ELECTRON MICROSCOPY OF LIVER CELLS IN CIRRHOTIC NODULES

I. The Lateral Cell Membranes

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The hepatocytes of the normal mammalian liver appear to be arrayed in one cell thick cords when observed in sections with the light microscope.[1,2] Since liver cells form secretory units enclosing a bile canaliculus, however, the cords must be at least two cells in width or thickness.[1] Elias[3] showed, in three-dimensional reconstructions, that the parenchyma of human liver is formed by anastomosing one cell thick plates. The occasional visualization of broad sheets of cells might be anticipated if the hepatocytes are indeed arranged in plates. According to Elias,[3] such broad sheets of cells are not seen, because the plates are extensively perforated, and because they are curved.

Liver cell plates more than one cell thick are commonly seen in the embryonic liver[2] and in the liver of infants and young children.[4] They are also seen in cirrhosis, where their presence is considered to indicate regeneration, and hence continued activity of the cirrhotic process. They are replaced by one cell thick plates in inactive cirrhosis.[2]

In the course of a study of the fine structural changes in human cirrhosis, we have encountered many examples of widened liver cell plates within cirrhotic nodules. It is the purpose of this paper to describe the fine structural modifications of the cell membranes of hepatocytes in the nodular parenchyma of advanced cirrhosis with emphasis upon some features of those cells which are completely separated from sinusoids in a given plane of section. The similarity of the findings to the structure of embryonic liver cells will be stressed.

Material and Methods

Biopsy Material. Liver biopsy specimens were examined from 10 patients with established cirrhosis, from 2 human embryos and from 5 normal adults. The cases of cirrhosis comprised 5 specimens from patients with postnecrotic cirrhosis; these were obtained surgically at the time of porto-caval shunt operations. Needle specimens were obtained from 4 patients with portal cirrhosis, and from another with hemochromatosis. The embryonic livers were obtained from 12-week-old fetuses after

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therapeutic abortion; in both instances the cord was pulsating when removed from the uterus. The normal control livers comprised 1 obtained by open biopsy and 4 by needle biopsy.

Histologic Techniques. Tissues for light microscopy were fixed in Stieve's fluid or in alcoholic formalin. Paraffin sections were stained with hematoxylin and eosin (H&E), by the periodic acid-Schiff (PAS) method, with and without saliva digestion for 30 minutes at room temperature, or with Wilder's reticulum stain.

Small fragments of tissue for electron microscopy were fixed in Palade's buffered osmium tetroxide (pH 7.4) containing 0.25 M sucrose.[5] Two ml of fixative were used for each sample. After insertion of the tissue, the vial containing the fixative was maintained at 4° C for 90 minutes and then kept for 30 minutes at room temperature. The tissues were dehydrated in a graded series of ethanol solutions, and embedded in Epon 812 by the method of Luft.[6] Sections were cut on Porter-Blum ultramicrotomes with glass knives, and "stained" with lead hydroxide by the method of Karnovsky,[7] or with phosphotungstic acid (PTA), 5 per cent alcoholic solution for 30 minutes. The sections were examined with an RCA-EMU-3E electron microscope, using a 100 kv acceleration potential.

Thin (0.5 to 1.0 μ) sections of Epon embedded tissues were stained by the method of Cardno and Steiner.[8]

Results

Gross and Light Microscopic Findings

A. Normal Adult Liver. In the normal adult liver, the parenchyma was composed of liver cell plates which appeared in sections to be one cell, or occasionally two cells, in thickness. The arrangement was such that each liver cell had at least one, and usually two, of its surfaces abutting upon sinusoids (Figs. 1 and 2).

B. Cirrhotic Liver. Since different types of cirrhosis were examined, the gross appearance and the microscopic features varied. They were, however, all examples of advanced cirrhosis with a diffusely nodular parenchyma.

In the cases of postnecrotic cirrhosis there was a coarse nodularity of the entire liver surface at laparotomy. Viewed by light microscopy, the nodules varied greatly in size, and were commonly separated by broad scars containing variable numbers of bile ducts and chronic inflammatory cells. Some nodules were devoid of central veins, but in others one or more were present. The liver cells of the hyperplastic nodules formed plates, which were commonly more than one cell thick (Figs. 7 and 8). Ball-like masses, three or more cells thick, were present in places; these were especially frequent at the periphery of the nodules. In these areas, many liver cells had no contact with the sinusoids in the plane of section. The hepatocytes appeared normal, aside from a watery appearance of the cytoplasm in H and E-stained sections in some cases. Such hepatocytes possessed a weak, diffuse PAS-positivity in the cytoplasm which was abolished after saliva digestion. Hepatic cell boundaries were distinct. Kupffer cells were inconspicuous. There was a peri-

nodular condensation of reticulin (Fig. 4), and reticulin fibers within nodules lined sinusoids, and surrounded groups of hepatocytes or individual cells (Fig. 5).

In the needle biopsies from portal cirrhosis and hemochromatosis, the parenchyma was also nodular. Although only portions of nodules could be seen, the diagnosis was readily made in each case. These specimens resembled the examples of postnecrotic cirrhosis described above, in that the plates of liver cells within the nodules were also widened.

C. Embryonic Liver. In the 12-week-old embryos, one cell thick plates of the normal adult liver were not found. The liver cells were arranged in broad, anastomosing plates, many cells in thickness (Figs. 9 and 10). The lobular pattern of adult liver was poorly defined. The rudimentary sinusoids were irregular in shape. Hematopoietic cells were present in large numbers. A tubular arrangement of hepatocytes was noted in some areas.

Electron Microscopic Findings

A. Normal Adult Liver. Normal liver cells were provided with microvilli on the surfaces which faced the sinusoids and on the surfaces which formed the walls of bile canaliculi. Short, wedge-shaped extensions of the perisinusoidal spaces between adjacent hepatocytes (perisinusoidal recesses) also had microvillous borders. Except for these specialized surfaces, the cell membranes of the hepatocytes were straight, parallel to each other, separated by a 100 Å intercellular space, and devoid of microvilli (Fig. 3). Reticulin fibrils were present in the space of Disse, but were sparse.

