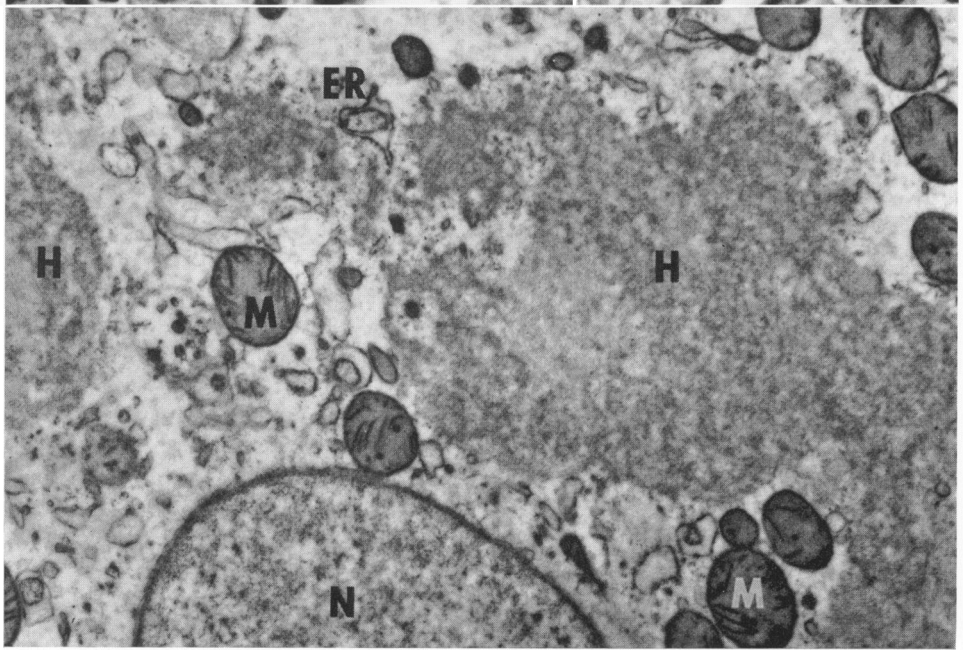

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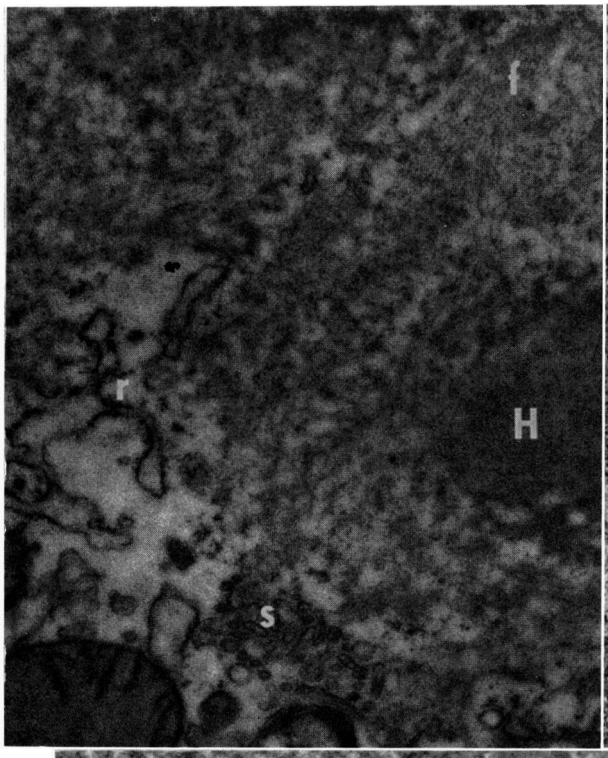
FIG. 4. A mass of hyalin (H) exhibits areas composed of the fine filamentous structures (f) and granular elements. Smooth (s) and rough (r) surfaced endoplasmic reticulum lies adjacent to the mass. × 48,000.

FIG. 5. A perinuclear hyaline mass exhibits a dense osmiophilic granular component (d) and, in addition, a typical pale fibrillar component (p). Nucleus, N. × 11,600.

FIG. 6. A portion of a perinuclear hyaline mass (H) consisting primarily of the pale component. Interspersed within it are numerous "myelin forms" (mf), consisting of multiple concentric rings of fine membranous material and rough endoplasmic reticulum (r). × 14,000.



