STUDIES ON THE FINE STRUCTURE OF THE TERMINAL BRANCHES OF THE BILIARY TREE

II. Observations of Pathologically Altered Bile Canaliculi

Jan W. Steiner, M.D., and John S. Carruthers, M.D.

From the Department of Pathology, University of Toronto, Toronto, Ontario, Canada

In the course of studies designed to elucidate the cause of intrahepatic biliary obstruction, changes were noted in the microvilli of parenchymal liver cells where these project into the lumens of biliary passages. It will be shown that widespread edema of the microvilli produces a subtotal or total obstruction of the lumens of bile canaliculi. Such alterations have not been recorded in previous studies of the fine structural changes in intrahepatic or extrahepatic cholestasis.[1-4]

In order to investigate the specificity of this change and the reciprocal behavior of parenchymal liver cells and their microvilli in bile canaliculi, tissues were subsequently examined from cases with extrahepatic biliary obstruction and from cases of human and experimental liver injury not associated with clinical evidence of cholestasis. It will be shown that in all such instances qualitatively similar alterations of the microvilli can be demonstrated. Their distribution is less widespread than in intrahepatic cholestasis.

Material and Methods

Biopsy tissue was examined from the following disorders; the diagnosis was based upon the clinical pattern in the patient or experimental animal.

Intrahepatic Biliary Obstruction. Two patients with intrahepatic cholestasis, both of whom were found to have no obstruction to their extrahepatic biliary tree at operation. The relevant microscopic finding in each case was plugging of bile canaliculi. A second biopsy specimen obtained from one patient showed early evidence of biliary cirrhosis. The cause of the original intrahepatic biliary obstruction could not be determined in either case.

Extrahepatic Biliary Obstruction. (a) Two patients with congenital biliary atresia which had progressed to biliary cirrhosis; (b) rabbits with early complete experimental extrahepatic biliary obstruction.[5]

Hepatic Lesions Not Associated with Clinical Evidence of Cholestasis. (a) An example of "fatty" liver in a human subject; (b) a patient with portal cirrhosis; (c) rabbits receiving portal vein injections of nonspecific soluble immune complexes. Some

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aspects of the histologic features in these lesions have been reported separately[6]; (d) rabbits receiving a prolonged series of systemic injections with heterologous protein (human gamma globulin).

Tissues obtained by surgical biopsy or, in the case of animals, immediately prior to sacrifice, were fixed, impregnated and stained by the methods outlined previously.[7] Care was taken not to pass judgment on any altered tissue which was inadequately preserved. The criteria of good preservation employed were the following: (a) even distribution of the karyoplasm in the central portions of parenchymal cell nuclei; (b) preservation of the reciprocal relationship between Kupffer cells and their trabeculae and the parenchymal microvilli where these project into the space of Disse; and (c) uninterrupted cell membranes.

In all the cases under review, sections stained with phosphotungstic acid (PTA) and with silver methenamine (SM), with or without prior periodic acid oxidation, were examined. The purpose of this was to facilitate distinction between bile canaliculi and sinusoids. The spaces of Disse surrounding the latter contained demonstrable reticulin fibrils and an amorphous ground substance-like material[4] whereas bile canaliculi were devoid of them. Despite these precautions, channels were found where it was not possible to make a distinction. These were ignored in arriving at conclusions.

Results

Alterations of Microvilli of Bile Canaliculi

It was noted previously that variations in size, shape and distribution of microvilli in the ultimate branches of the biliary tree occurred even normally.[7] Only marked deviations were considered to be of pathologic significance.

Edema of Microvilli. This was seen in all cases under review though the distribution and severity of this change varied (Figs. 10 to 16). In the cases of intrahepatic biliary obstruction, almost total or total occlusion was seen frequently. In all other instances such alterations were patchy and involved only a very few areas and often only a single villus in an occasional canaliculus. Observations of large numbers of swollen microvilli indicated that the evolution of the edema was not uniform. In many it commenced in the tip, producing a clublike dilatation of the extremity, a narrow neck of cytoplasm connecting it to the main parenchymal cell mass (Fig. 11). In other instances the edema began near the base of the villus, producing a broad-based structure with a delicate finger-like projection at the extremity (Fig. 10). Finally, the entire villus became edematous and progressively more broad-based (Figs. 13 to 15). Occasionally such a large villus filled the entire lumen (Fig. 14). This was seen more frequently in bile pre-ductules and ductules (Fig. 21) than in canaliculi, in which the obstruction was usually caused by more numerous interlocking microvilli. The internal structure of the edematous microvilli differed little from their normal counterparts except for a reduction in over-all electron density (Fig. 12). Occasional small smooth-surfaced vesicles (Fig. 11) or aggregates of crystals, thought to be cholesterol (Fig. 7), were found in the cytoplasmic matrix