B. Cirrhotic Liver. In the nodules relatively low power electron micrographs showed that many of the parenchymal liver cells had microvilli on their contiguous lateral surfaces (Figs. 6 and 11). Very rare segments of the lateral cell surfaces with random distribution had plasma membranes of adjacent cells arranged in parallel to each other, and these were the usual 100 Å apart. The normal arrangement was invariably retained immediately adjacent to bile canaliculi, that is in the location of the terminal bars. Desmosomes were few in number, except immediately adjacent to the anti-luminal extremity of the terminal bars, where one was present occasionally. Where the lateral cell surfaces of hepatocytes were provided with the anomalous microvilli, their plasma membranes were separated by a space 4200 to 5500 Å in width. The microvilli which projected into the space were shorter and less erect than those which projected into the space of Disse. These surface changes were particularly elaborate in areas where the liver cell plates were several cells thick. In these areas, widened intercellular

spaces lined by microvilli often surrounded the entire perimeter of hepatocytes. Bile canaliculi were often absent from the surfaces of cells which lay in areas remote from sinusoids in the central portion of the widened plates.

The widened intercellular spaces provided with microvilli were of two varieties. Some communicated with a perisinusoidal space of Disse; these were designated perisinusoidal canals. Others lacked an obvious communication with the space of Disse, and were designated intercellular canals. Occasional sections showed that the intercellular and perisinusoidal canals communicated with each other. Three-dimensional reconstructions suggested that both were in direct communication with sinusoidal lumens through the usual gaps in the endothelial lining of the sinusoids. Where cells abutted upon the connective tissue of portal tracts or of septa, the canals opened directly into their ground substance.

The perisinusoidal spaces of Disse showed considerable variation in width. They contained an abundant amorphous, homogeneous material of moderate electron opacity. This basement membrane-like material blended imperceptibly with the usual contents of extremely low electron opacity within the space of Disse (presumably plasma). In a few instances, short segments of well formed basement membrane, usually 100 Å in thickness, were also present within the space. In PTA-"stained" sections, the perisinusoidal spaces were seen to contain substantial aggregates of reticulin fibrils, usually polarized in parallel arrays and often in thick bundles. Basement membrane-like material, basement membranes and reticulin fibrils were also found in the perisinusoidal and intercellular canals (Fig. 6). Well formed basement membranes were, however, much rarer in this location, and reticulin fibrils were scattered, and usually lacked polarization into bundles. Since bile canaliculi contained none of these metaplasmic substances or structures and since the cell membranes adjacent to bile canaliculi were provided with terminal bars, bile canaliculi could be readily distinguished from the other channels provided with microvilli. This distinction was most obvious in PTA-"stained" sections.

The canals lined by microvilli were very numerous in all the cases of cirrhosis, but some hepatocytes with normal lateral cell membranes could be found particularly in those nodules in which liver cell plates appeared to be only one cell thick. In yet other areas, where the cells were isolated by connective tissue into small groups, the appearance of cell membranes was variable, but pericellular canals lined by microvilli were quite numerous.

The hepatocytes of the cirrhotic nodules were essentially normal. The only constant abnormality was a more irregular arrangement of profiles

of the endoplasmic reticulum and a diffuse distribution of polyparticulate rosettes of glycogen (Fig. 13). The latter change could be correlated with the diffuse, weak PAS-positivity of the cells in conventional sections.

C. Embryonic Liver. The lateral cell membranes of embryonic hepatocytes were frequently provided with microvilli. Some hepatocytes remote from sinusoids in the multi-layered cell plates were provided with microvilli around their entire perimeter (Fig. 12). Other hepatocytes had cell borders similar to those of normal adult liver. Hematopoietic cells were located predominantly at the sinusoidal borders. The surfaces of hepatocytes in contact with hematopoietic cells were usually smooth and devoid of microvilli.

Discussion

Liver cells of the normal adult mammalian liver are unique in that, with the exception of some species [9,10,11] plasma of the circulating blood has direct access to their vascular pole through gaps between the endothelial lining cells which delimit the space of Disse.[12] Short, wedge-shaped extensions of the spaces of Disse, perisinusoidal recesses,[13] are found where the rounded corners of adjacent hepatocytes form the lining of the perisinusoidal spaces. The lateral cell membranes of hepatocytes are usually arranged in parallel around a 100 Å gap. In the normal adult liver the lateral cell membranes are on rare occasions provided with microvilli. This gives rise to deep perisinusoidal canals which penetrate between adjacent liver cells to within a short distance of bile canaliculi. Cossel [14] suggested that these perisinusoidal canals are the result of physiologic, structural variations, which reflect the need for temporary increases of the absorptive or secretory activity of the liver cells. Be this as it may, the microvillation of lateral cell membranes is an uncommon and inconstant phenomenon in normal, adult mammalian livers.

In the nodules of cirrhosis examined, the presence of microvilli on contiguous lateral surfaces of the hepatocytes was an almost constant finding. This change was associated with a considerable widening of the 100 Å lateral intercellular space. The resulting canals communicated with the perisinusoidal spaces of Disse, with the connective tissue of portal tracts or septa, or with both. The change was particularly elaborate where liver cells were isolated from the sinusoids as a result of a widening of liver cell plates, or as a result of the ingrowth of connective tissue into the parenchyma. In places where the liver cell plates were more than two cells thick, intercellular canals lined by microvilli often surrounded the entire perimeter of hepatocytes.

Associated with this finding was an increase in reticulin in the nodular parenchyma. The amount of reticulin present was difficult to assess by light microscopy. Our findings corroborated those of Popper, Paronetto, Schaffner and Perez,[15] that in normal human liver reticulin fibers were not demonstrable as frequently as expected from light microscopic studies. Popper and his colleagues[15,16] described the electron microscopic appearance of pericellular fibrosis. In the nodules of cirrhosis they found areas of focal increase of reticulin, ascribed to collapse, intermixed with focal rarefaction of the reticulin associated with regeneration. We observed by light and electron microscopy an increase of reticulin fibers and fibrils in cirrhotic nodules. Our cases differed from those of Popper and co-workers[15,16] in that the pericellular fibrosis was diffuse and generalized because of an increase of reticulin fibrils in areas in which the fibrils are normally present (spaces of Disse) and in abnormal locations (perisinusoidal and intercellular canals).