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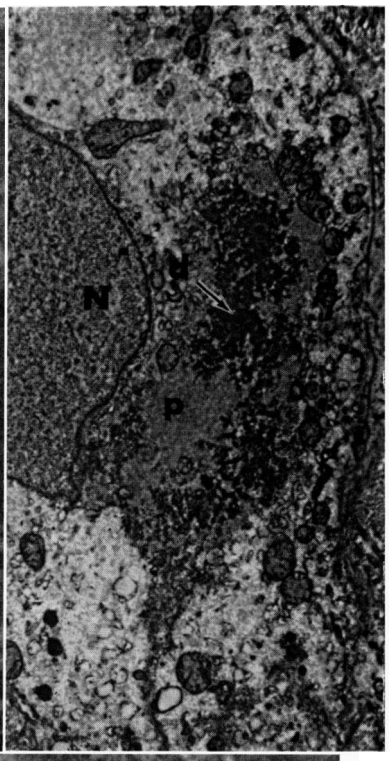

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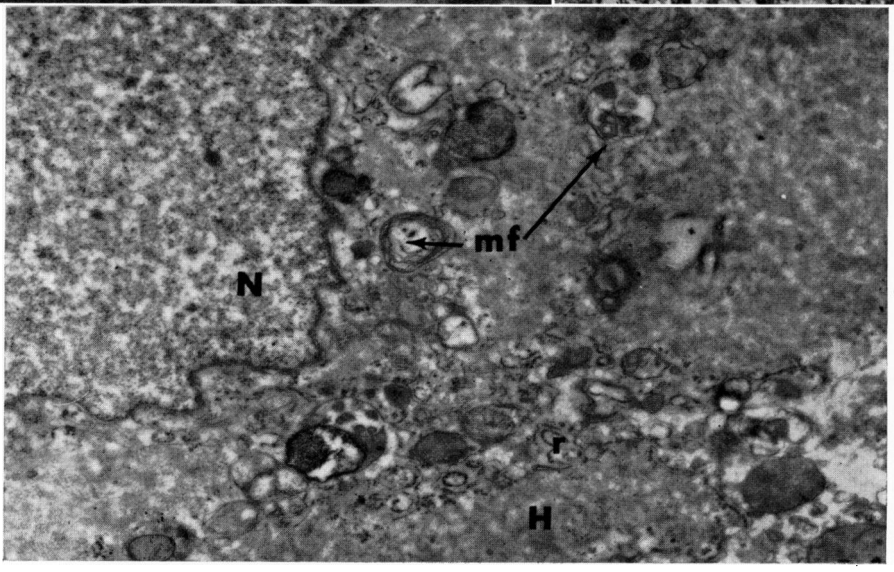

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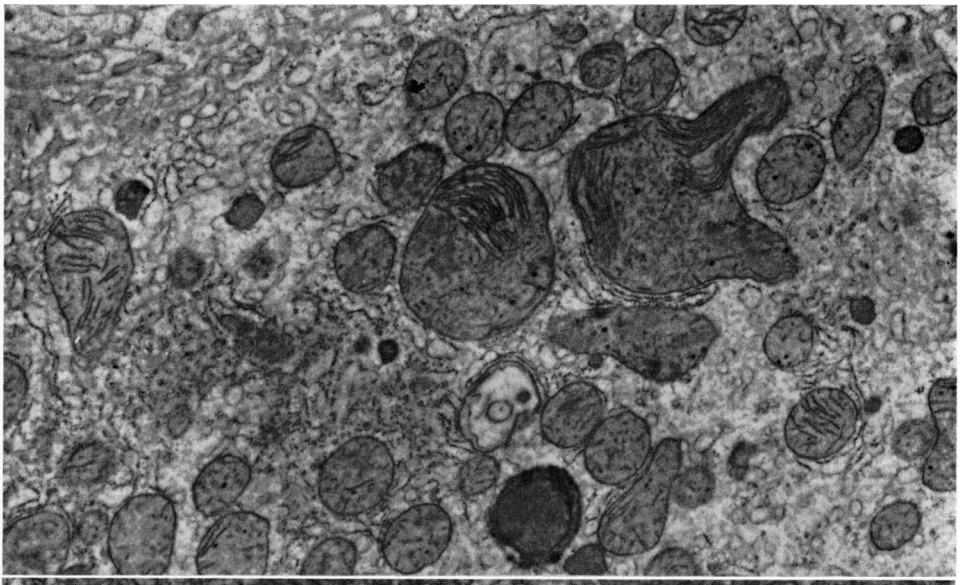

