Acute Manganese Overload

A New Experimental Model of Intrahepatic Cholestasis

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THE BILE CANALICULI and their microvilli, together with the Golgi apparatus, show characteristic ultrastructural alterations in many instances of cholestasis.[1] The mechanisms by which these ultrastructural abnormalities develop, and their precise physiologic connotations have not been elucidated. During a study of the effects of manganese overload on the liver, we have found the constant occurrence of similar abnormalities of the bile canaliculi and the Golgi apparatus in hepatic cells. Studies of bilirubin clearance carried out on these animals have confirmed the presence of physiologic cholestasis. This report describes these morphologic and physiologic observations and discusses their potential significance.

Materials and Methods

For morphologic studies, 26 male Sprague-Dawley rats weighing between 200 and 400 gm. were used as test animals and 10 similar rats were used as controls. Test animals were injected via a tail vein with a freshly prepared aqueous solution of either 10% or 20% manganese sulfate in amounts of 0.1 ml./100 gm. body weight. Food and water were withheld from test and control animals following injection until they were killed. Fourteen test animals were killed approximately 20 hr. after injection, 6 were killed 12 hr. after injection, and 6 were killed 6 hr. after injection. Control animals were also killed at each interval.

Immediately after the animals were killed, fragments of liver were minced in Millonig's fixative, fixed in this solution for 90 min. at 0–4° C., dehydrated, and embedded in Epon 812. Sections of these blocks were subsequently cut at 1 μ and stained with toluidine blue. Specific blocks were chosen for thin sectioning and examination in the electron microscope, and the thin sections were stained with uranyl acetate and lead citrate. At the time of sacrifice, pieces of liver were also fixed in formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

For the measurements of bilirubin clearance, 10 control and 11 test animals were used. The conditions of manganese administration were similar to those

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used in the morphologic studies, except that all animals were given 20% manganese sulfate and all bilirubin infusions were carried out at 5 hr. after manganese injection. After anesthesia with Nembutal, a jugular vein was cannulated for infusion purposes and the bile duct was cannulated for bile collections. Test and control animals were infused with a bilirubin solution (unconjugated) at a rate of 0.66 mg./min. This solution was freshly prepared and contained 0.52 gm. of sodium chloride, 0.25 gm. of sodium carbonate, and 0.20 gm. unconjugated bilirubin per 100 ml. Bile was collected for 5-min. periods and the total bilirubin collected during each period was determined by the method of Weinbren and Billing [2] with readings at 540 mμ on a Coleman Jr. spectrophotometer.

Results

Morphologic

Light Microscopy. Within 6 hr., loss of cytoplasmic basophilia was evident. At this time most animals given 20% manganese sulphate showed multiple small foci of hepatocellular necrosis. Similar changes, although less extensive, were seen in animals given 10% manganese sulfate.

Twelve hours after manganese injection, all animals showed hepatocellular necrosis involving either single cells, or, more commonly, patchy areas.

After 20 hr., the foci of necrosis were generally still larger, and occasional animals showed subtotal hepatic necrosis, although this was uncommon. Necrosis was randomly scattered throughout the acinar and lobular units, although a predilection for midzonal localization of the necrosis was sometimes evident (Fig. 1).

Electron Microscopy. In order to study the fully developed lesions as well as minimal changes, electron microscopic studies were carried out on animals 20 hr. after manganese injection. This interval was chosen because both severely involved and minimally involved areas were visible in light microscopic sections at this time, often in the same animals. By studying 1-μ-thick sections of Epon-embedded material, areas showing changes varying from minimal alterations to frank necrosis could readily be found and examined in the electron microscope.

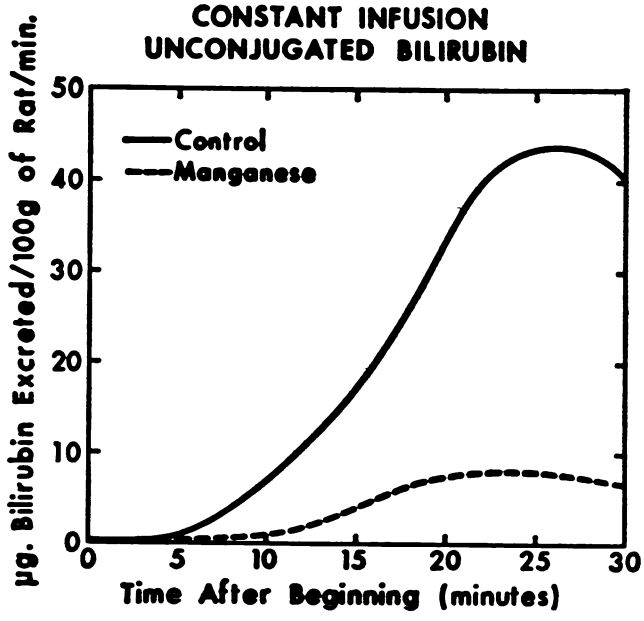
A number of nonspecific ultrastructural changes, seen in many toxic liver injuries, were widespread in the hepatocytes 20 hr. after manganese injection. These included loss of glycogen, a marked reduction in numbers of parallel arrays of granular endoplasmic reticulum, and an increased proportion of smooth endoplasmic reticulum profiles, many of which were circular and dilated. There was an increase in numbers of clearly distinct "dark" and "light" hepatocytes, but no definite ultrastructural basis for the differences in appearance between the latter two groups of hepatocytes was evident.