of the enlarged microvilli. Particles of ribose-nucleoprotein (RNP) were seen rarely in their cytoplasm (Fig. 10).

Reduction and Distortion in the Number of Microvilli. Apart from the reduction of microvilli in those canaliculi where a few edematous villi were found filling the lumen (Figs. 13 to 16), decrease in the number of microvilli was noted most frequently in dilated canaliculi (Figs. 3 and 4). Only very marked reduction was considered to be of pathologic significance since variations in the density distribution of these structures were found even normally.[7] Reduction in the number of microvilli contributed occasionally to the impression that canaliculi were dilated (Figs. 3 and 4). Many microvilli in dilated and occluded channels were found to be stunted or distorted (Figs. 4 and 13 to 16).

Changes of Canalicular Size

Markedly dilated bile canaliculi were found in all cases under review. In general, their numbers were greater and their presence ubiquitous in a given lobule in instances associated with clinical evidence of cholestasis (Figs. 1 to 5). In all other cases their distribution was patchy, and often they could be found only after a prolonged search. Assessment of pathologic dilatation of bile canaliculi could only be made in electron micrographs not exceeding an original magnification of approximately 6,000 times (Figs. 1 and 2). At higher magnifications, where a comparison between the average size of cells and of bile canaliculi could not be made, conclusions could not be reached on the existence of this form of alteration. Moreover, dilatation may be merely apparent in those channels in which the number of microvilli projecting into the lumen is reduced (Figs. 3 and 4).

The Relationship of Dilated to Occluded Canaliculi

In general, in intrahepatic cholestasis the number of occluded canaliculi exceeded the number of dilated ones, and the opposite was observed in extrahepatic biliary obstruction. It was, however, of importance to note that both pathologically dilated and occluded canaliculi were found in all the material under review. Despite attempts to localize exactly the site and distribution of the obstruction and dilatations respectively in a given lobule, no definitive conclusions could be reached on this point. It is noteworthy that in intrahepatic biliary obstruction, dilated canaliculi were often found in the marginal zones of lobules. In these instances the cause of the obstruction was occasionally found to be occlusion of the lumens of bile pre-ductules or ductules as a result of edema of their microvilli (Fig. 21).

In extrahepatic cholestasis, dilatation of bile canaliculi and of bile

ductules was not always accompanied by a dilatation of the intervening pre-ductule (duct of Hering).

Disturbances of Bile Canalicular Topography

As a result of both distention of most and opening up of some pre-existing partially or totally closed canaliculi, the topographic relationship between the spaces of Disse, perisinusoidal recesses and canals and bile canaliculi was found to be altered. They were often found to be in closer contact with each other than normally, yet actual communications could not be demonstrated. In well-preserved tissue, canalicular ruptures were not observed.

Numbers of Bile Canaliculi

In animals with experimentally induced complete extrahepatic biliary obstruction, an apparent increase in the number of bile canaliculi was observed. This was thought to be due to an opening up of channels which were normally partially or totally closed.[7] A reduction in the number of bile canaliculi was not seen in any of the cases under review.

Morphologic Evidence of Bile Stasis

It was stated previously that small quantities of a faintly electron-opaque material could be found occasionally in the lumens of bile canaliculi under normal circumstances.[7] In general, the presence of more highly electron-dense or particulate material in bile canalicular lumens was most marked in instances associated with clinical evidence of cholestasis. However, even in cases of total extrahepatic biliary obstruction, completely empty bile canaliculi were found (Fig. 2).

