ACUTE ALCOHOLIC HEPATITIS

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In the last decade particular attention has been focused on a clinical pattern of acute hepatic insufficiency occurring in individuals with chronic alcoholism.[1-9] Although it is realized that the morphologic hepatic alterations appear more variably than the rather uniform symptoms, signs and laboratory findings, the frequency of acute inflammatory and degenerative changes in liver biopsy specimens would justify the term acute alcoholic hepatitis, used first by Beckett, Livingstone and Hill[7] and more recently employed by other investigators.[8,9] Previous clinico-morphologic studies, however, have included a large and rather heterogeneous group of patients in whom the only common factor was prolonged excessive intake of alcoholic beverages. Several morphologic alterations have been described in acute alcoholic hepatitis by light[1-8] and electron microscopy.[9] The wide variability of changes reported would seem to correspond in part to a lack of uniformity in the group with chronic alcoholism.

In order to seek more precise information on the ultrastructural hepatic changes occurring in this condition we have confined our study to only 3 cases. In these there have been uniform historical and clinical manifestations. The specimens were selected from a large number of biopsy tissues obtained from female patients with alcoholism. The tissues from our strictly selected cases showed a remarkable similarity in their histologic features. This consistency helped to compensate for the small number of cases reported and the lack of serial biopsy in the same patients.

MATERIAL AND METHODS

Liver biopsy specimens, taken with the Vim-Silverman or Menghini needles, were obtained shortly after the admission of 3 women, 50, 48 and 40 years of age, with manifestations of hepatic disease. All three admitted to recent drinking, had long histories of heavy alcohol intake (more than 1½ quarts of wine or beer and more than a pint of spirits daily for 20 to 30 years) and had poor food intake. Common features in all patients at the time of biopsy were hepatomegaly (4 to 8 finger

This study was partially supported by Grant USPH 62-420.

Presented in part at the Symposium on Cellular Injury of The American Association of Pathologists and Bacteriologists, Chicago, Illinois, April 5, 1964.

Accepted for publication, December 17, 1964.

breadths below the costal margin), slightly elevated serum bilirubin values, abnormal sulfobromophthalein retention, increase of serum globulin, elevation of serum alkaline phosphatase, transminase and lactic d-hydrogenase, positive cephalin flocculation tests and increased thymol turbidity levels.

For electron microscopy a portion of each biopsy specimen was fixed by immersion in Dalton's chrome-osmium solution,[10] at 5° C for 1 hour, dehydrated in graded dilutions of ethanol and then embedded in Epon 812 [11] or in Maraglas.[12] Sections were cut with a Porter-Blum ultramicrotome using glass knives. In order to select the areas to be studied by electron microscopy, unstained sections (cut at 1 to 2 μ) were examined by phase microscopy; others were stained with borax-buffered toluidine blue [13] or Nile blue A [14] for bright field microscopy. In some, adjacent thin sections from preselected areas were stained by flotation on uranyl acetate or on phosphomolybdic acid by the method of Watson [15] and mounted on uncoated copper electromesh grids. Others were stained with lead using the Millonig or Karnovsky methods.[16–17] Sections were examined with either an RCA-EMU-3F or a Phillips 200 electron microscope at various magnifications.

For light microscopy the other portions of each specimen were fixed in 3:1 acetic acid-methyl alcohol and embedded in paraffin. Sections were stained with hematoxylin and eosin, oil red O, McManus periodic acid-Schiff (PAS) (with and without diastase digestion), Verhoeff-Van Gieson, Masson trichrome, phloxin methylene blue, Mallory's aniline blue, Perls' iron stain, and Luxol fast blue techniques.

Results

Light Microscopy

All sections from the 3 cases exhibited similar changes, the differences being mainly a matter of degree. Hepatic architecture was extensively altered, and conformed generally to a portal type of cirrhosis. Diagnostic limitations frequently encountered in needle biopsies, however, were also experienced in our cases. In some areas for example, liver cell plates were arranged haphazardly between irregularly placed efferent veins and small, non-fibrosed, portal triads, a pattern more characteristic of coarse nodular cirrhosis. Imperfect arrangement of the radial plates, due in part to fatty cyst formation, appeared here and there. Rather extensively the plates were multicellular (two or more cells in thickness) and lacked fatty changes. These were considered portions of regenerative nodules. The fibrosis was, nevertheless, predominantly of the stellate type extending from the portal tracts into the lobule parenchyma (Fig. 1). Thick fibrous trabeculae were absent in all.

Most conspicuous were various manifestations of hepatocellular degeneration chiefly associated with fatty changes. Fat droplets of varied size were encountered in most hepatocytes, except in regenerating areas. Smaller droplets (1 to 2 μ) were scattered throughout the cytoplasm, although they were most frequently encountered in the portions facing the sinusoids; larger, coalescent drops (5 to 10 μ) which displaced nuclei pointed the transition to advanced stages when single large globules (up to 40 μ) occupied almost the entire cytoplasm (Fig. 2). In this stage hepatocytes were enlarged to 3 or 4 times their normal size (average

normal diameter, 25 μ). Their cytoplasm even when reduced to a thin peripheral rim, contained normal concentrations of glycogen as evidenced by the distribution of PAS-positive, diastase-digestible granules. The basophilic cytoplasmic material (basophilic bodies) was slightly reduced in amount in most such degenerated cells. Finally, numerous fatty cysts represented the late stage of the progressive fatty changes occurring in these livers. The cysts measured up to 200 μ in diameter and were most frequently located periportally (Fig. 3). All other degrees of fatty change also were usually most prominent in this location. Occasionally fatty cysts were entrapped within the connective tissue of portal spaces although trabecular fat was usually found within macrophages. The majority of identifiable Kupffer cells contained varying amounts of visible fat irrespective of their location.

The histochemical characterization of soluble lipids by light microscopy was possible in Epon and Maraglas-embedded and stained tissue, while insoluble lipids were readily identified in paraffin-embedded sections stained with oil red O. In toluidine blue-stained sections the lipid material displayed various shades of green, droplets in hepatocytes staining less intensely than those of Kupffer cells. In Nile blue A-stained sections the affinity of the variously dispersed lipids was faint and more uniform in either Epon or Maraglas-embedded material. In paraffin-embedded sections stained with oil red O most of the lipid material which had accumulated in the Kupffer and other mesenchymal cells, was insoluble, staining to a moderate degree. In unstained sections examined with the fluorescent microscope, the characteristic yellowish-bronze autofluorescence displayed by the lipofuscins was demonstrable. From these and other histochemical reactions performed (Ziehl-Neelsen, PAS and performic acid-Schiff stains) it was concluded that the lipoid pigment in these littoral and mesenchymal cells was ceroid. On several occasions it was especially well noted in the Epon and Maraglas stained sections that ruptured fatty cysts had released some of their fat into adjacent sinusoids.

Other degenerative changes encountered included loss of cytoplasmic basophilia with varying degrees of acidophilia or "hyalinization" and the presence of Mallory bodies (Fig. 4). In Epon and in Maraglas-embedded thin sections stained with toluidine blue the configuration of alcoholic hyalin was polymorphic with confluent perinuclear clumps displaying various shades of blue. These parenchymal alterations were, however, not extensive but focal, and were generally restricted to periportal positions. These foci were invariably accompanied by inflammatory mesenchymal cells, particularly eosinophils, although mast cells were not infrequently found (Fig. 5). Necrosis of individual hepatocytes

was also observed within such inflammatory foci. Cholangiolar proliferation of mild degree appeared in the moderately fibrosed portal tracts (Fig. 6). The amount of iron detected histochemically was not greater than that encountered in livers with cirrhosis generally. The light microscopic features are summarized in Table I.

TABLE I

LIGHT MICROSCOPIC ALTERATIONS IN ALCOHOLIC HEPATITIS *

Case #	I	II	III
Alteration of hepatic architecture	++	+++	+++
Regeneration	++	+++	+++
Fibrosis	+++	++	++
Hepatocellular fat	+++	++	++
Fatty cysts	+++	++	++
Ceroid	+	+	++
Loss of basophilia	++	++	+
Mallory bodies	+	+	+
Necrosis	++	+	+
Intralobular inflammation	++	++	++
Glycogen content	Normal	Normal	Normal
Cholestasis	+	+	+
Cholangiolar proliferation	++	++	+
Iron	+	+	+

* +, slightly increased or occasionally seen.
++, moderately increased or frequently seen.
+++, markedly increased or very frequently seen.

Electron Microscopy

Since the light microscopic features of the hepatic architecture in the biopsy tissues have been described, the electron microscopic findings will be grouped for purely descriptive purposes under the following headings: hepatocytes; Kupffer cells; and sinusoids.