Schaffner and Popper[17] described basement membranes in the space of Disse with conversion of the sinusoids into capillaries in advanced, longstanding cirrhosis. In our cases of advanced cirrhosis, basement membrane-like material and short segments of basement membranes were present, but conversion of sinusoids into capillaries was not seen. Our findings resembled those in the cases of "nodular parenchyma without fibrosis and necrosis," described by Schaffner and Popper,[17] in which capillarization was not found. These authors[17] did not comment upon the lateral cell membranes, but stated that where the sinusoids were replaced by capillaries, the adjacent hepatocytes had few microvilli on their vascular poles. We were unable to confirm this observation.

Anomalous microvillous borders, similar to those found in this study, have been described by Hosokawa, *et al.*,[18] in cirrhosis, and by Theron and Liebenberg[19] in kwashiorkor. Hosokawa, *et al.*,[18] attributed the change to the increased anabolic demands of the enhanced collagen production in cirrhosis. Theron and Liebenberg[19] interpreted it as "an attempt by the cell to increase the total area exposed to the lowered oxygen tensions in the blood and tissue fluid." These interpretations seem to us inadequate. It is unlikely that anoxia alone could account for this change. All authors who dealt with the alterations of liver cells in experimental anoxia and ischemia[20–29] emphasized the development of membrane-bound "hypoxic vacuoles" in the cytoplasm, but none mentioned the development of anomalous microvillous borders. "Hypoxic vacuoles" were not seen in the liver cells in our cases. It seems equally unlikely that the purpose of the anomalous microvillous borders is to facilitate the elaboration of connective tissue fibrils, since there is no evidence to suggest that hepatocytes produce collagen or its precursors.

It seems reasonable to suggest that the presence of intercellular canals in communication with the space of Disse might facilitate the access of all substrates to remote cell surfaces, thus maintaining the structural integrity of hepatocytes and allowing the formation of plates two or more cells thick. Indeed, it needs to be stressed that many of the cells in the cirrhotic nodules were in all respects normal, thus testifying to the efficacy of this arrangement. The development of anomalous microvilli on lateral intercellular borders may be a primary abnormality of the cells in the cirrhotic nodules. The consequent development of intercellular canals in communication with the space of Disse would in this way provide a means for the formation of thicker liver cell plates. Since the majority of the cells in the cirrhotic nodules are thought to arise by regeneration, it may be that the development of anomalous microvillous borders is a property of regenerating cells. None of the studies of the ultrastructure of regenerating cells after partial hepatectomy, however, have referred to this feature.[30–37] It could be also argued that the ingrowth of connective tissue, the formation of basement membranes and the deposition of a basement membrane-like material in the spaces of Disse might act as a barrier to diffusion of substrates from sinusoids, and lead secondarily to the development of anomalous microvillous borders as an adaptive response on the part of the hepatocytes.

Finally, it is possible that in cirrhotic nodules liver cells revert to an embryonic behavior pattern. In human embryonic livers, in which active proliferation of hepatocytes is taking place, thick liver cell plates are common, and it is of interest that many of these cells also possess microvilli on all cell surfaces, and that the intercellular spaces in these areas are also widened. The findings add substantial support to the view of Popper and Schaffner[2] based upon light microscopic studies that the proliferation of hepatocytes in cirrhosis resembles that which occurs during embryonic development.

Summary

The fine structural changes of hepatic cell membranes in human cirrhosis have been described. A striking feature of the cells in the hyperplastic nodules was the presence of anomalous microvillous cell borders which lined elongated perisinusoidal and intercellular canals. It was suggested that the development of the canals in communication with the spaces of Disse might facilitate the access of substrates to remote cell surfaces, thus maintaining the structural integrity of the cells, and allowing the growth of 2- or 3-cell-thick cords of liver cells. It was concluded that the most likely explanation for this phenomenon was a reversion of liver cells to an embryonic pattern of growth.

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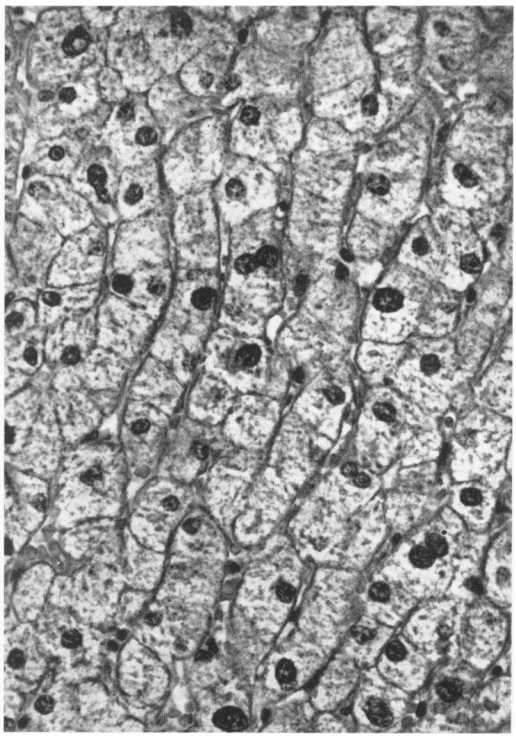
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Normal liver parenchyma. Conventional 5 μ section showing hepatocytes arranged in one cell thick trabeculae. The intervening sinusoids are difficult to discern (compare with Fig. 2). Hematoxylin and eosin stain. × 260.

FIG. 2. Normal liver parenchyma. A 0.5 μ section of epon-embedded tissue shows more clearly than the conventional 5 μ section (Fig. 1) the arrangement of hepatocytes in one cell thick trabeculae. Each liver cell abuts with at least one of its surfaces upon a sinusoid. PAS-toluidine blue stain. × 260.