The retained products of bile in the canaliculi assumed several forms: (a) a diffuse, moderately electron-dense material partially or totally filling the lumen (Fig. 5); (b) a coarsely granular amorphous material of a fairly marked electron density, less evenly distributed and often intermingled with the material described under (a) above (Figs. 3 and 4); (c) precipitates with a crystalline structure forming rosettes of needles. Small ringlike structures of the same material were occasionally found in the vicinity of the crystalline aggregates. It was thought that this material represented cholesterol (Fig. 8); (d) aggregates of lipid of a variable, but usually marked, electron density, closely resembling the so-called peribiliary and lipofuscin bodies. These intraluminal aggregates will be referred to as lipid emboli since they were never found to adhere to the lining of canaliculi and therefore could not be designated as bile thrombi (Fig. 5). The mode of entry of these lipid bodies into the lumen was never observed at a canalicular level, but was seen fairly

frequently in bile pre-ductules and ductules. Figure 6 shows the extrusion of such material from a biliary epithelial cell across its cell membrane.

Alterations of Parenchymal Liver Cells

The main cytoplasmic mass of parenchymal liver cells, which formed the boundary and microvilli of pathologically altered bile canaliculi, was frequently found to behave independently of the pathologic processes in the latter. Markedly dilated canaliculi with reduced numbers of microvilli or with edematous microvilli were often found to be bounded by otherwise normal or almost normal parenchymal cells (Figs. 2 and 13 to 16). On the other hand, cells with severe degrees of vacuolar (hydropic) degeneration were frequently provided with microvilli of average dimensions protruding into bile canaliculi of average appearance (Fig. 9). In many instances, particularly in extrahepatic biliary obstruction, both changes in canaliculi and in the adjacent parenchymal cells were found to have developed simultaneously.

The changes of parenchymal cells which were noted in relation to altered canaliculi can be summarized as follows:

Condensation of Peri-canalicular Cytoplasmic Matrix. A band of dense cytoplasmic matrix devoid of organelles surrounded the canalicular lumen (Fig. 2). A similar condensation may be noted even in normal tissues.[7] However, in SM-stained sections of pathologically altered tissues, the zone of condensation assumed an intense argentophilia, possibly as a result of the presence of carbohydrate-containing material (e.g., glycoprotein) in this region (Fig. 2). In sections stained with lead hydroxide, the zone was usually found to be entirely devoid of RNP granules (Fig. 12).

Increase of Peribiliary and Lipid Bodies. Pericanalicular, "dense," lipid, and lipofuscin bodies were often markedly increased in number in the vicinity of pathologically altered bile canaliculi (Figs. 3 and 4).

Vacuolar (Hydropic) Degeneration. As was previously noted, this change was seen frequently in the parenchymal cells adjoining altered canaliculi. It consisted of a dilatation of the endoplasmic reticulum, thus forming spaces usually devoid of any electron-dense material in the lumen. Neither the change itself nor the size of dilated sacs bore any obvious direct relation to the severity of the canalicular changes.

Alterations of Mitochondria. In the vicinity of altered bile canaliculi from any cause, abnormal mitochondria were found in the parenchymal cells (Figs. 17 to 20). These changes were patchy in distribution and usually had to be searched for. They may be summarized as follows: (a) an over-all increase in size of the corpuscles; (b) a distortion of the

shape of the entire mitochondrion (Fig. 17); (c) an elongation of the cristae mitochondriales, thus forming long, tortuous partitions within the mitochondrial matrix (Fig. 20); (d) formation of "ring-shaped" cristae which surrounded a lumen containing part of the mitochondrial matrix (Fig. 19); and (e) an increase in size of the usual opaque or dense bodies within the matrix (Fig. 18).

Development of Abnormal Resorptive or Secretory Cell Surfaces. In most of the cases under review in which bile canalicular alterations were demonstrated, microvilli were found on surfaces of liver cells where they are not normally present. Thus, cells were frequently found to be provided with microvilli on all surfaces in addition to those which faced the space of Disse and bile canaliculi (Fig. 22). Such aberrant microvilli were particularly numerous on those surfaces which were in direct contact with portal tracts or with fibrous connective tissue septums in regenerating lobules. Lipid and bile-laden macrophages were often found in these areas in direct apposition to such microvillous borders, suggesting that their predominant function was to provide an alternate pathway of bile secretion.

The various changes in parenchymal liver cells will be analyzed in detail later.