More striking and specific alterations were also present at this time, including widespread dilatation of the bile canaliculi, with loss and occasional swelling of their microvilli (Fig. 3 and 5–7). Bile canaliculi and microvilli in control animals appeared normal (Fig. 2 and 4). The Golgi apparatus was frequently prominent in cells showing these alterations in the canaliculi (Fig. 6 and 7). Occasionally, irregular "lakes" apparently representing coalesced and dilated Golgi vesicles and containing a material of variable electron density were seen (Fig. 8). There was no clear correlation between the extent of these "cholestatic" changes and the severity of necrosis in the particular section or in the particular animal. The ultrastructure of the bile ducts was within normal limits (Fig. 9).

Necrotic cells and cells adjacent to areas of frank necrosis showed varying degrees of mitochondrial swelling with loss of cristae (Fig. 10). This was accompanied by a rather mild degree of lipid accumulation, and, occasionally, striking dilatation of agranular endoplasmic reticulum.

Physiologic

Results of the studies of the clearance of unconjugated bilirubin are illustrated in Text-fig 1. The maximum clearance (Tm) of unconjugated



TEXT-FIG. 1. Biliary clearance curves of unconjugated bilirubin infused in control and manganese-loaded rats. Average maximum clearance (Tm) in control animals is 43 μg./100 gm. body weight per minute, whereas Tm in manganese-loaded animals is 7.5 μg./100 gm. body weight per minute.

bilirubin per 100 gm. of rat per minute for control animals was 43 mg. and for manganese-loaded animals, 7.5 mg. Both peaks were achieved 25 min. after beginning the infusion. The results indicate a significant depression of the maximum clearance (Tm) of unconjugated bilirubin in manganese loaded animals. The Tm for control animals was somewhat lower than the values often reported in the literature,[3,4] but has been constant in the strain of rat used. There was no correlation between the extent of necrosis and the reduction of maximum Tm. These results confirm the presence of physiologic cholestasis accompanying the characteristic ultrastructural alterations created by acute manganese overload.

Discussion

Manganese overload causes the constellation of ultrastructural changes considered to be "characteristic" of many states of cholestasis, with dilatation of the bile canaliculi, loss and swelling of the microvilli of the canaliculi, and increased prominence of the Golgi apparatus. On the basis of such changes, measurements of unconjugated bilirubin clearance have been carried out in manganese-loaded animals, and they have established the presence of functional cholestasis following acute manganese overload. Since manganese overload rapidly and regularly creates such ultrastructural alterations, this overload appears to be valuable as a model for the study of the mechanisms of cholestasis.

Functional cholestasis and characteristic ultrastructural alterations are known to occur on the basis of "obstruction" to bile flow at a variety of sites, including extrahepatic.[1] The presence of morphologically normal bile ducts in manganese overload at a time when both functional and ultrastructural alterations of cholestasis are present, points to the hepatocyte itself as the critically involved site in such intoxication, indicating that manganese overload is a model of intrahepatic cholestasis at the level of the hepatocyte.

The precise mechanism of manganese-induced cholestasis is not yet evident. Although hepatocellular necrosis is a feature of manganese overload, functional cholestasis has not proved to parallel the extent of the necrosis, and cholestasis is severe when visible necrosis is minimal. Moreover, although many substances are known to cause hepatocellular necrosis, only a relatively small number of these are known to cause functional and morphologic cholestasis. It is unlikely, therefore, that the manganese-induced hepatocellular necrosis per se plays a significant role in the development of cholestasis, and it seems probable that a relatively specific disturbance of the bilirubin transport, conjugation,

or excretory apparatus, possibly in combination with a disturbance of bile salt formation and/or excretion,[5,6] operative at the level of the hepatocyte, forms the basis for manganese-induced cholestasis. Studies to delineate the mechanism(s) of manganese-induced cholestasis are underway in our laboratory.

The development of severe mitochondrial injury and hepatocellular necrosis with acute manganese overload is not surprising, since Maynard and Cotzias [7] have shown that manganese tends to localize in hepatocellular mitochondria and since several divalent cations, including iron [8] and calcium,[9] are known to be associated with mitochondrial injury.

Summary

The intravenous injection of manganese sulphate in rats causes the rapid and regular development of hepatic abnormalities, including, ultimately, hepatocellular necrosis. This necrosis is accompanied by marked swelling of the hepatocellular mitochondria and lipid accumulation in these cells. Ultrastructural studies also reveal widespread alterations in bile transport and secretory apparatus of the hepatocytes, with increased prominence and dilatation of the Golgi apparatus and dilatation of the bile canaliculi with loss and swelling of the microvilli of the canaliculi. Bile ducts show normal ultrastructure at this time. Measurements of unconjugated bilirubin clearance in these animals demonstrates the presence of severe cholestasis. Since these morphologic and physiologic changes can be induced readily and regularly, manganese overload appears to be a potentially valuable model for the study of the mechanisms of intrahepatic cholestasis.