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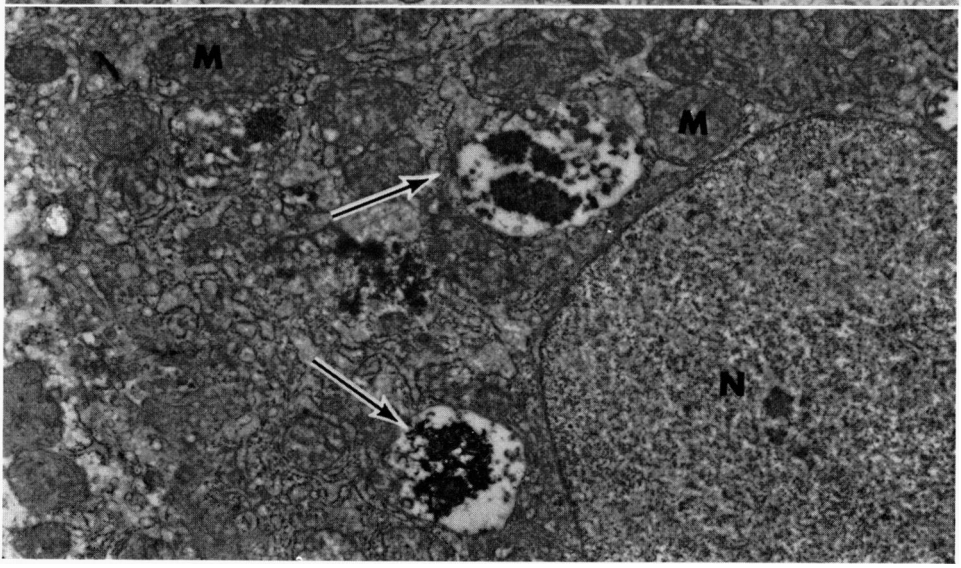

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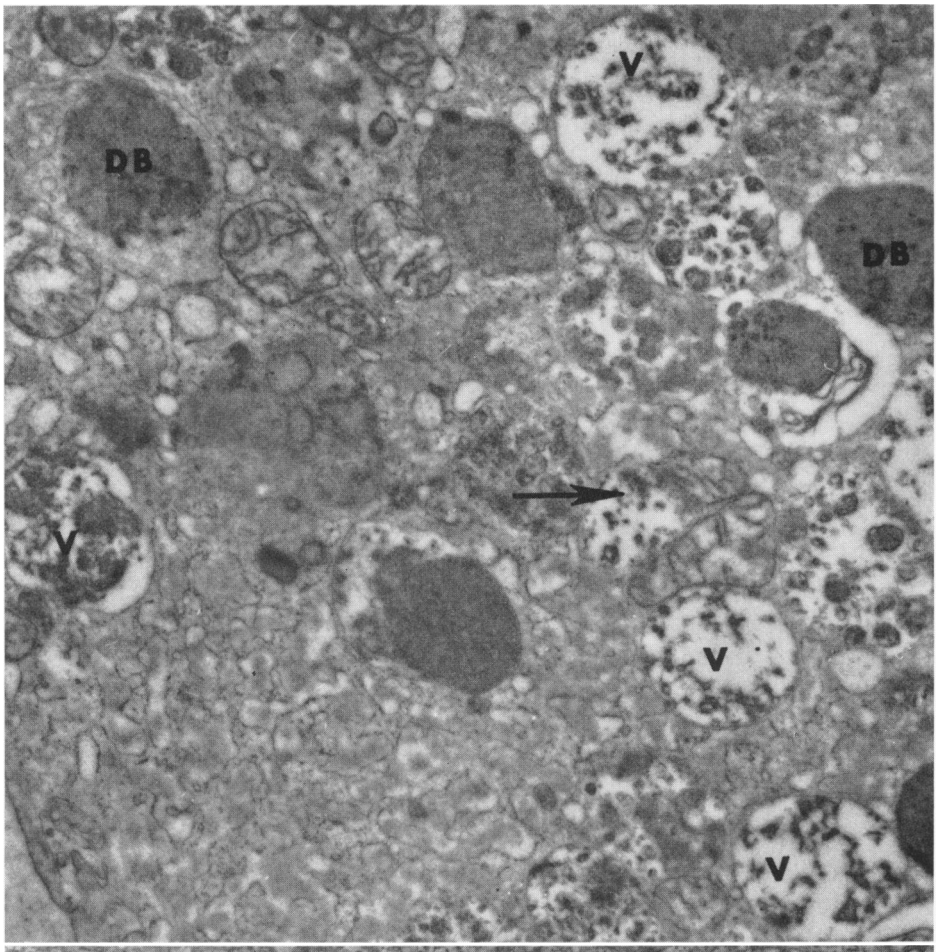
FIG. 7. A portion of a hepatic cell exhibits several abnormally large irregular mitochondria. The cristae are closely packed in some regions and some have circular profiles. The remainder of the cell is unremarkable. × 16,000.