FIG. 3. Normal liver parenchyma. Portions of 3 liver cells form the wall of a bile canaliculus (bc). The lateral cell membranes (converging unlabelled arrows) are arranged in parallel around an intervening 100 Å intercellular space. A terminal bar (tb) can be seen immediately adjacent to the canalicular lumen; nuc, nucleus; lys, lysosomes; m, mitochondrion; er, endoplasmic reticulum. Lead hydroxide "stain." × 5,500.



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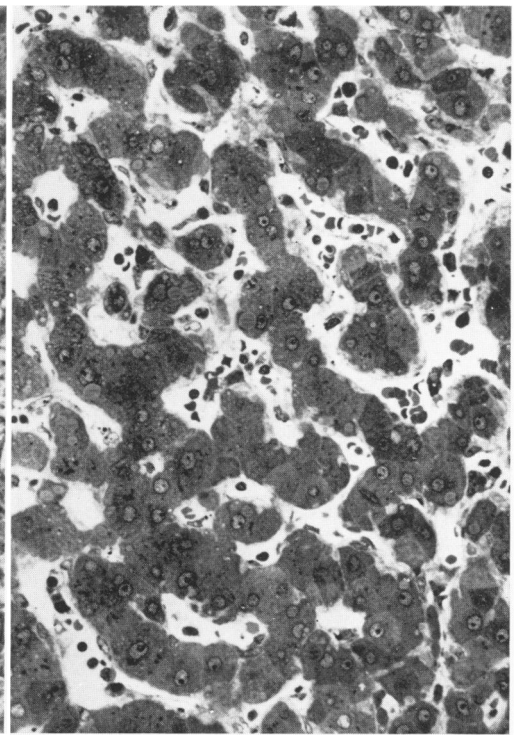

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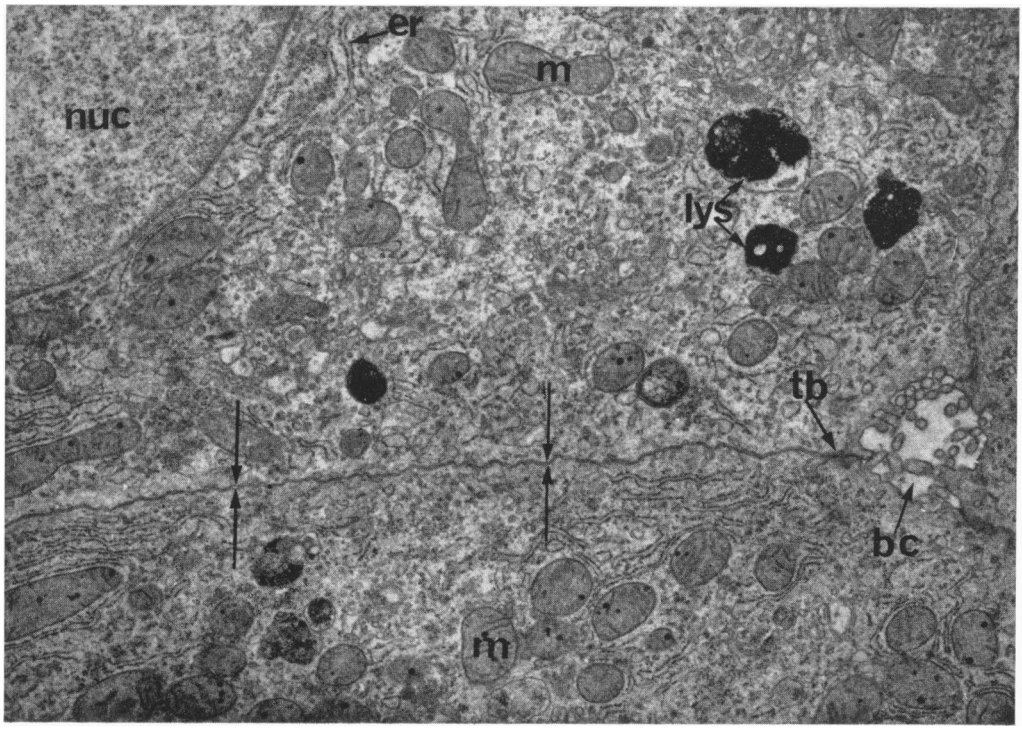

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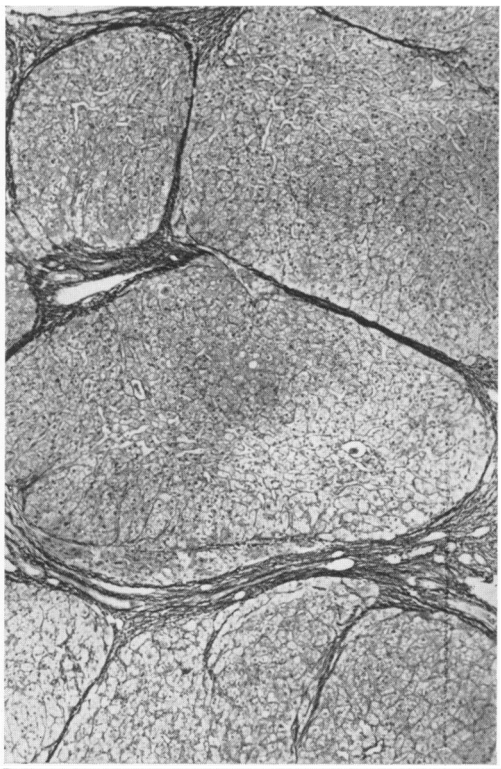
FIG. 4. Cirrhotic nodules. There is perinodular condensation of the hepatic reticulum (compare with Fig. 5). Wilder's stain. × 50.

FIG. 5. Part of a cirrhotic nodule. Reticulin fibers surround small groups of cells or individual cells. The fibers are present on surfaces adjacent to sinusoids as well as on those remote from vascular channels. Wilder's stain. × 130.