Hepatocytes. Although extensive quantitative and qualitative variation in the ultrastructural alterations of the hepatocytes was seen in all the specimens, 3 types were most prominent. First, hepatocytes containing droplets of fat (coalescent or not); second, those forming parts of the walls of fatty cysts; and third, hepatocytes without visible fat, at least within the plane of section. In either Epon or Maraglas-embedded material the electron density of the lipid droplets was usually faint or at best moderately dense and they displayed a rather amorphous configuration irrespective of size. In Maraglas sections fat droplets easily contracted under the electron beam, even at low intensity. This distortion was, however, not so great as to disguise its nature and only occasionally created minimal and readily identified artifacts in the surrounding cytoplasmic organelles. Small fat droplets (1 to 3 μ in diameter) were most

frequently located within the cytoplasm of hepatocytes beneath the sinusoid margins (Figs. 7 and 8). The droplets, usually grouped closely together, were partially surrounded by a limiting membrane. This membrane was believed to have been derived from smooth-surfaced endoplasmic reticulum because, in general, it lacked characteristic ribosomal granules in uranyl acetate-stained sections. Instead it almost invariably showed a close relation to glycogen granules (500 Å in diameter) which surrounded fat droplets in a single concentric row. Moreover, rough-surfaced endoplasmic reticulum was absent in the vicinity of lipid droplets. Coalescence of small droplets into large ones was suggested in areas in which droplets were separated only by single tenuous septa; these were found in many cases to be disrupted at points of maximal stress (Fig. 9). Still larger fat globules occupying nearly all of the hepatocyte and surrounded only by slim rims or crescentic remnants of cytoplasm were also seen. Finally, large pools of fat filled cysts were not infrequent (Fig. 10).

Intracystic fat was limited by plasma membranes of hepatocytes forming the walls of the cysts. At certain points these membranes were extremely attenuated or completely absent so that fat lay in direct contact with the cytoplasmic elements. In several instances the fat lacked any interposed membrane and appeared to be in direct contact with the sinusoid lumen.

The nuclei of hepatocytes containing variously sized fat droplets, including even those extremely compressed or forming walls of cysts, appeared essentially unaltered. Other cytoplasmic organelles were, however, often conspicuously altered, although in any one cell not all components were simultaneously affected.

The most striking change in hepatocytes was a bizarre configuration of the smooth endoplasmic reticulum associated with intensely electron-dense particles of glycogen (Figs. 11, 12 and 13). Paired, parallel smooth-surfaced membranes arranged either in straight or concentric arrays and occasionally adopting a form not unlike "fingerprints" were frequently seen. Between pairs of these smooth-surfaced ER membranes, although not attached to them, were dense, roughly spherical granules measuring an average of 500 Å in diameter usually aligned in single rows and separated by an average distance of 200 Å. Both in Epon and Maraglas-embedded tissue the glycogen granules could be deeply stained by lead hydroxide, phosphomolybdic and phosphotungstic acid; this was not the case in uranyl acetate-treated sections.

The complex of membrane-particulate arrays was most frequently located within peripheral parts of the cytoplasm adjacent to sinusoids and was only occasionally seen in other areas. More than 30 per cent of

the hepatocytes affected by fatty change, including those forming the walls of fatty cysts, showed this peculiar type of configuration. It was found in fewer than 5 per cent of hepatocytes without visible fat droplets in the plane of section. Apart from these rather localized arrangements, a more diffuse and more frequent disposition of the smooth ER was found in fatty as well as in non-fatty hepatocytes. It consisted of round or oblong cisternae of various sizes. The vesicles of these cisternae were usually surrounded by a corona of glycogen granules similar to those lying in the parallel arrays. In these cases the single glycogen particles maintained their individuality, showing but slight tendency to form clusters. The clusters, however, were more common here than between the parallel arrays of endoplasmic reticulum.

Finally, in some focal cytoplasmic areas well developed rough-surfaced ER was found. It was not associated with glycogen granules but with very distinctive and very electron-opaque ribosomal granules ($\cong$ 150 Å) attached to the outer surface of the lamellae. This rather well preserved ergastoplasm with orderly arrangement was not only particularly prominent in the vicinity of nuclei but was also frequently encountered in pericanalicular areas of the hepatocyte. In some instances the peribiliary ergastoplasm was dilated to such a degree that the spaces bounded by the smooth sides of the lamellae gave a vesicular aspect to these portions of the hepatocytes. A homogeneous substance of low electron density was seen within such spaces while the interspaces limited by the granular side of the lamellae was occupied by a dense material still devoid of glycogen granules.

Although mitochondria of normal appearance were seen in many otherwise altered hepatocytes, their overall configuration was markedly changed. Mitochondrial enlargement leading to megamitochondria measuring up to 15 μ in diameter was the feature most commonly observed. There were three types of enlarged mitochondria. (1) In hepatocytes with little fat the mitochondrial enlargement was rather widely distributed throughout the cytoplasm although the largest, giant organelles were preferentially located near the sinusoidal border. These were spherulated and the density of their matrices was uniformly increased along with a reduction in the number of opaque granules (Fig. 14). The largest mitochondria were generally limited by very distinctive outer and inner membranes and their cristae were shortened even to the point of disappearance. The cristae of moderately enlarged mitochondria, however, particularly those in their centers, frequently extended from one side to another suggesting, on occasion, a complete division of the organelle. Intervening spaces between cristae were sometimes widened or focally ballooned and their matrices were paler than those in the main body of

the mitochondrion (Fig. 15). (2) In other hepatocytes only a few mitochondria were obviously enlarged but these assumed bizarre shapes (Fig. 16). Their abnormally dense matrices frequently contained focal areas of rarefaction. A prominent feature was a striking increase in the number of cristae. In addition to short peripheral cristae in these misshapen mitochondria were others of varying lengths usually orientated in stacks of parallel lamellae, occasionally simulating myelin figures. The predominant location of these enlarged mitochondria varied from cell to cell although they were seen most frequently near the bile canaliculi. (3) Another type of enlarged mitochondrion found near the sinusoids in some hepatocytes was represented by rod-shaped organelles of normal density and width but of extreme length (Fig. 17). Peripheral cristae of these mitochondria were curved or coiled. Central ones were of moderate length and associated with conspicuous parallel arrays of thinner membranes of less electron density which were more closely interspaced than the cristae ($\cong$ 200 Å). These structures also encountered in other types of misshapen mitochondria were considered to be true myelin figures (Fig. 18). Such crystalline-like inclusions were also encountered in some giant misshapen mitochondria and occasionally extended from one pole to another but at other times occupied a number of separate loci within the same mitochondrion. The membranes or bands composing these inclusions were in general uniformly electron dense in longitudinal section although occasionally in certain places they appeared to have a regularly alternating dense and light periodicity. Cross sections of these "myelin figures" showed a regular hexagonal lattice structure. Finally, other mitochondrial changes included the loss of the double limiting membrane, diffuse or focal swelling with decreased density of the matrices, marked whorling of cristae or even more bizarre alterations such as those which lead to "compartmentalization" of the matrices (Fig. 19). A variety of profound degenerative changes of mitochondria were seen in fragments of organelles incorporated within autophagic vacuoles (*vide infra*).

In many hepatic parenchymal cells, particularly those with little or no visible fat, the presence of focal cytoplasmic degradation appeared to indicate that a rather localized and severe injury had occurred. In most instances these areas of varied size were surrounded by a distinct limiting membrane suggesting a sequestration from the rest of the cytoplasm. For descriptive purposes and in keeping with the nomenclature widely used at present we have termed these sequestered cytoplasmic areas autophagic vacuoles. They were surrounded by definite limiting membranes and contained still recognizable yet variously altered cytoplasmic constituents (Fig. 20). Frequently in the vicinity of these

vacuoles numerous pleomorphic membrane-bounded bodies of various sizes (0.2 to 4 μ) were found to contain laminated electron dense structures along with pale homogeneous masses and other more heterogeneous or vacuolated inclusions (Fig. 21). The latter lysosome-like bodies were usually located in the pericanalicular region (Fig. 22). On occasion, large polymorphic bodies (up to 15 μ), only partially limited by a membrane, were seen in pericanalicular regions or in other areas of the cytoplasm. The structure of the large bodies was more compact and more homogeneous, although in some areas and particularly at the periphery they frequently contained laminated figures and recognizable mitochondria in different stages of degradation (Figs. 23 and 24). The main constituent was a moderate electron dense granular or finely fibrillar substance.

These large structures, often branched or confluent, were correlated with Mallory's alcoholic hyaline bodies seen by light microscopy. A clear cut difference between the larger and the smaller lysosome-like bodies, however, did not exist in most cases. From the electron micrographic evidence in our material we concluded that this hyalin was probably made up of lysosome-like bodies composed of more homogeneous material and in most instances was only partially surrounded by a limiting membrane. The presence of some degraded but still recognizable cytoplasmic constituents at the periphery of the bodies together with the altered cytoplasmic organelles found in the vicinity, such as markedly degenerated mitochondria and disorganized endoplasmic reticulum suggested that any of the altered cytoplasmic constituents could probably participate in the formation of the Mallory bodies.