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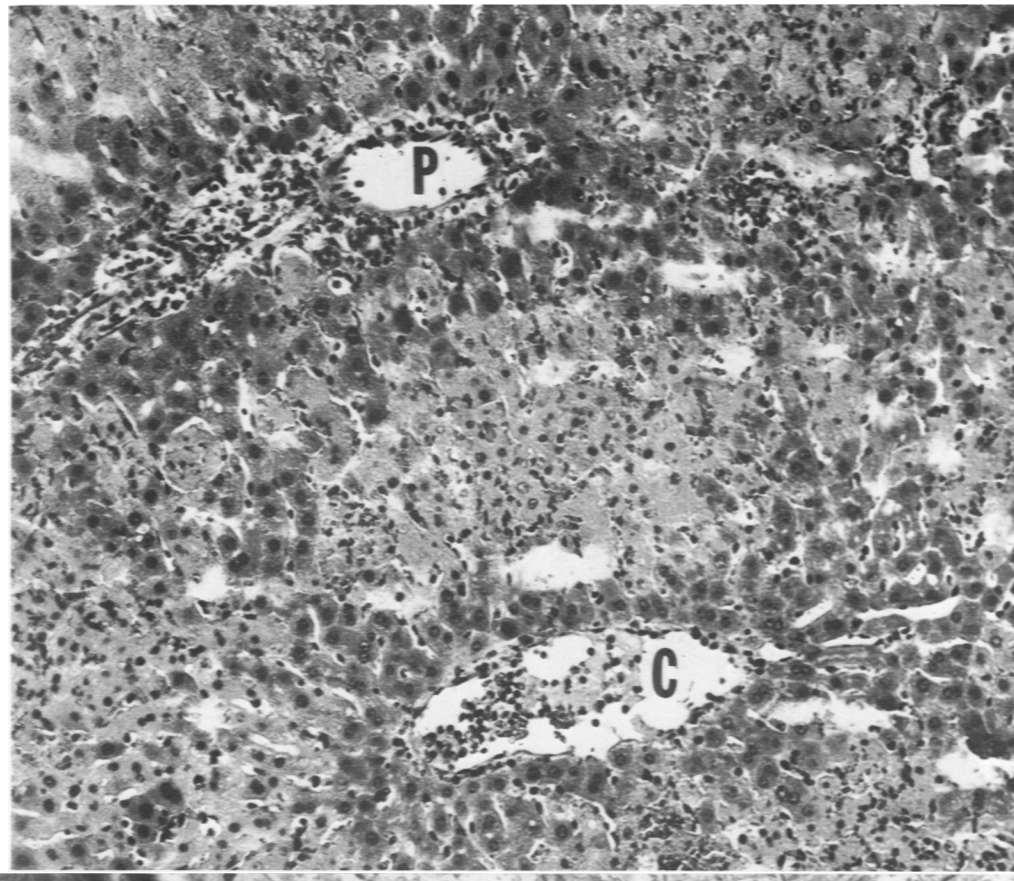
Legends for Figures

All illustrations except Fig. 1 are electron micrographs.

Fig. 1. Liver from animal given manganese sulfate 20 hr. previously. Extensive midzonal necrosis is present, sparing only a thin layer of cells around portal tract (*P*) and central vein (C). × 200.

Fig. 2. Appearance in cross section of two bile canaliculi (*B*) in control animal. A relatively large number of microvilli are evident within canaliculi, which are not dilated. Golgi apparatus, G. × 15,000.



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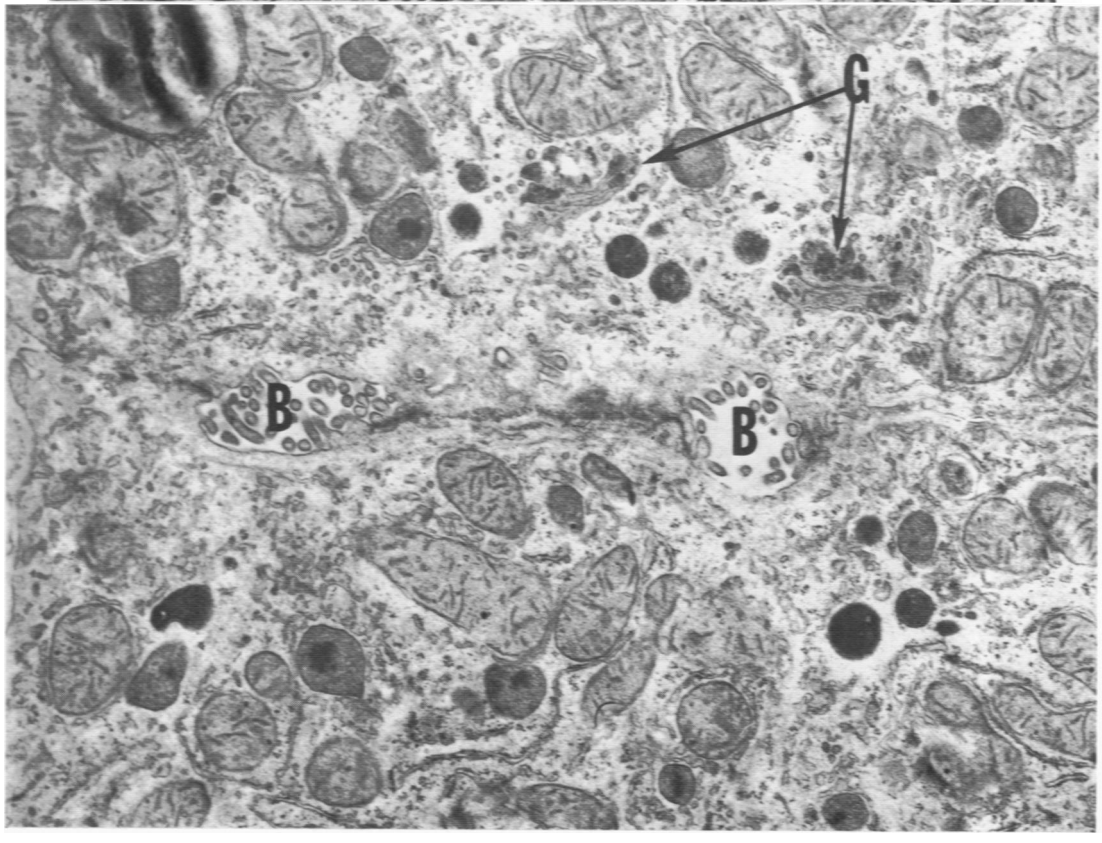