FIG. 6. Parts of 3 liver cells in a cirrhotic nodule. Microvilli project into intercellular canals (ic) between them. Reticulin fibrils (unmarked arrows) are scattered in the canals either singly or in small bundles; m, mitochondria. Phosphotungstic acid "stain." × 20,520.



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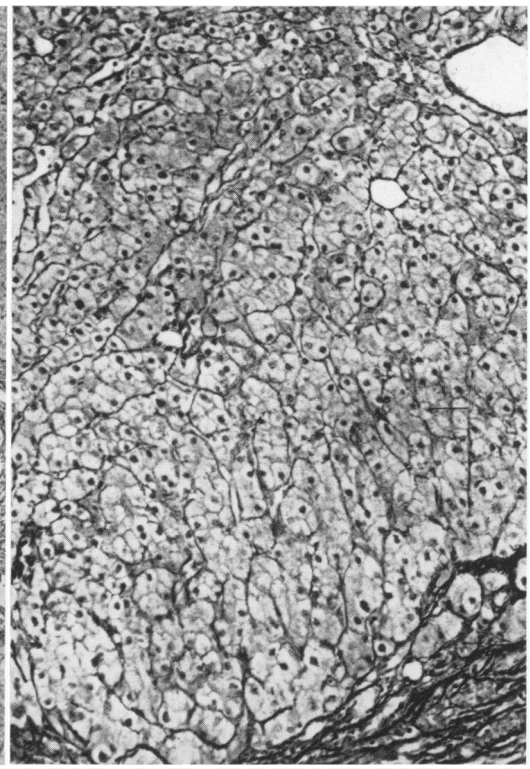

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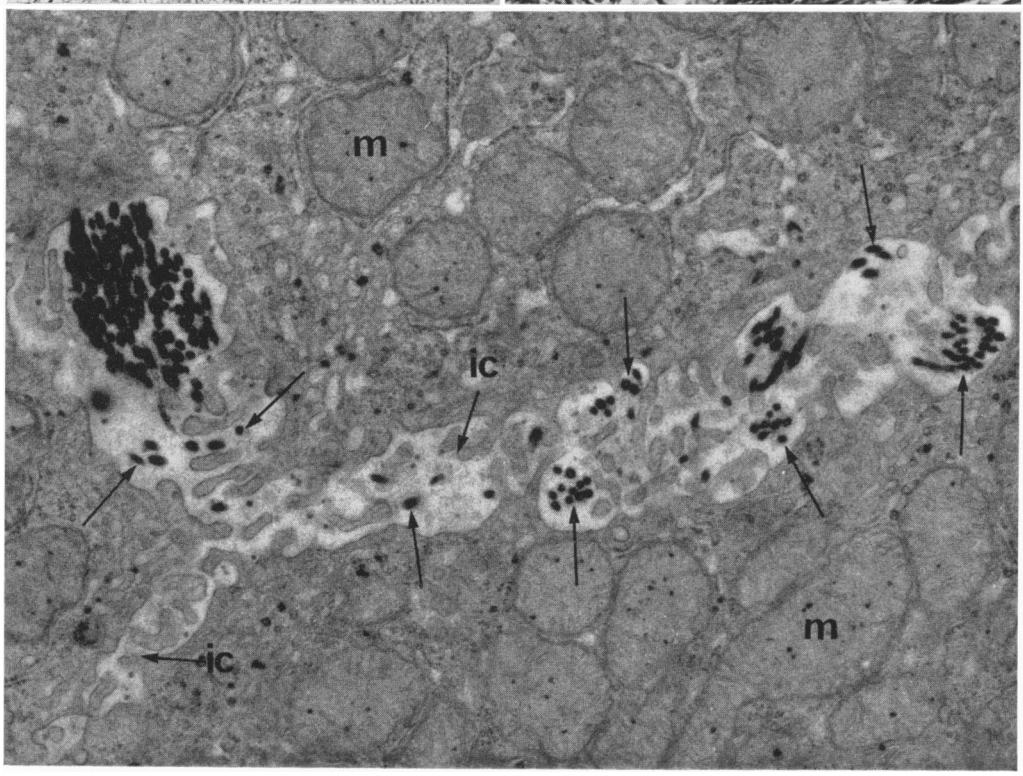

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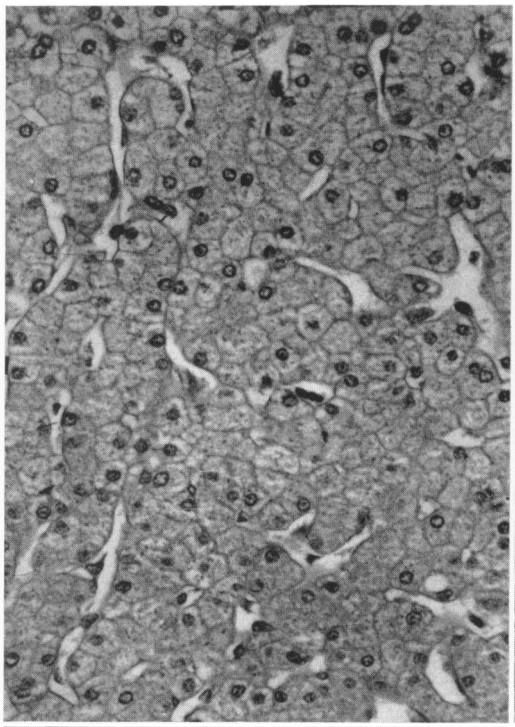
FIG. 7. Cirrhotic nodule. A conventional 5 μ section shows liver cells arranged in broad sheets (compare with Figs. 1 and 8). Many liver cells within the widened trabeculae are remote from sinusoids. Liver cells appear to be closely apposed to each other. Hematoxylin and eosin stain. × 260.

FIG. 8. Cirrhotic nodule. An 0.5 μ section of epon-embedded tissue shows the broad trabeculae of liver cells in the nodule. Some cells are in contact with sinusoids on one surface only and others lack such contact in the plane of section. Unlike the situation in conventional sections, the liver cells appear to be bordered on their lateral surfaces by a clear zone separating them from their neighbors (compare with Fig. 11). PAS-toluidine blue stain. × 420.