FIG. 8. Two focal areas of cytoplasmic change are manifest within a hepatic cell (arrows). These comprise densely osmiophilic, irregular masses. The foci are well circumscribed and appear membrane-limited. × 17,000.

FIG. 9. A portion of a damaged hepatic cell exhibits a variety of altered cytoplasmic organelles. Numerous membrane-limited vacuolar structures (V) contain irregular fragments; one portion of a degenerating mitochondrion consists of similar material (arrow). A variety of denser bodies of uncertain nature (?lysosomes) are also present (DB). × 30,000.

FIG. 10. Several well-circumscribed foci of cytoplasmic degeneration (large arrows) appear within a liver cell. These consist of irregular amorphous dense bodies, membranous structures and myelin forms. Some of the larger bodies have a distinctly fibrillar character (small arrow). × 15,000.



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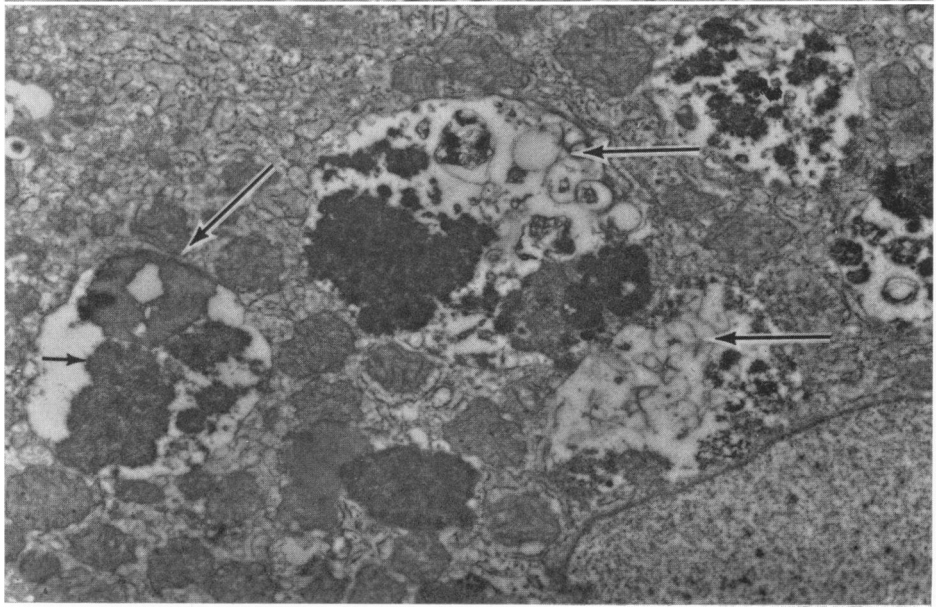