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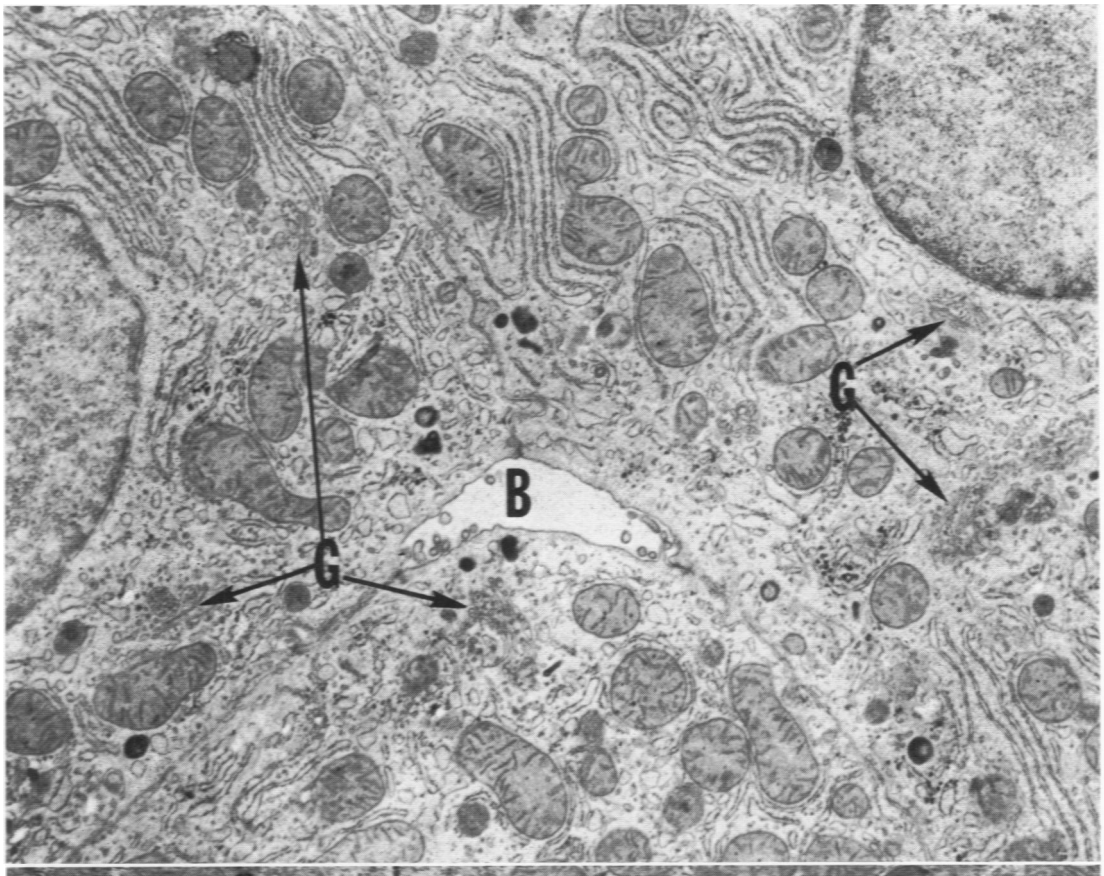
Fig. 3. Bile canaliculus (*B*) in animal given manganese sulfate 20 hr. previously. Canaliculus is dilated and the number of microvilli reduced. Golgi apparatus (*G*) is prominent in adjacent cells. × 10,000.

Fig. 4. Longitudinal section of bile canaliculus (*B*) from control animal. Numerous microvilli are present. A number of lysosomes are present adjacent to canaliculus. × 8750.