FIG. 9. Liver parenchyma in a 12-week-old human embryo. A conventional 5 μ section shows broad trabeculae of hepatocytes, some of which are isolated from sinusoidal lumens either by adjacent hepatocytes or by hematopoietic cells. There is a similarity of the arrangement with that seen in the cirrhotic nodule in Figure 7. Hematoxylin and eosin stain. × 400.

FIG. 10. Liver parenchyma in a 12-week-old human embryo. An 0.5 μ section shows the broad trabeculae of liver cells intermingled with hematopoietic cells. The liver cells are bordered on their lateral surfaces by clear zones which correspond to intercellular canals and perisinusoidal canals seen in electron micrographs. (Compare with Fig. 12). There is a similarity between the embryonic arrangement of liver cells and the arrangement of hepatocytes in the cirrhotic nodule (Fig. 8). PAS-toluidine blue stain. × 400.



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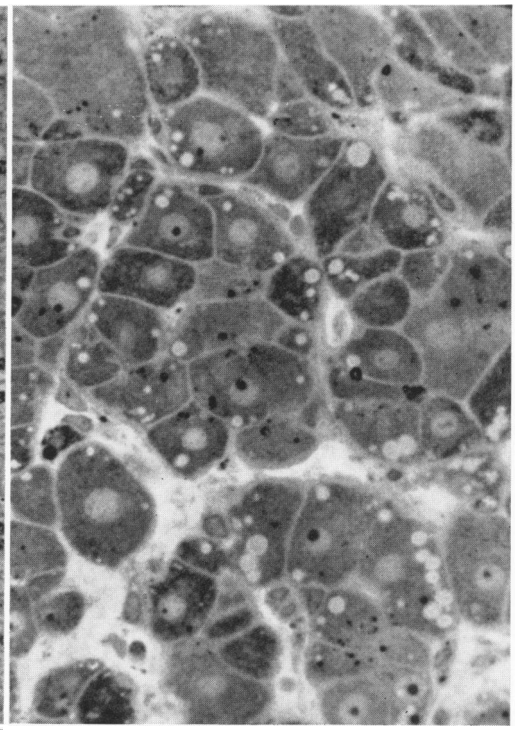

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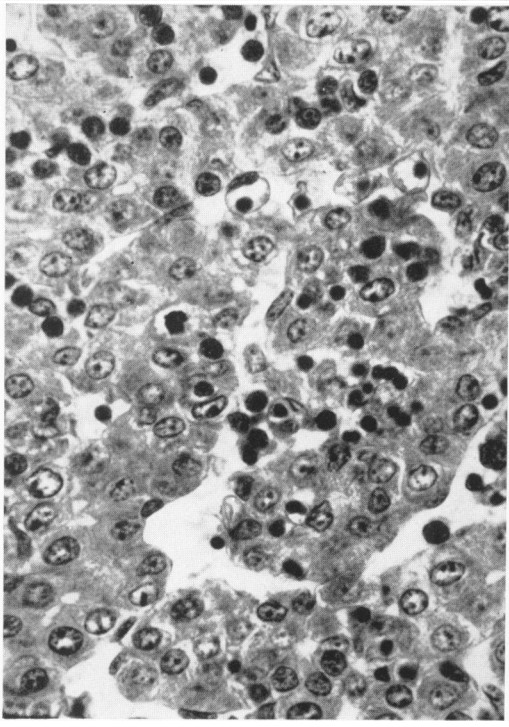