Kupffer Cells. In areas where the hepatic architecture was altered there was an increase in the number of Kupffer cells and macrophages. Both types of cells were usually heavily loaded by masses (0.5 to 25 μ or more in diameter) of lipoid pigment histochemically characterized as ceroid and usually surrounded by a limiting membrane (Fig. 25). The internal configuration of these pigment granules varied from solid homogeneous electron dense material, coarse granules or multivesicular or multivacuolar structures. Individual masses usually maintained a uniform internal structure throughout the entire residual body in which they were contained. In this way some ceroid was composed exclusively of coarse granular material while other deposits were formed chiefly by multivacuolar material. A few residual bodies combined these forms to suggest transitional stages from compact or coarse granular to multivacuolar material or *vice versa.* The largest masses of ceroid were in general those with multivacuolar structure and probably represented intermediate stages of the pigment formation.

Several ceroid-laden mesenchymal cells were usually grouped focally particularly near portal spaces. Accumulation of fibrous tissue was also more prominent in and around these areas although no constant relation could be found between the two. These collections of macrophages might well have obstructed sinusoidal flow thereby contributing to alteration of hepatic architecture.

Sinusoids. As implied above, proliferation of Kupffer cells and focal conglomerations of other mesenchymal cells distended by pigment so altered sinusoidal arrangement that obstruction of the sinusoid lumen could be postulated. The lumens of most sinusoids, however, were normal or occasionally slightly dilated. The most striking change was found in the spaces of Disse which were, in general, dilated, sometimes widely and often contained rather dense bundles of connective tissue fibrils displaying typical collagen periodicity (Fig. 26). Very frequently the bundles extended to adjoining hepatocytes in which deep indentations suggested that the connective tissue had dug into the cytoplasm. The number of hepatocellular microvilli was reduced at these points and a fairly continuous basement membrane of low electron density was observed immediately beneath the endothelial lining. The spaces of Disse so affected were mainly those located near portal triads.

Discussion

Previous studies [7,8] have shown that fatty and inflammatory changes are the most consistent features of alcoholic hepatitis. Although these changes are frequently superimposed on more chronic lesions such as fibrosis and coarse nodular cirrhosis as in our cases, they are not necessarily accompanied by chronic liver disease. The fatty liver *per se* may produce symptoms of acute hepatic failure, particularly in patients with alcoholism,[1,3,18] yet it is usually latent and benign, giving rise to few or no symptoms.[19] The hepatomegaly present in our patients could be related to the fatty alterations although inflammatory infiltration, liver cell regeneration and fibrosis also played roles.

Currently available data from animal experiments and human biopsy studies indicate that alcohol produces a fatty liver which may eventually progress to cirrhosis. The pathogenesis of this disorder is still the subject of much debate. For years it has been a difficult task for pathologists to reproduce the hepatic lesions encountered in the liver of chronic alcoholism in experimental animals.[20] In 1949, Best, Hartroft, Lucas and Ridout [21] succeeded in the production of the hepatic lesions, providing evidence that the chronic administration of alcohol to rats increases the choline requirements; they suggested that this effect was due to the increased caloric intake provided by alcohol. Subsequent studies by

Klatskin, Krehl and Conn [22] and Klatskin and Krehl [23] have confirmed these findings but demonstrated that the lesions are not wholly dependent on food consumption or caloric intake. More recent work by Lieber, Jones, Mendelson and De Carli [24] suggest that alcohol might have a more direct effect on the liver irrespective of lipotropic requirements. Although acute ethanol intoxication in rats appears to be a rather separate experimental entity, the fatty liver produced under these circumstances seems also to be the result of a more direct effect of alcohol. It was shown by Di Luzio,[25] and confirmed in Hartroft's laboratories,[26] that choline chloride has no effect in this acute ethanol-induced fatty liver. It was also demonstrated,[27] however, that the lobular hepatic distribution of the abnormal stainable fat was periportal, in sharp distinction to the centrilobular distribution associated constantly with early dietary choline deficiency and chronic alcoholism in rats.

It is interesting to note that while lipotropic substances have no effect on triglyceride accumulation in acute experimental alcoholism, more recent experiments [28,29] have proved that the administration of certain antioxidants reduce or completely prevent the hepatic fatty changes. In addition Porta and Hartroft have shown [30] by combined biochemical and electron microscopic approaches that vitamin E and other antioxidants not only prevent the fatty changes but also reduce or completely prevent the ultrastructural changes in mitochondria and endoplasmic reticulum induced by alcohol under these conditions. Since the constituents of these membranous organelles are rich in unsaturated fatty acids it seems now that a possible mechanism of action of antioxidants could be the prevention of lipid peroxidation damage. In support of this assumption, biochemical data have indicated [31–36] that lipid peroxidation produces severe damage to mitochondrial and lysosomal enzymes. Furthermore, the presence of ceroid pigment in macrophages found in the livers of individuals with chronic alcoholism [37] as well as in the 3 cases herein presented, would indicate that peroxidation occurs to a certain extent in this condition since the pigment is the product of peroxidation of unsaturated lipids.[38–44] At any rate, the possible effect of antioxidants on the liver in chronic alcoholism in human beings and experimental animals is at present unknown but offers a fruitful field for further study.

The cirrhogenic nature of the fatty liver has been clearly shown by Hartroft and Ridout [45] in experimental cirrhosis induced in the rat by dietary choline deficiency, and the progression of fatty liver into cirrhosis in man has been demonstrated by Popper, Szanto and Elias [46] as well as by others.[47,48] According to Hartroft [49,50] the rapidly enlarged intracellular fat globules in the rat break the plasma membranes in such

a way that the fat is released into the extracellular space. The pools of fat do not lie freely but are contained in epithelial cysts whose walls are formed by the parent hepatocytes. A report published by Lombardi [51] indicated that the cysts were actually formed by Kupffer cells rather than hepatocytes. Electron microscopic studies of fatty cysts in rats,[52] however, and the present investigation in human subjects show that the walls of these cysts are indeed formed by hepatocytes with varying ultrastructural cytoplasmic changes although with little or no intracellular fat. The fat contained in the cysts is in immediate contact with the plasma membranes of the hepatocytes forming the wall. These membranes are focally attenuated or absent suggesting the passage of fat from the cytoplasm into the fat pool or *vice versa*. It could also represent ruptures at points of maximum stress in the progressive fusion of one fatty cyst with another or with single fat-laden hepatocytes. This seems to be the case in Figure 10.

The rupture of cysts, their collapse and eventual disappearance, according to Hartroft,[49,50] would produce the condensation of the persisting reticulin into strands or bands which would thereby form trabeculae. The mechanisms which appear to be of major importance in experimental dietary cirrhosis may not be the only ones linking the fatty liver to cirrhosis. Elias and Popper [53] have emphasized that tissue stress fissures and actual membrane formation after inflammation may play an important role in the development of the human cirrhosis of Laennec type. Electron microscopic studies by Popper, Paronetto, Schaffner and Perez [54] have shown further that hepatic fibrosis resulting from either accumulation of preformed fibers or from the new formation of fibers occurs in the portal tracts, around hepatic cell plates and around proliferated cholangioles with varying contributions of each mechanism to the different forms of cirrhosis.

Another possible and more controversial mechanism in the development of fibrosis has been advanced by MacDonald.[55] This author has suggested that the earliest change leading to the development of fibrous tissue is the proliferation of endothelial cells from central and portal veins and from cells lining the sinusoids. The obliteration of these vessels would result later in the formation of dense fibrous bands.

We have not been able to determine in our material which of the aforementioned mechanisms is the commonest in the development of fibrosis. The study of serial biopsy specimens from patients with alcoholic liver damage not as advanced as in our cases would be necessary before any conclusion could be drawn. On the other hand we were impressed by the frequent finding of considerable amounts of fibrous tissue around hepatic cell plates. It is known from previous electron microscopic studies [54,56–58]

that few collagen fibers are normally found in the spaces of Disse. It has been also shown by Popper and co-workers [54] that in chronic fatty metamorphosis with liver cell damage or in cirrhosis, these fibers are greatly increased in number. In our biopsy material many spaces of Disse were markedly widened by the accumulation of numerous connective tissue fibrils arranged in parallel fashion. Although this type of fibrosis was usually seen near portal spaces it was also found in other locations. The presence of fibrils in areas where the hepatic plates were arranged in orderly manner and the preservation of relationships to Kupffer cells, suggested that the pericellular aggregation of fibrils occurred without actual loss of cells. The nearby hepatic parenchymal cells, however, always showed obvious ultrastructural changes and might have been possible stimuli for fibrogenesis. Fibroblasts were not seen in the vicinity of the sinusoidal alterations but Kupffer cells were increased in number supporting the hypothesis that the latter may act as fibroblasts forming the ground substance.[54] The fibrosis in the spaces of Disse together with the presence of a basement membrane immediately adjacent to the Kupffer cells in some of the altered sinusoids as in our cases, has been previously reported by Schaffner and Popper [59] in patients with various liver disorders. These changes have been designated "capillarization of hepatic sinusoids" and the authors have suggested that their presence render less effective the parenchymal blood flow already reduced by shunts and sinusoidal obstruction. In this way pre-existing hepatocellular insufficiency may be aggravated.