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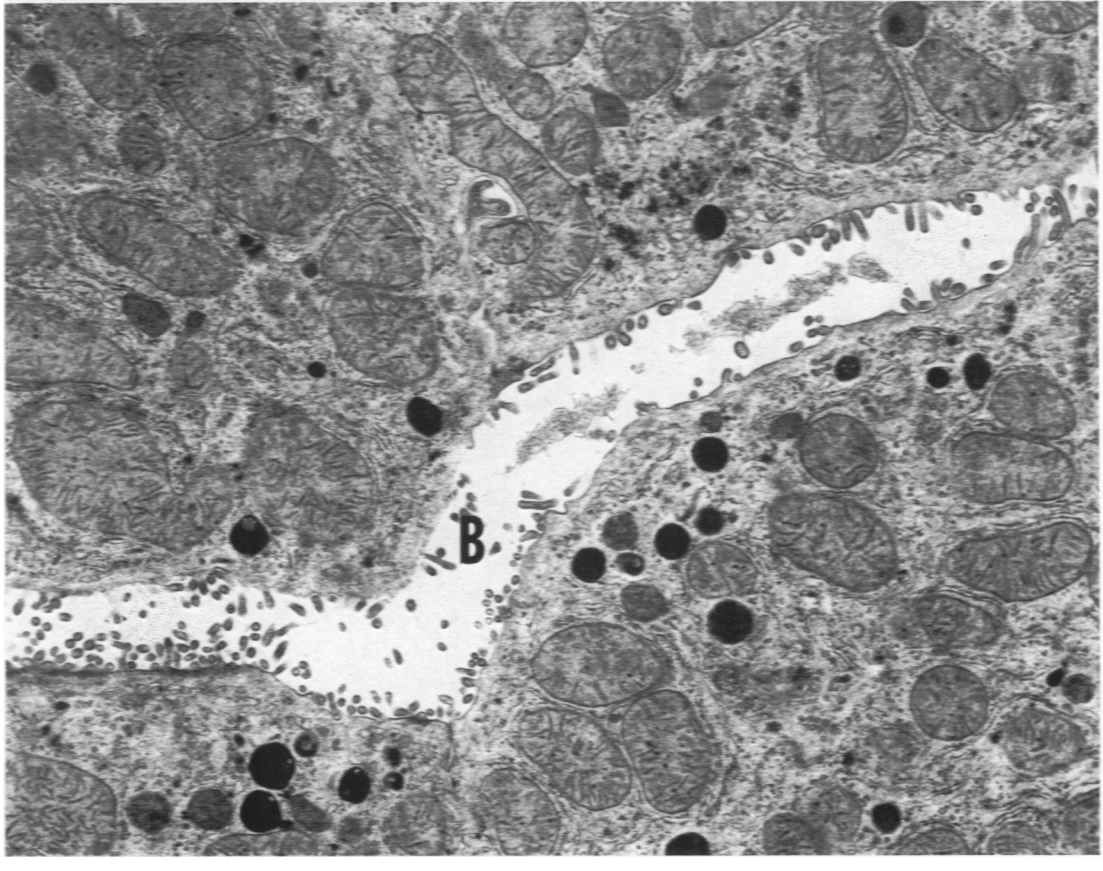

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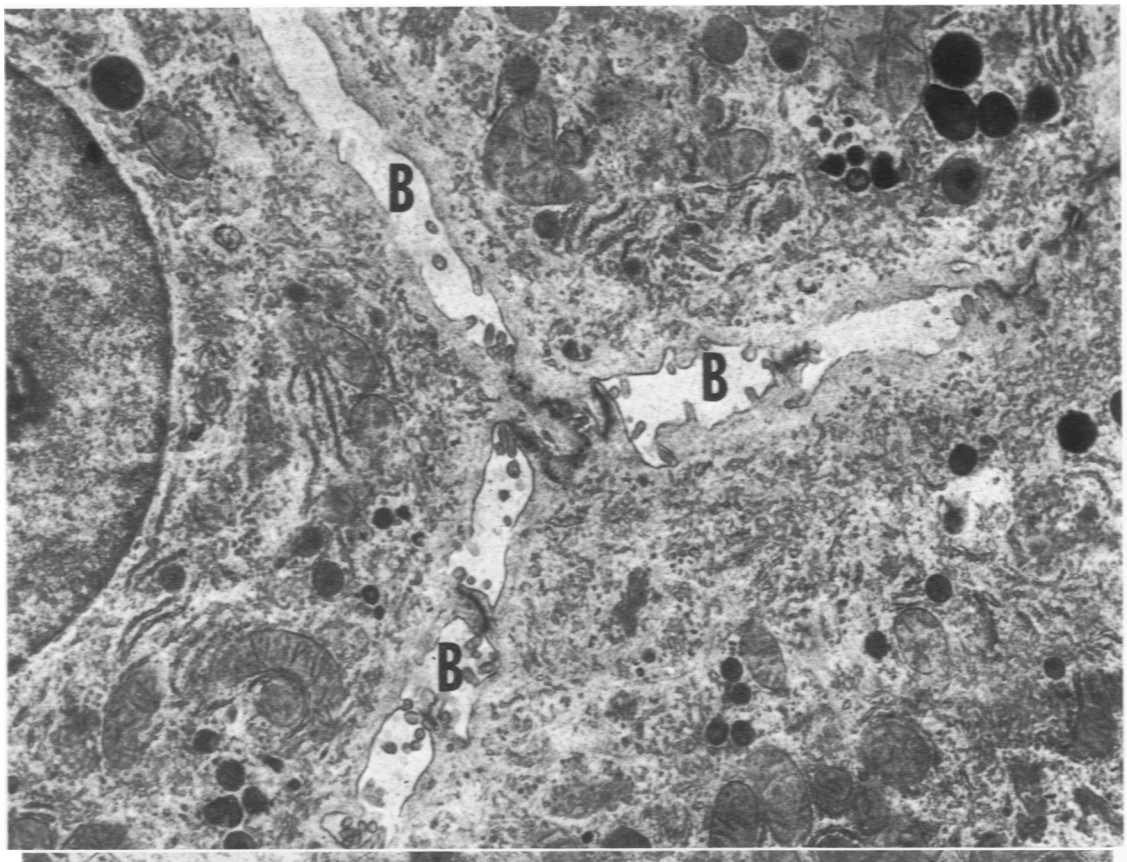
Fig. 5. Longitudinally sectioned bile canaliculi (*B*) from animal given manganese sulfate 20 hr. previously. There is marked dilatation of canaliculi with loss of microvilli. × 8000.