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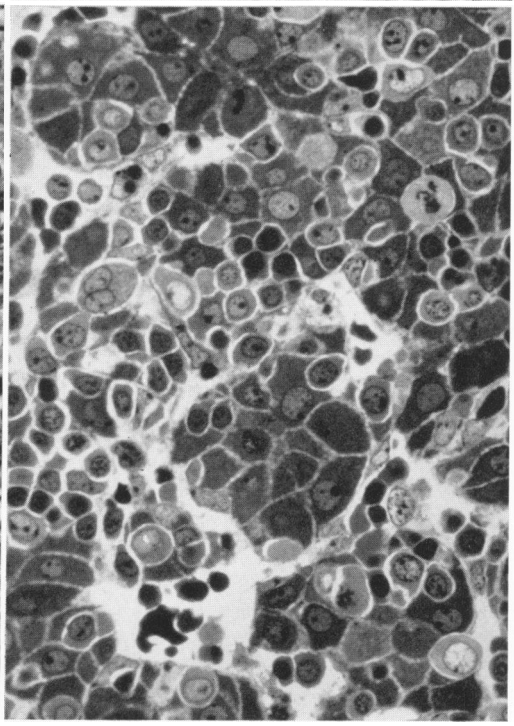
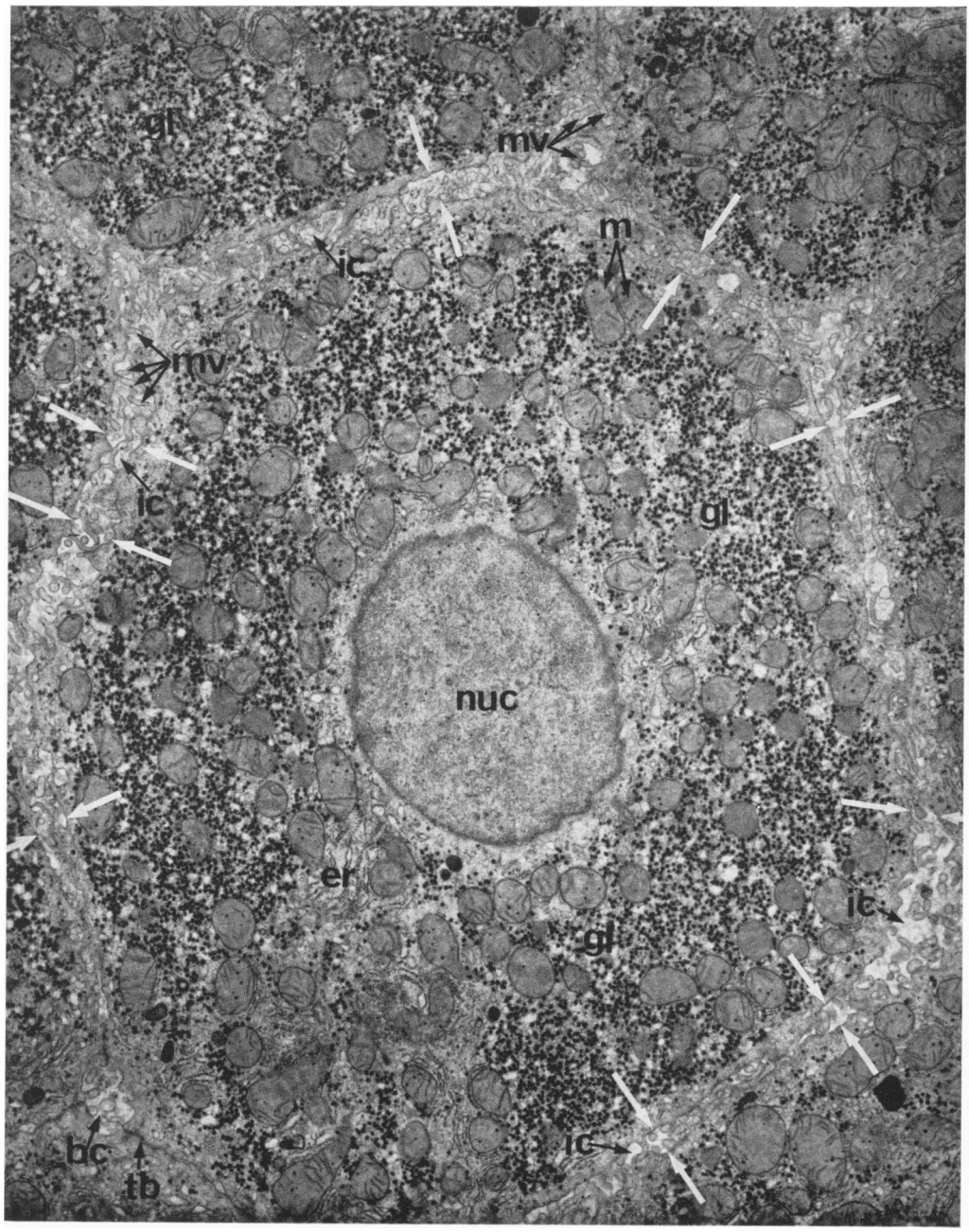