Since the hepatic blood flow in human fatty liver is reportedly normal,[60] and it is usually normal or slightly low in different types of cirrhosis [61–63] it now seems possible that the abnormal sulfobromophthalein retention found in similar conditions is, at least in part, directly related to the sinusoidal alteration and in this way overshadows parenchymal hepatic damage in non-jaundiced patients.

The sequential relationship between fat and fibrosis in animals with dietary cirrhosis has also been questioned by Handler and Dubin.[64] These authors have suggested that fat and fibrosis are independent manifestations of the cirrhogenic nature of the diet, choline being responsible for the fatty changes while methionine for the fibrosis. More recent experiments by Grisham and Hartroft,[65] using two diets with different levels of protein but so adjusted that both were equally deficient in choline and methionine, indicate that under these circumstances the amount of dietary protein (not merely methionine) is the key factor in the development of cirrhosis. In these experiments it was shown that in the absence of adequate protein the fat is cirrhogenic but high levels of dietary protein prevent the development of cirrhosis. Due in part to

the well known difficulties in obtaining a satisfactory nutritional history from individuals with alcoholism it would be premature to draw any conclusions for man, but it is interesting to note that alcoholic patients with a history of poor food intake are those most likely to develop cirrhosis.[48] On the other hand, excessive alcohol consumption and a poor nutritional intake have been repeatedly found in cases of acute alcoholic hepatitis.[1,8] It has been reported that ceroid is absent or scanty in human cirrhosis.[66,67] With adequate staining techniques and by electron microscopy, however, considerable amounts of this pigment can be readily and consistently identified in the liver. In our cases ceroid was detected mainly in Kupffer and other mesenchymal cells while little was found in hepatocytes.

Previous histochemical studies have provided evidence that ceroid might be a product of auto-oxidation of unsaturated lipids. The mechanisms of its formation have been extensively studied by experiments *in vitro* and *in vivo*. Hartroft and his group have shown transition forms between red cells and ceroid aggregates in hemorrhagic fatty cysts in the livers of choline deficient rats,[68] in the mesenteric and epididymal hemorrhagic fat pads,[69–71] and with studies *in vitro* by incubating a mixture of fresh blood with highly unsaturated lipids.[69–72] Inasmuch as similar transitional forms were encountered in Kupffer cells and other macrophages in our specimens, it now appears that the same mechanisms can be postulated for the pathogenesis of ceroid in alcoholic cirrhosis.

Ceroid accumulation in cirrhosis in rats can be reduced by either supplementing the choline deficient diet with alpha-tocopherol or replacing the unsaturated fats in the diet with saturated oils.[67] Vitamin E may act to inhibit ceroid formation by virtue of its role as an antioxidant. Although it is intriguing to see that alpha-tocopherol and other antioxidants also prevent the acute ethanol-induced fatty liver in rats, it is premature at present to interpret this finding in relation to a possible but not proved vitamin E deficiency in alcoholic cirrhosis.

The large spectrum of mitochondrial alterations in alcoholic hepatitis can not be considered specific for this condition since similar changes are encountered in lesions produced by a great variety of injurious hepatic agents[73,74] including dietary deficiencies.[75] On the other hand the enlargement and myelin degeneration of mitochondria which other investigators[9,76,77,78] have also found consistently in alcoholic hepatitis, if not pathognomonic, are at least very prominent features of this condition. These two mitochondrial alterations which occur in hepatocytes with or without fatty changes, may possibly bear a relation to the effect of excessive amounts of alcohol intake. It has been postulated that this effect is mediated through intermediate nutritional factors.[77] In this

regard, Porta, Hartroft and Meyer[79] have observed spherulation and enlargement of mitochondria in the livers of choline deficient rats, but this type of change has now been reported by others in various chronic nutritional abnormalities in experimental animals.[75] More recently, our studies in acute ethanol intoxication of rats[27,30] have clearly shown a dramatic but transient mitochondrial alteration in size and shape. These changes, which were not corrected by diet supplementation with choline, were almost completely preventable by the oral administration of alpha-tocopherol. This, again, suggests the possibility of a role for this vitamin in the development of lesions induced by alcohol.

The peculiar complex of smooth endoplasmic reticulum and glycogen particles, arranged in annular or "fingerprint-like" fashion, appeared to be a common feature in alcoholic hepatitis because it was found in many hepatocytes in our patients and in similar cases previously studied by Schaffner.[80] These arrays have also been observed by Salomon[81] in hepatocytes of rats chronically intoxicated with thioacetamide and by Steiner, Miyai and Phillips[82] after chronic ethionine administration. The latter authors considered the alterations to be regenerative rather than degenerative lesions and probably related to the glycogen-storing capacity of hepatocytes in hyperplastic nodules. They also suggested a possible role of these lesions in the neoplastic transformation of the cells.

We do not know the significance of these changes in alcoholic hepatitis. Since they were found more frequently in degenerated hepatocytes, however, and were improbably related to areas of regeneration, we have considered that they might represent morphologic expressions of cellular decay. Although it seems possible that the enzymatic mechanisms controlling the synthesis and breakdown of glycogen could be altered in alcoholic hepatitis, further investigations are necessary to elucidate this point.

The alcoholic hyaline degeneration (Mallory bodies,)[83] commonly associated with alcoholic cirrhosis,[84–86] may rarely be encountered in non-alcoholic human cirrhosis[87,88] as well as in non-cirrhotic human livers with other disorders.[89] Although its incidence in acute alcoholic hepatitis has been variously reported[1,8,9] it is generally considered a common feature of this condition and its presence has been associated with a poor clinical prognosis by some authors.[1,90] Based on histochemical and electron microscopic observations the "Mallory bodies" found in the dietary cirrhosis of rats on low-choline diets were initially assumed by Hartroft[91] to be derived from degenerated mitochondria that became enlarged and clumped together. This author proposed the same pathogenesis for the hyaline degeneration appearing in human conditions and

his opinion is shared by others.[9,87,92,93] Some workers,[94,95] however, with similar histochemical features but rather different electron microscopic observations have postulated an origin from ergastoplasm. Recently Flax and Tisdale [76] have concluded that both degenerated mitochondria and altered lysosomes contribute to the formation of intracellular alcoholic hyalin. These authors observed that focal cytoplasmic alterations, supposedly a sort of immature hyalin, appeared to be membrane-limited, while the final, mature bodies were not. In our own material we found focal areas of cytoplasmic alterations, in many instances only partially surrounded by a membrane, and frequent transitional forms between moderately sized autophagic vacuoles (lysosomes) and extensive areas of degradation not membrane-bound. It, therefore, appears possible that Mallory bodies may be derived from any cytoplasmic constituent via lysosomes. It is obvious that one of the greatest difficulties in the electron microscopic identification of the Mallory body stems from the fact that its histochemical characterization in tissues embedded in the resins currently used, is still to be accomplished. Toluidine blue staining is not enough. Thus, all the conclusions or suggestions on the origin of these bodies have been made only by inference.

The fate of Mallory bodies is at present unknown. The existence of these focal alterations, probably related to lysosomal digestive mechanisms would not necessarily predicate total hepatocyte necrosis. The body could be extruded from the cell, as in other focal degenerations (Councilmen bodies, etc.), leaving behind a viable hepatic parenchymal cell.

Summary

The ultrastructural features of strictly selected liver biopsy specimens from 3 women with alcoholism exhibiting similar clinical features and showing uniform pathologic changes by light microscopy were examined. Fatty hepatocellular alterations leading to the formation of fatty cysts were one of the most consistent findings. More than 30 per cent of the hepatocytes affected by fatty changes, including those forming the wall of fatty cysts showed bizarre "fingerprint-like" aggregates of parallel arrays of membranes studded with glycogen particles. Mitochondrial enlargement, the most common feature in these organelles, was accompanied by alterations of cristae and matrices and abundant crystalline-like inclusions (myelin figures). Focal cytoplasmic degradation encountered in some hepatocytes suggested a relationship to the formation of Mallory bodies and it appeared that any of the altered cytoplasmic constituents could probably participate in the formation of these structures. Kupffer cells and reactive mesenchymal elements contained large

amounts of lipids which in most instances had the ultrastructural configuration of ceroid in different stages of evolution. Fibrosis appeared mainly as thin connective tissue fibril bands located in the spaces of Disse frequently appearing to abut into hepatocytes and also probably contributing to sinusoidal "capillarization."

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The authors wish to express their thanks to Professor W. Stanley Hartroft for his advice in the preparation of the manuscript.

LEGENDS FOR FIGURES

FIG. 1. Fibrous tissue near a portal space extends into the lobular parenchyma. Verhoeff-Van Gieson stain. × 500.

FIG. 2. Droplets of fat in hepatocytes. Epon-embedded thin section stained with toluidine blue. × 500.