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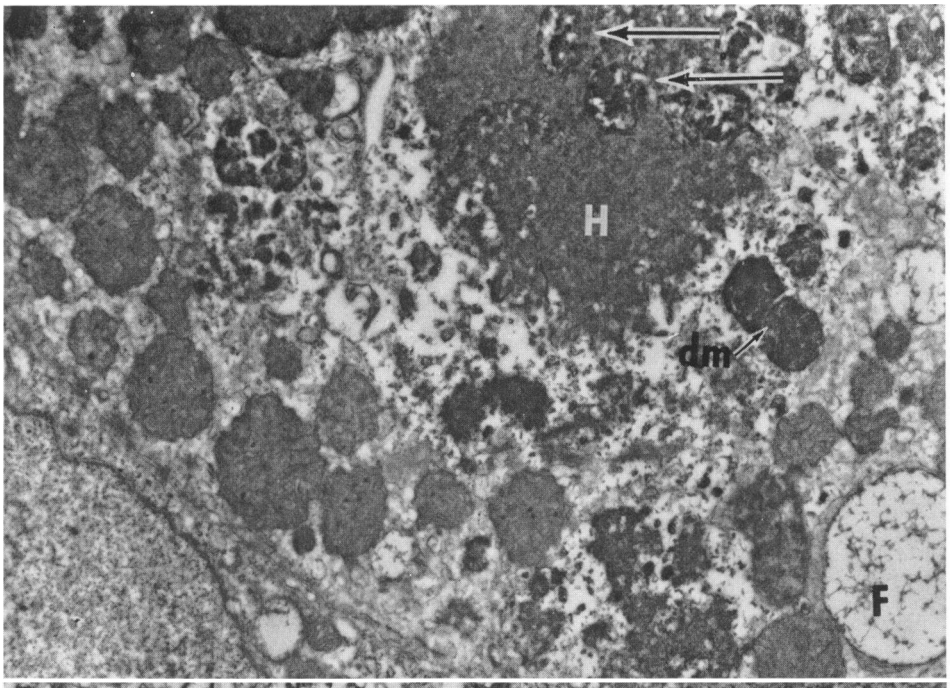
FIG. 11. At the upper right is a portion of hepatic cell cytoplasm showing an advanced stage of focal degeneration. At its upper margin is a large mass of amorphous fibrillar material (H), morphologically identical to the hyaline bodies described above. Incorporated within it are several denser bodies resembling damaged mitochondria (arrows); similar organelles, more clearly mitochondrial in origin, are present in an adjacent area (DM). The remaining degenerating area consists of fragmented membranous structures and amorphous dense aggregates. Small fat droplet, F. Peripheral areas of the same cell, and the adjacent one (lower left) appear normal. × 17,000.

FIG. 12. A hepatic cell contains several large foci of cytoplasmic vacuolation. The one on the left contains two large fibrillar hyaline masses (H) with adjacent fragmented membranous structures and irregular osmiophilic debris; its margin is not clearly demarcated from the adjacent cytoplasm. The other area contains osmiophilic bodies and scattered granular material; it is sharply delimited. × 17,000.



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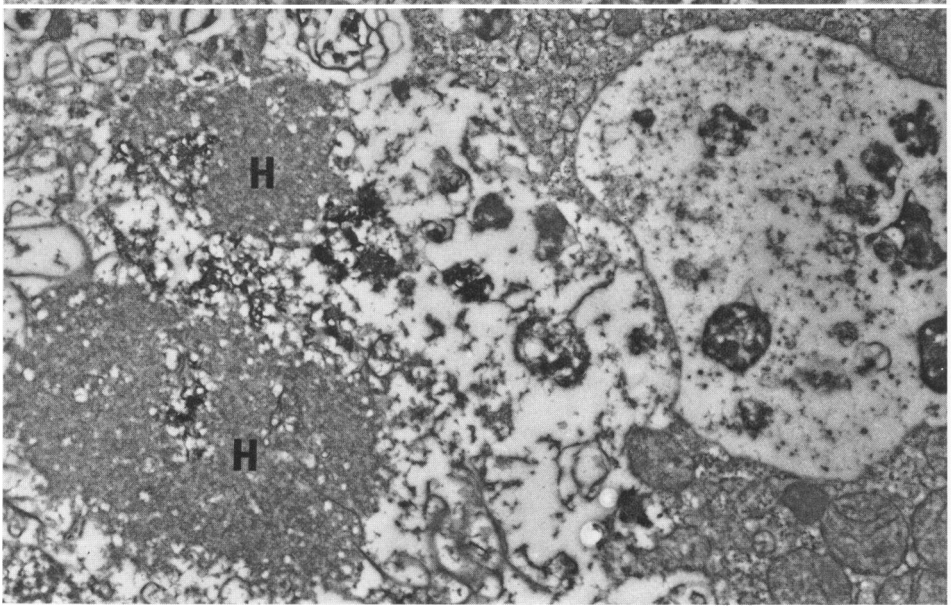

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