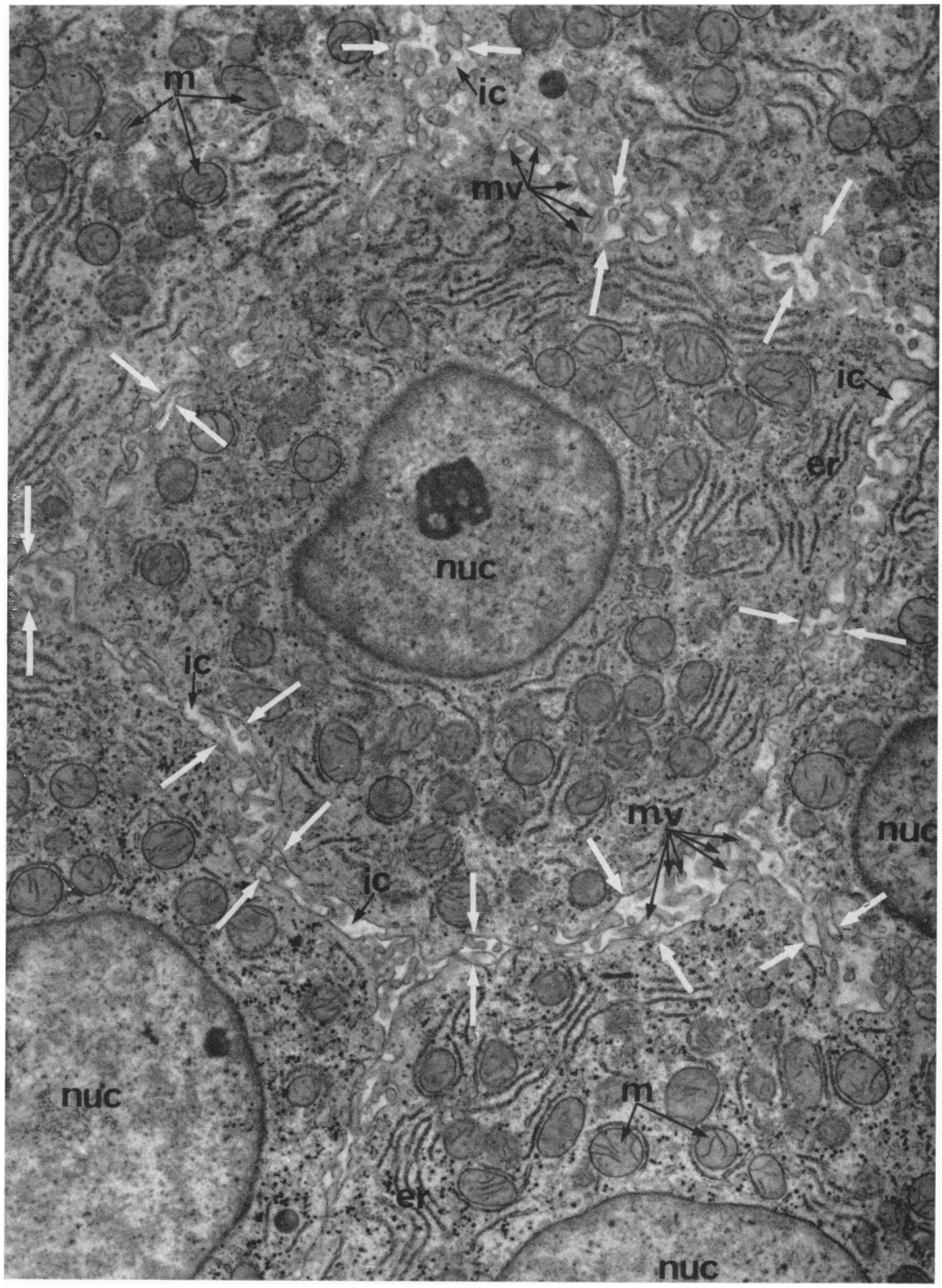
10

FIG. 11. Liver cells in a cirrhotic nodule. Parts of 7 cells surround a single cell in the center which was located in an area remote from sinusoids in the plane of section. The cell is entirely surrounded by intercellular canals (ic) outlined by white arrows. Only a single canaliculus (bc) can be seen in the left lower corner. Microvilli (mv) project into the canals. The structure of the cytoplasm of the cell is essentially normal; m, mitochondria; er, endoplasmic reticulum; gl, glycogen; tb, terminal bar; nuc, nucleus. Lead hydroxide "stain." × 4,750.



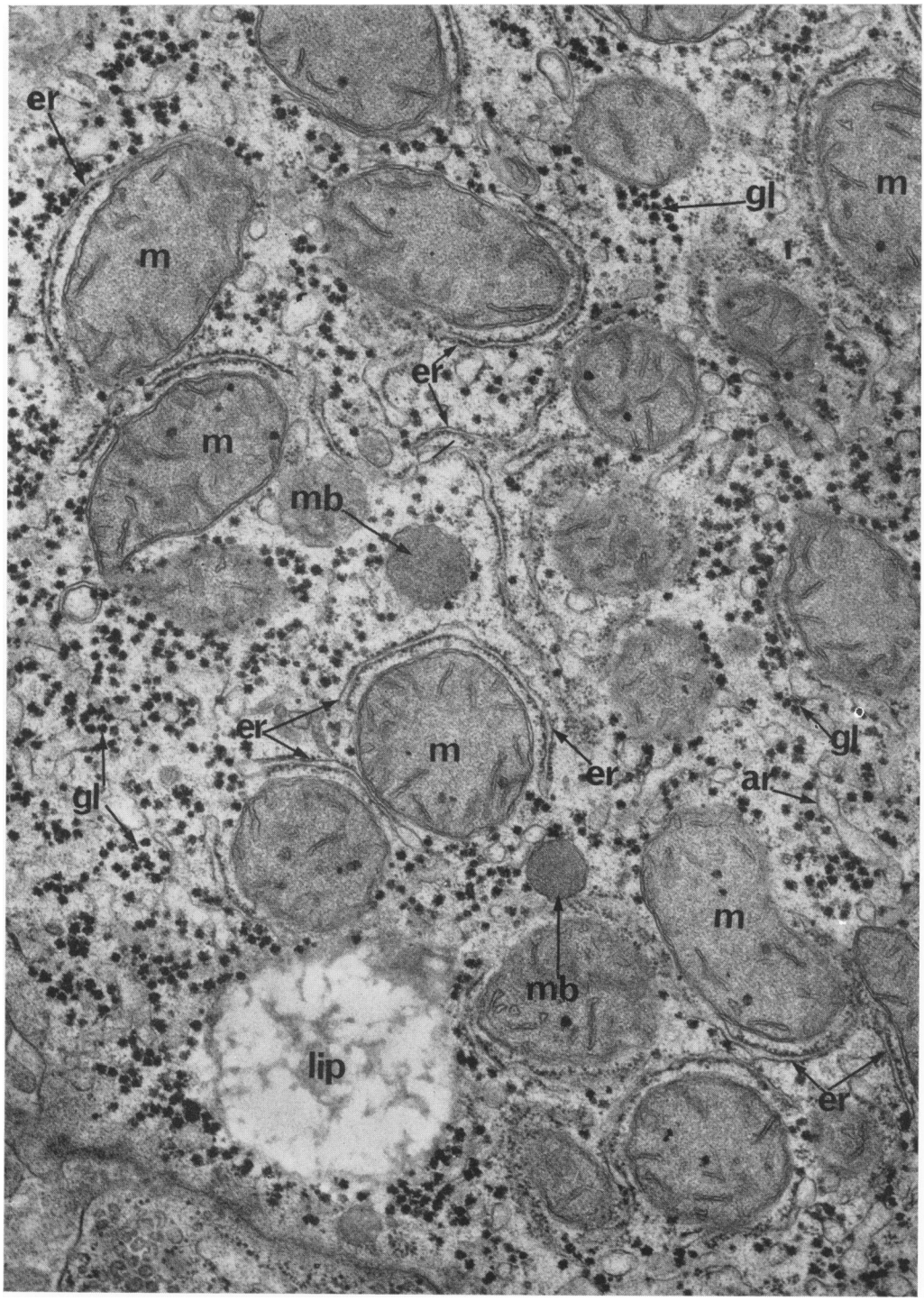
11

FIG. 12. Liver cells in a human 12-week-old embryo. Parts of 5 liver cells are seen to be surrounding a centrally located hepatocyte. This cell was remote from sinusoids in the plane of section. The cell is completely surrounded by wide intercellular canals (ic) outlined by white arrows into which project microvilli (mv) of the hepatocytes. There is a similarity of this arrangement with that shown in the cirrhotic nodule in Figure 11; nuc, nuclei; er, endoplasmic reticulum; m, mitochondria. Lead hydroxide "stain." × 6,500.



12

FIG. 13. Detail of the cytoplasm of a liver cell in a cirrhotic nodule. The mitochondria (m) are of average appearance. The endoplasmic reticulum (er) lacks the usual arrangement in parallel arrays, and the cisternae show a tendency to encompass mitochondria. Glycogen (g) is dispersed throughout the cytoplasm. The amount of glycogen is rather sparse in this cell. Agranular cisternae of the endoplasmic reticulum (ar) are few. mb, microbody; lip, lipid droplet. Lead hydroxide "stain." × 26,320.



13