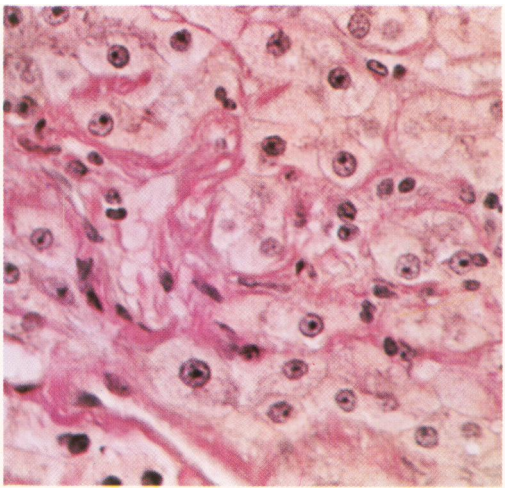
FIG. 3. Fatty cysts. Epon-embedded thin section stained with toluidine blue. × 500.

FIG. 4. Mallory bodies (arrows). Masson trichrome stain. × 500.

FIG. 5. Parenchymal alterations and inflammatory infiltration. Masson trichrome stain. × 200.

FIG. 6. A portion of a portal space exhibits fibrosis and cholangiolar proliferation. Epon-embedded thin section stained with toluidine blue. × 500.

1



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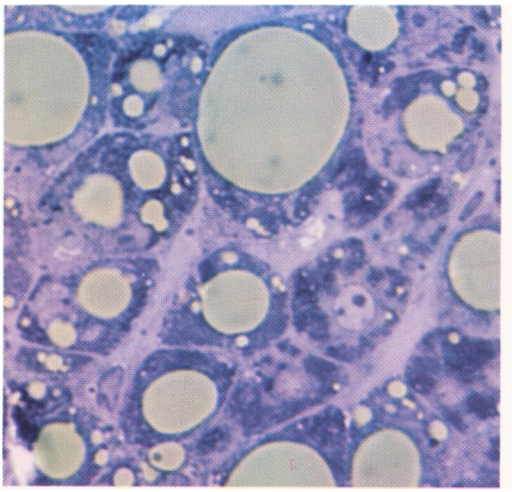

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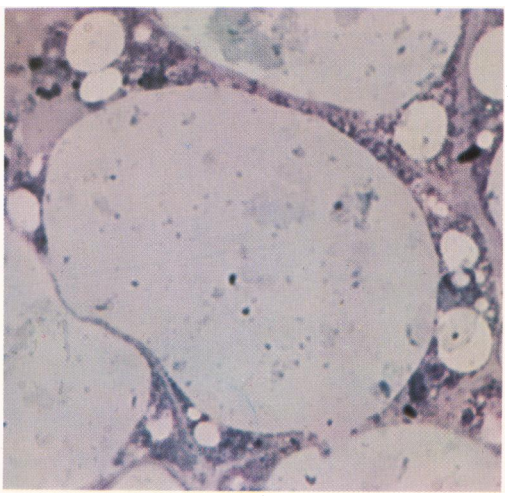

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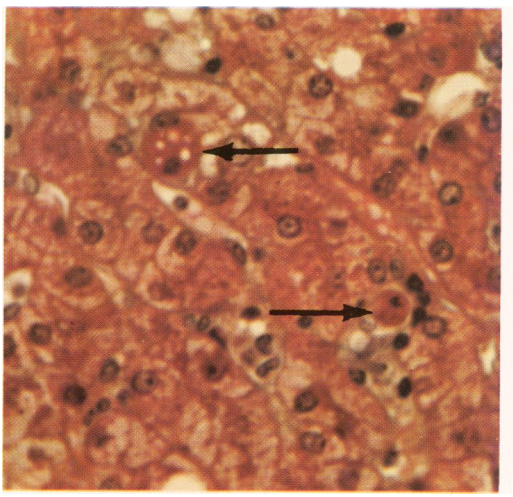

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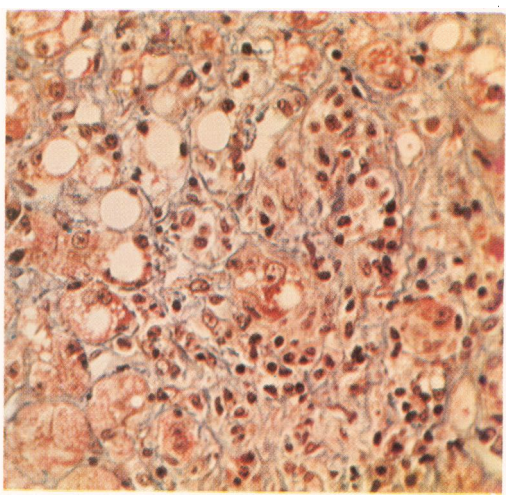

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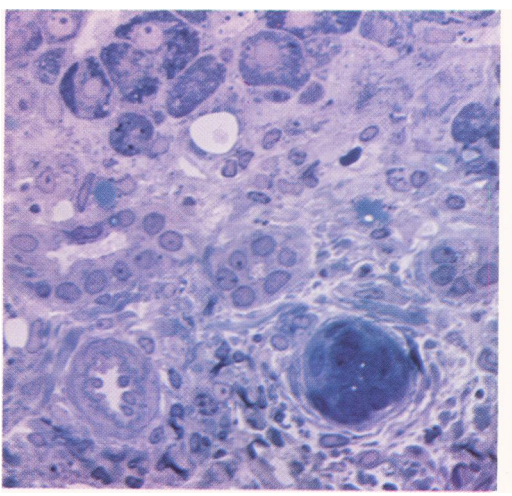


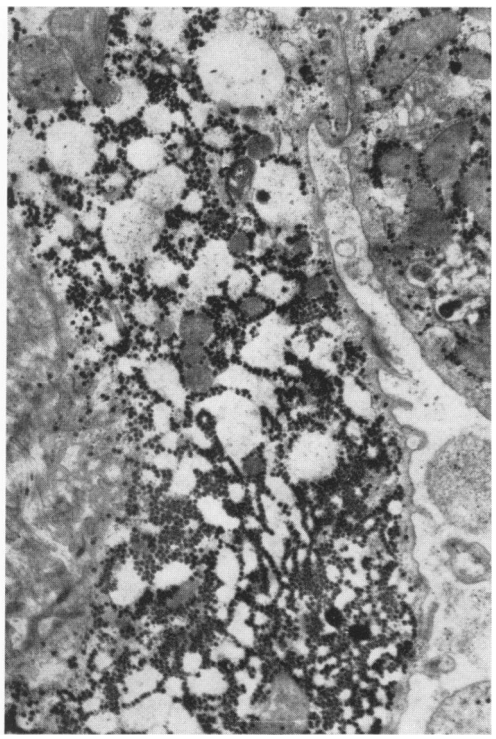
Unless otherwise stated electron micrographs were prepared from epon-embedded tissues stained with lead.

FIG. 7. Small fat droplets in a hepatocyte are surrounded by granules of glycogen. × 10,000.

FIG. 8. A medium-size fat droplet in a hepatocyte. × 11,600.

FIG. 9. Coalescent droplets of fat in a hepatocyte. × 10,000.

FIG. 10. A portion of the wall of a fatty cyst. Maraglas embedded, stained with lead. × 13,000.



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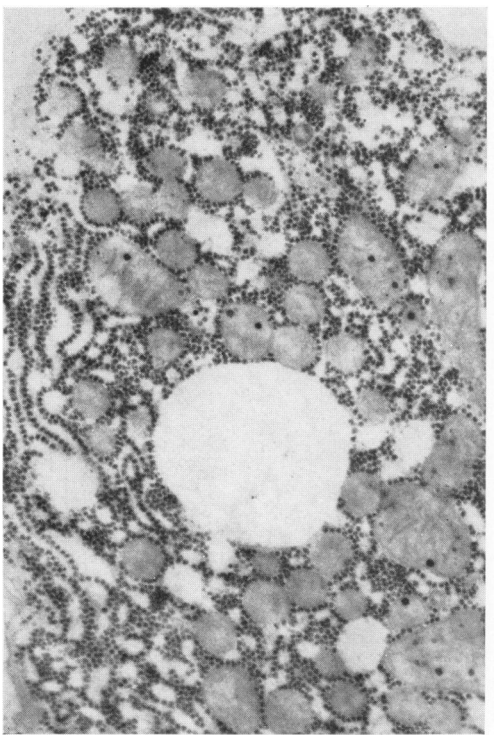

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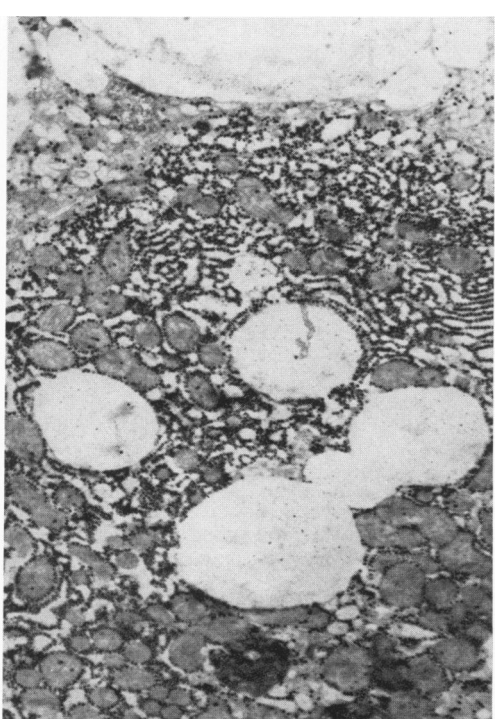