Fig. 6. Dilated bile canaliculus (*B*) from animal given manganese sulfate 20 hr. previously. Microvilli are reduced in number and one of the remaining microvilli is swollen (*small arrows*). Golgi apparatus (G) in adjacent cells is prominent, and several lipid droplets (*L*) are present. × 7000.



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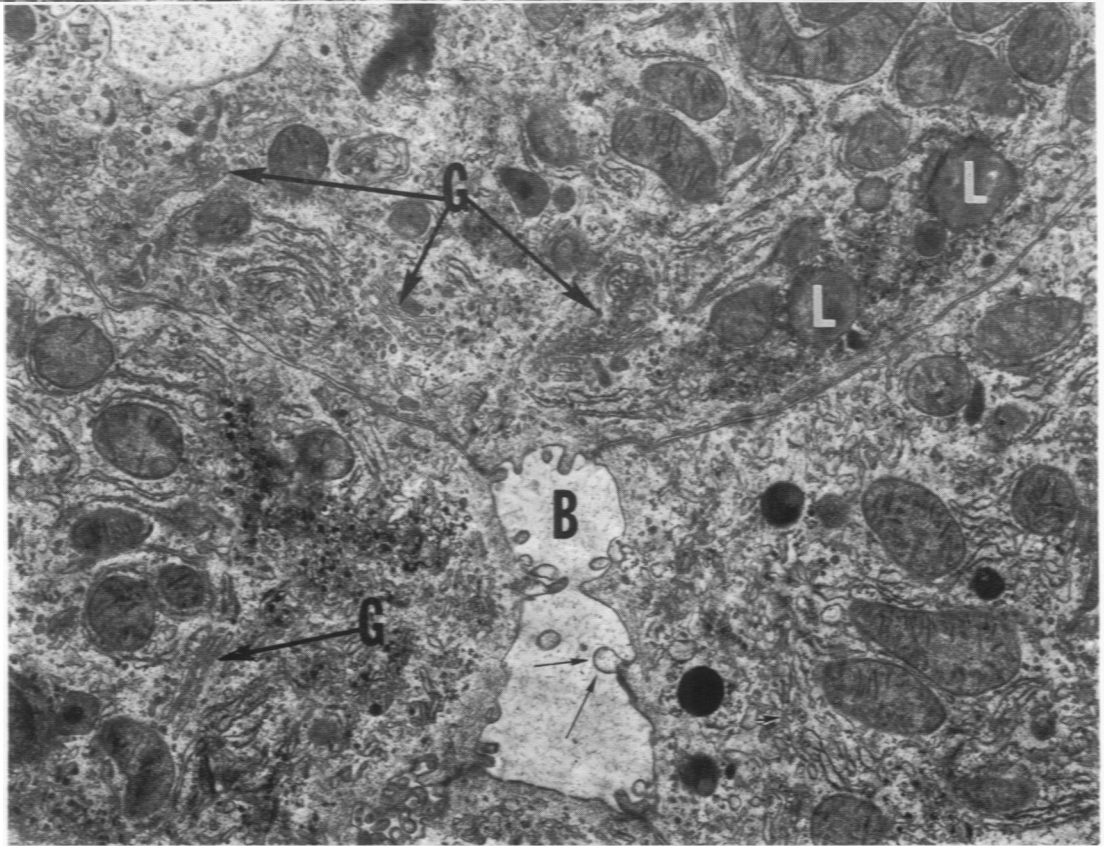

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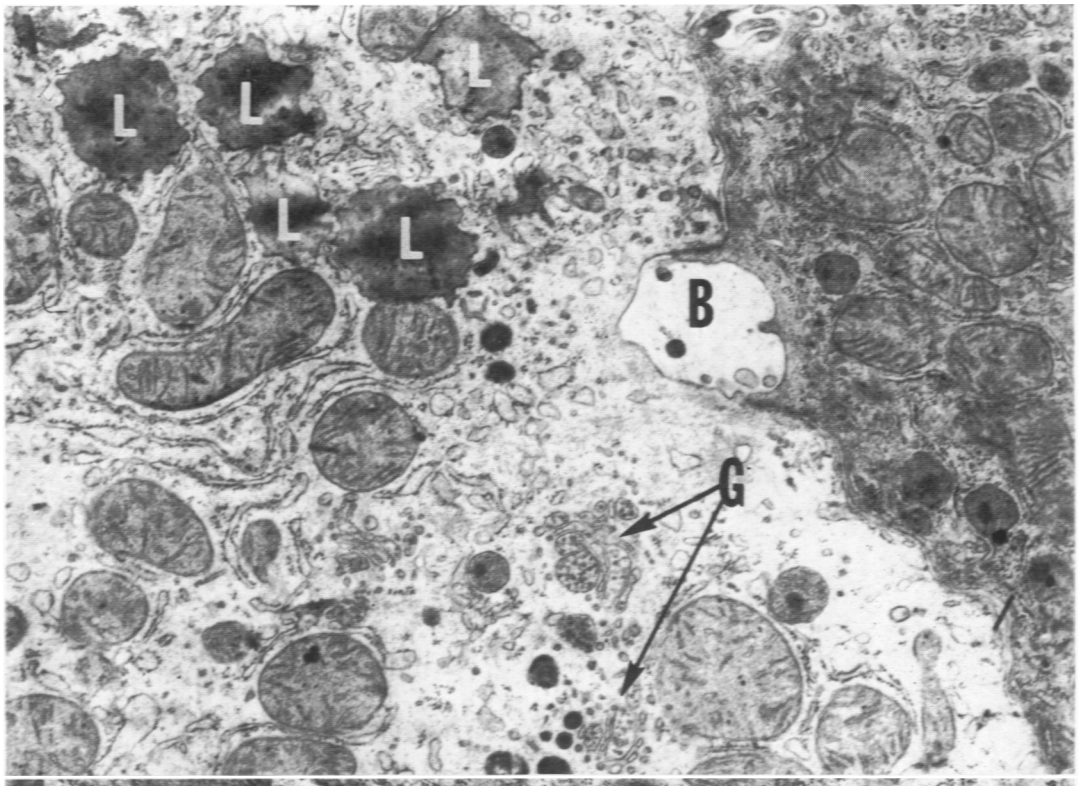
Fig. 7. Portions of liver cells from animal given manganese sulfate 20 hr. previously. "Light" cell is at left and "dark" cell at right. Bile canaliculus (*B*) between these two cells is dilated and has diminished numbers of microvilli. A number of lipid droplets (*L*) are present in the "light" cell, and several Golgi complexes (G) are evident. Mitochondria are slightly swollen in the "light" cell. × 15,000.