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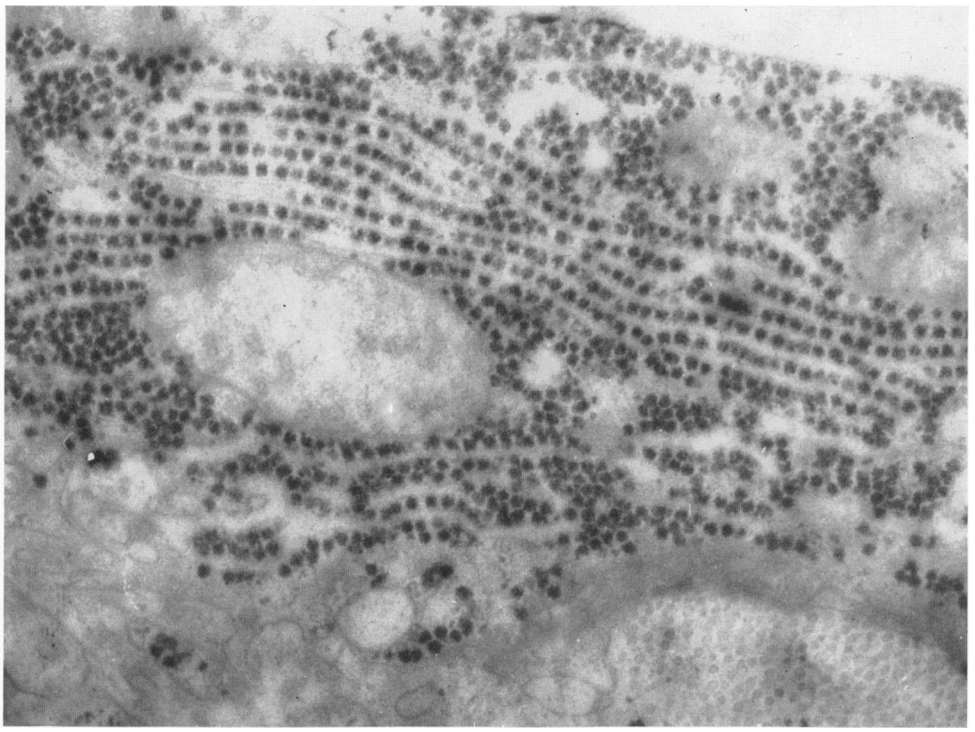
10

FIG. 11. Parallel, straight arrays of smooth endoplasmic reticulum are associated with particles of glycogen. × 30,000.

FIG. 12. Concentric arrays of smooth endoplasmic reticulum and glycogen granules are essentially arranged in single rows. Maraglas-embedded section stained with lead. × 26,000.

FIG. 13. A portion of a concentric array with glycogen granules arranged in 2, 3 or more rows. × 28,000.

11



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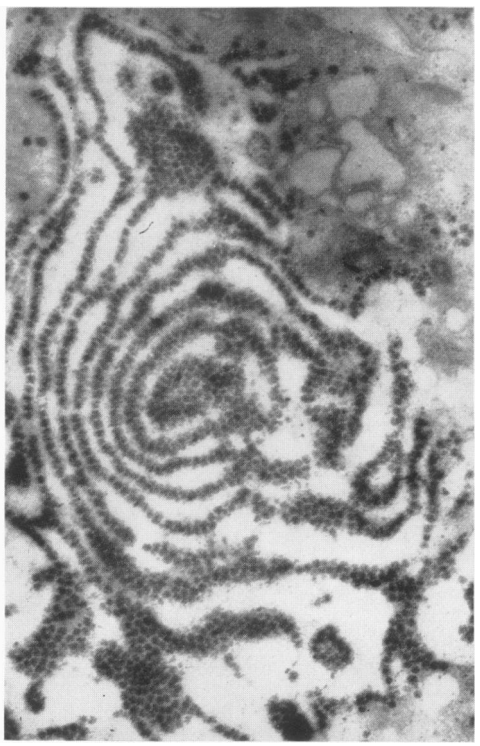

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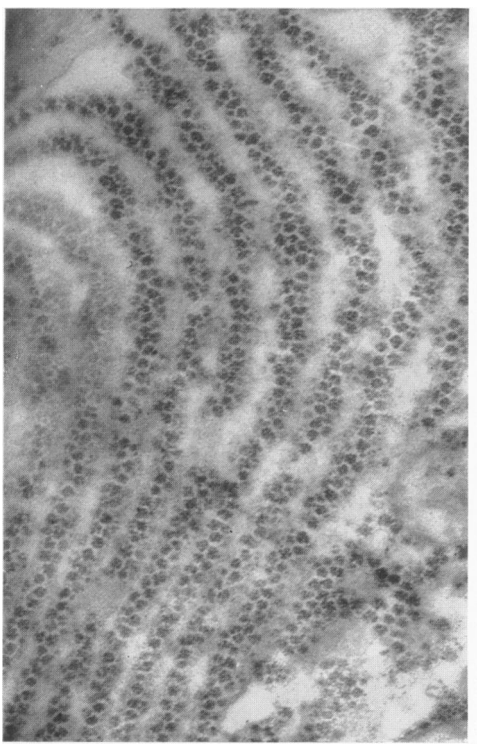

FIG. 14. Spherulated megamitochondria exhibit increased density of their matrices. × 10,000.

FIG. 15. Moderately enlarged mitochondria show alterations of their cristae. × 13,000.

FIG. 16. Bizarre-shaped giant mitochondria exhibit increase in the number of cristae. × 18,000.

FIG. 17. Rod-shaped mitochondria contain abnormal cristae and myelin figures. × 21,000.

FIG. 18. Transverse and longitudinal configurations of crystalline-like inclusions (myelin figures) appear within mitochondria. Maraglas-embedded section. Lead stain. × 35,000.

FIG. 19. Markedly whorled mitochondrial cristae are associated with "compartmentalization" of their matrices. × 18,000.

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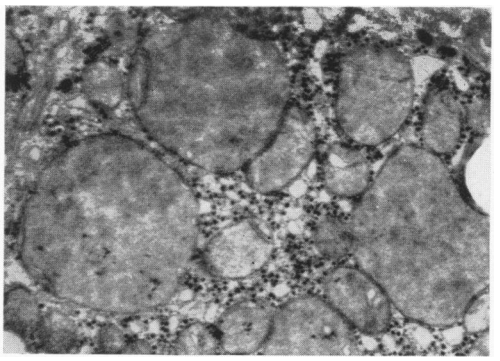

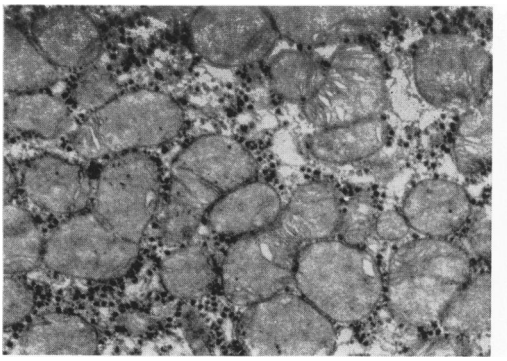

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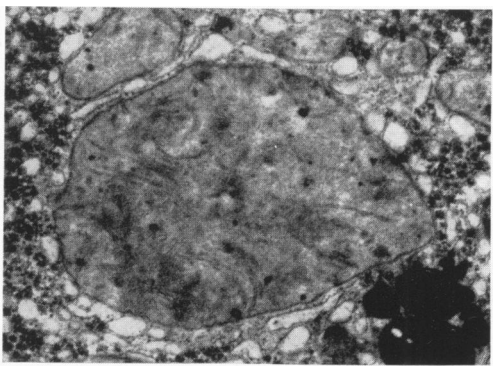

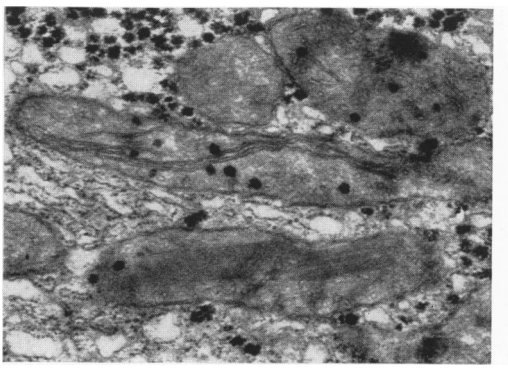

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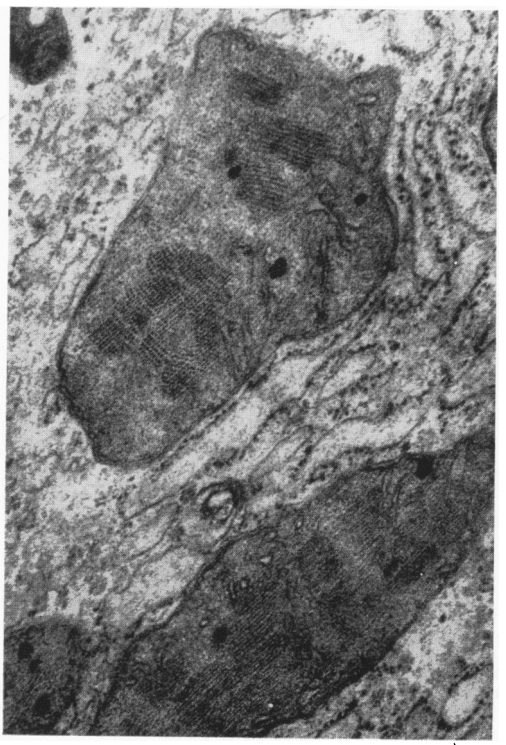

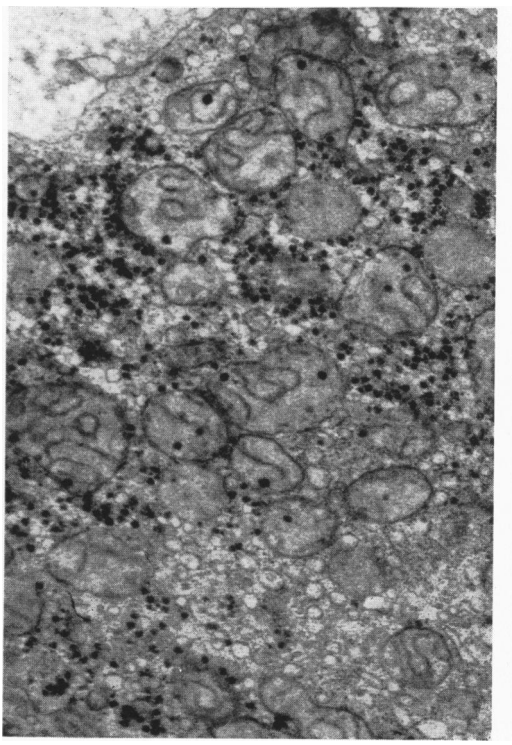

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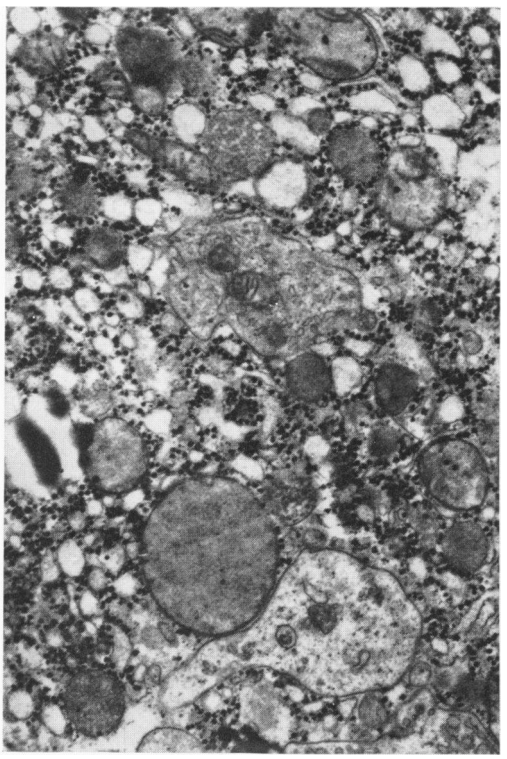
FIG. 20. Autophagic vacuoles in hepatocytes. Maraglas-embedded section. Lead stain. × 16,000.

FIG. 21. Autophagic vacuoles and lysosomes in a hepatocyte. × 15,000.

FIG. 22. Lysosomes, some with ferritin granules appear in the pericanalicular region of a hepatocyte. × 15,000.



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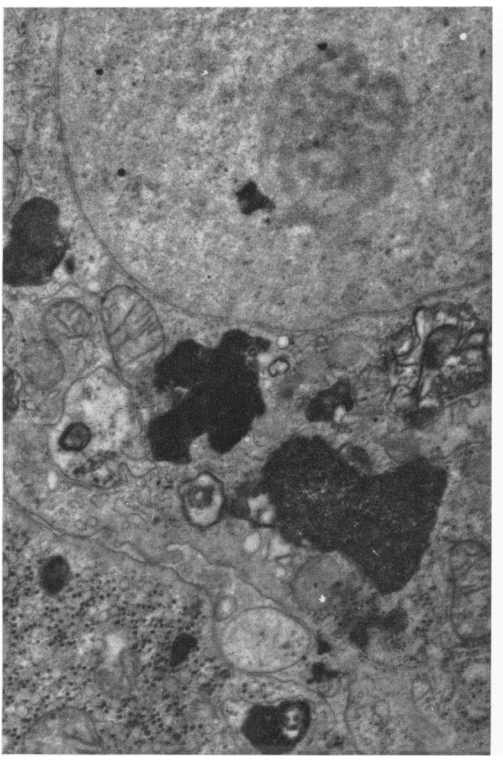

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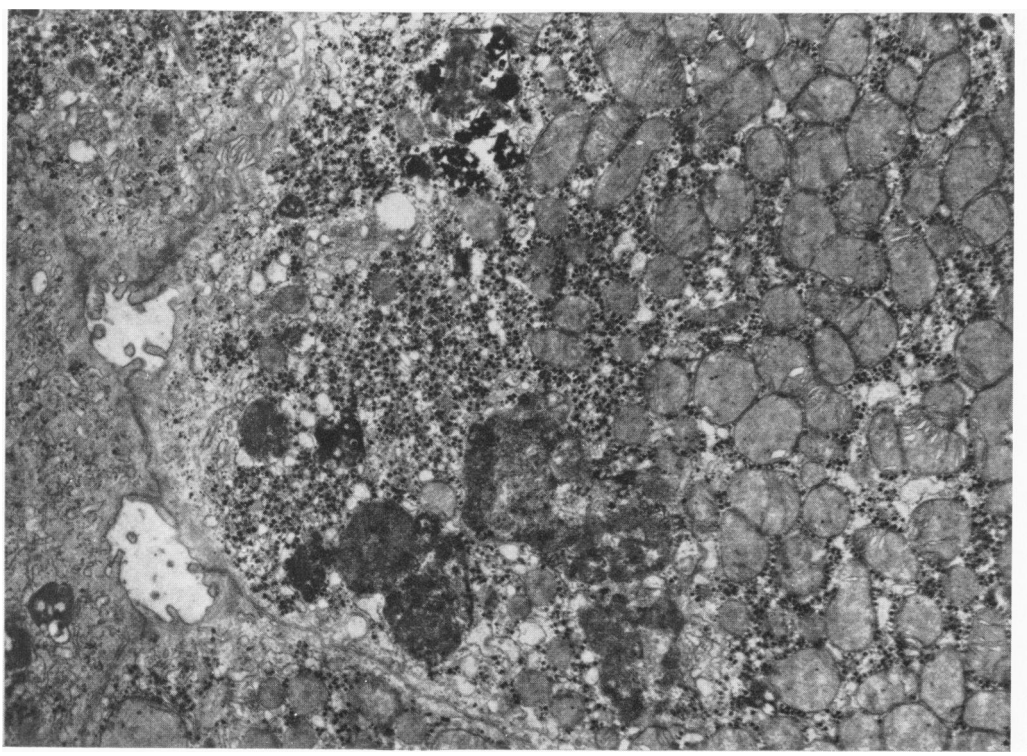

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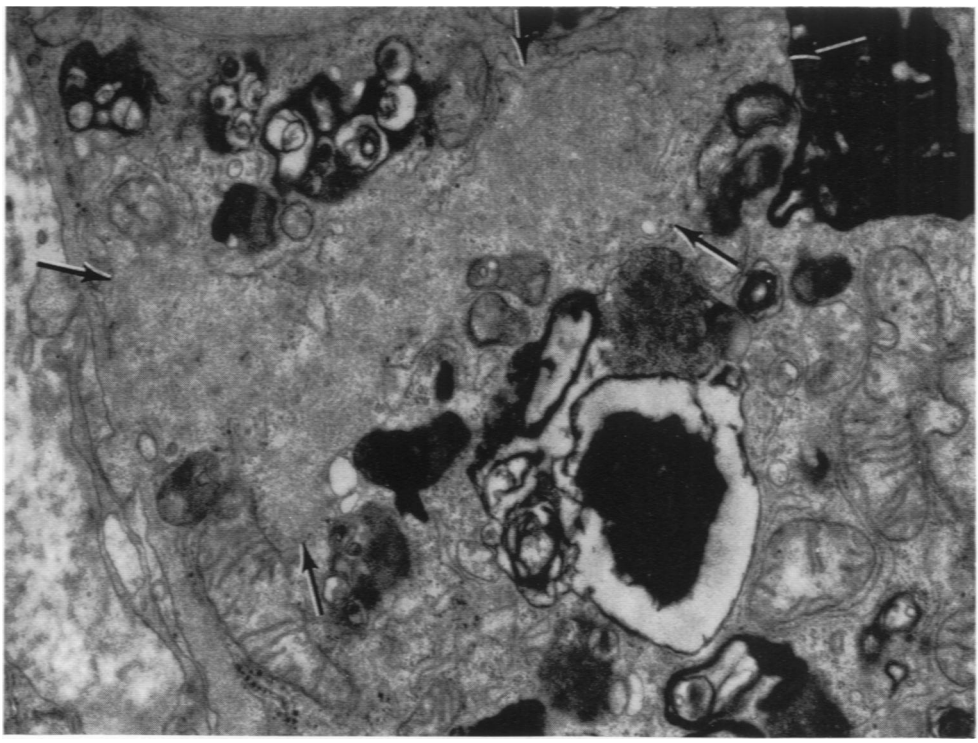
FIG. 23. The poorly delimited paranuclear area of a hepatocyte (arrows) is surrounded by lysosomes and degraded organelles. × 20,000.