Fig. 8. Portion of liver cells from animal given manganese sulfate 20 hr. previously. Bile canaliculus (*B*) is within normal limits, but adjacent to canaliculus (*arrows*) are dilated and coalesced Golgi vesicles containing material of relatively low electron density. Several dense clusters of smooth endoplasmic reticulum (*SR*) are present nearby. Mitochondria are slightly swollen. × 12,000.



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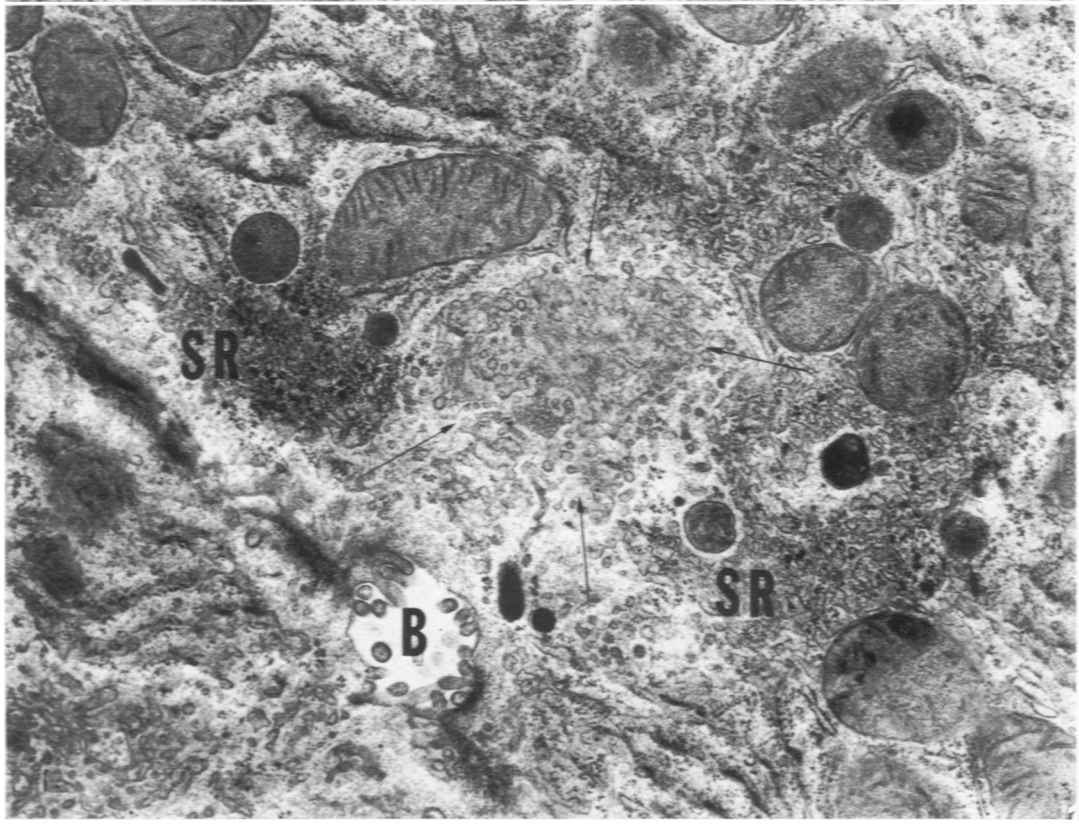