FIG. 24. Mallory body. Maraglass-embedded section. Lead stain. × 35,000.



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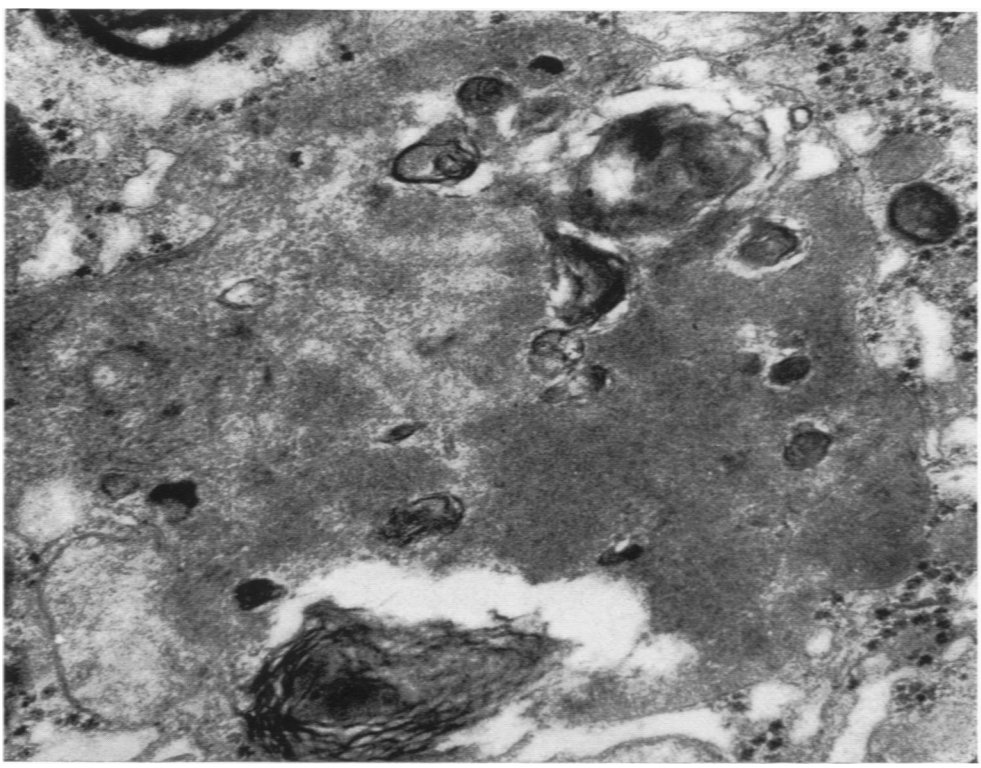

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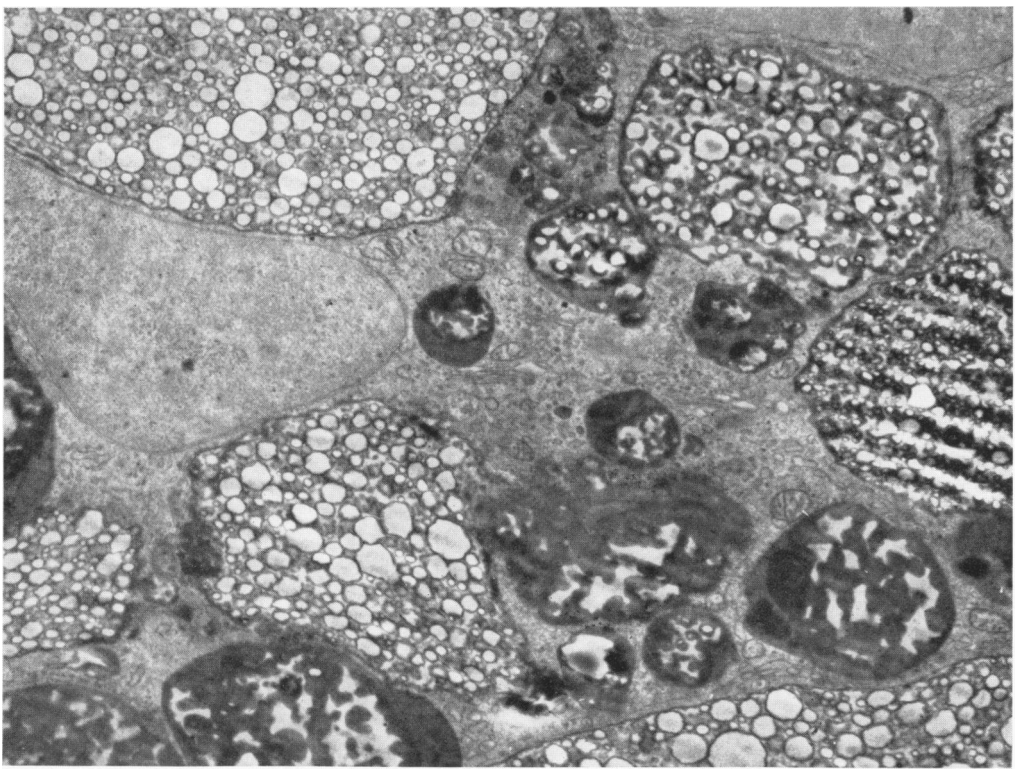
FIG. 25. The internal configuration of ceroid granules is shown in Kupffer and other mesenchymal cells. Maraglas-embedded section. Lead stain. × 10,000.

FIG. 26. A dilated space of Disse contains numerous connective tissue fibrils and a tenuous basement membrane (arrows) beneath the endothelial lining. Maraglas-embedded section. Lead stain. × 15,000.



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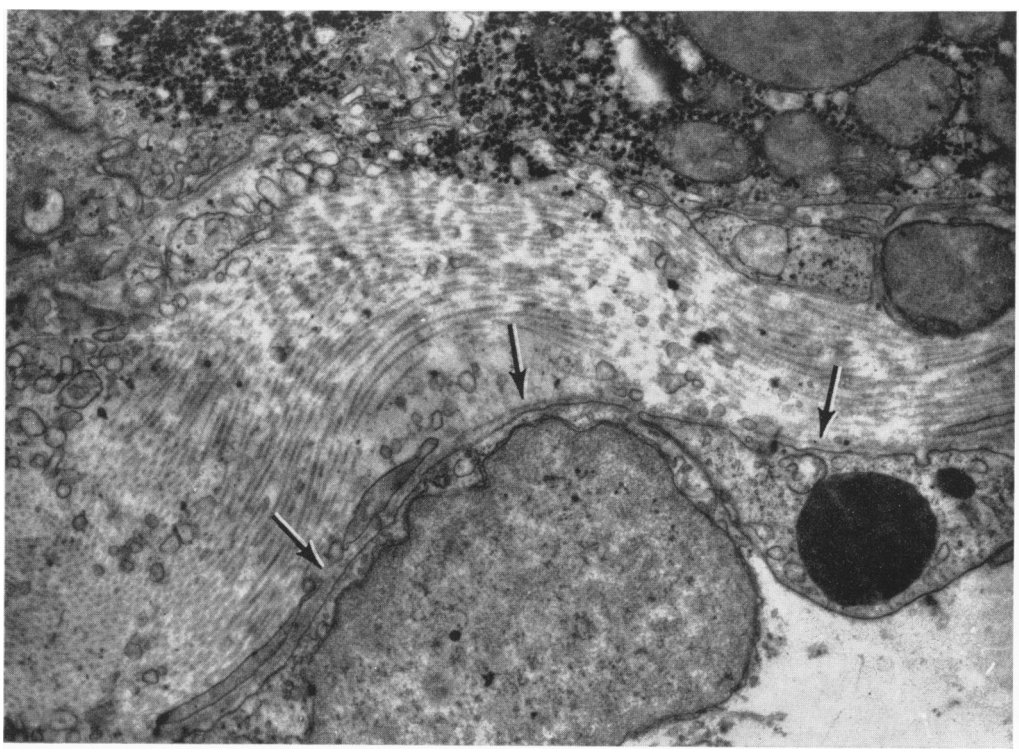

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