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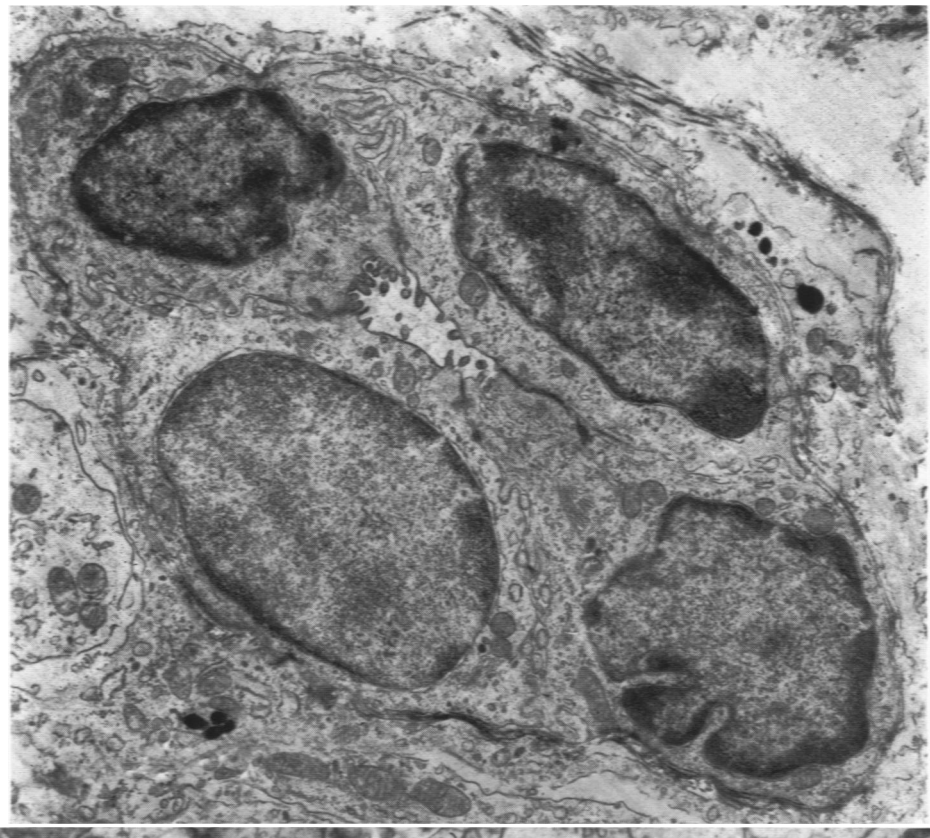
Fig. 9. Bile duct in manganese-loaded animal. Duct epithelium is morphologically within normal limits. × 6000.

Fig. 10. Portions of two liver cells (C^1 and C^2) 20 hr. after manganese sulfate administration. Both show dilatation of endoplasmic reticulum. In C^2 are lipid droplets (*L*) and mitochondria in all stages of swelling with loss of cristae. × 11,200.



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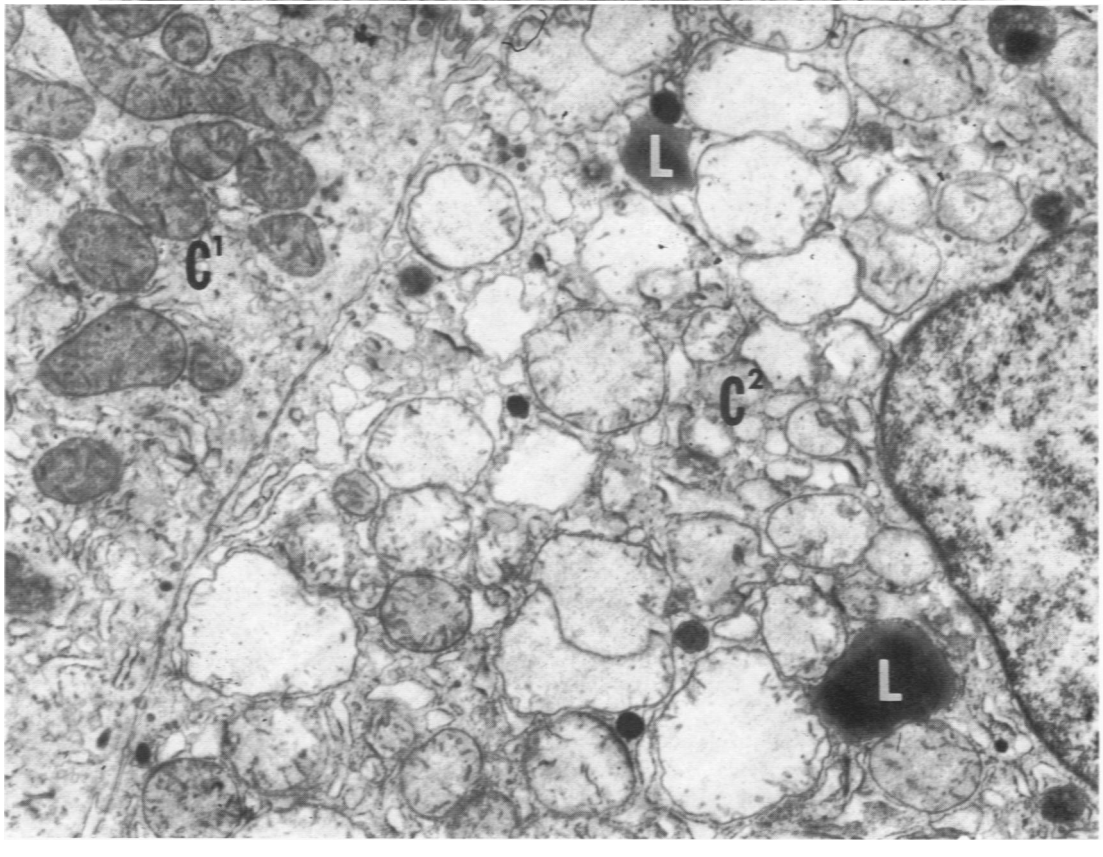

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