Discussion

This paper reports morphologic observations of pathologically altered bile canaliculi in intrahepatic cholestasis. These changes consisted of edema of microvilli, leading to subtotal or total obstruction of the canaliculi. In other areas the canalicular lumens were widened and their over-all topography was consequently disturbed so that they came to lie in close proximity to the perisinusoidal spaces of Disse. The contents of the dilated canaliculi were altered and were seen to consist of more or less particulate bile constituents.

In order to establish the specificity of these changes, human and experimental animal tissues were examined from cases with extrahepatic biliary obstruction and from others with liver injury not associated with clinical evidence of cholestasis. In these, changes were found which were qualitatively identical with those described in intrahepatic cholestasis. It is one of the purposes of this paper to show that alterations of bile canaliculi occurred not only in conditions associated with clinical evidence of cholestasis but also in its absence. This point seems to us of importance since interpretation of canalicular alterations based upon observations of a very few altered canaliculi in a given liver biopsy specimen cannot be used as definite evidence of a specific form of cholestasis.

The changes in canaliculi were extremely variable, and it was only by an assessment and estimation of their reciprocal frequency and distribution that some attempt could be made to arrive at a diagnosis. Since quantitation of changes at an electron microscopic level of observation is extremely difficult, the results serve mainly to advance the frontiers of microscopy to areas which could not be previously resolved and studied.

With the above considerations and limitations in mind, bile canalicular occlusion by edematous microvilli in intrahepatic cholestasis is seen considerably more frequently than canalicular dilatation. In extrahepatic biliary obstruction the reverse holds true. In the latter condition and in others not associated with clinical evidence of cholestasis, occlusions of lumens by edematous microvilli were seen only sporadically, though edema of individual microvilli was encountered frequently.

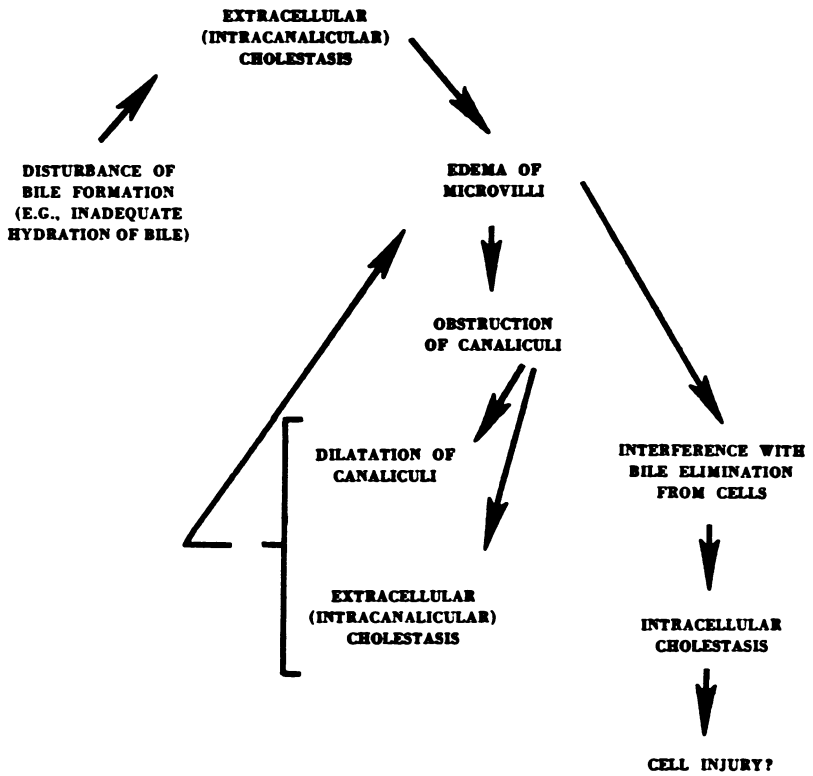
It has been suggested that precipitated bile plugs may be the cause of bile canalicular obstruction in intrahepatic cholestasis.[1,3] Our observations failed to substantiate this view. Where particulate material was observed in canalicular lumens, it was never of a consistency or size adequate to account for total obstruction. We considered it probable that bile plugs were a secondary phenomenon resulting from either hydrodynamic disturbances or from changes in the composition of bile. The edema of microvilli may be the primary cause of intrahepatic biliary obstruction. In view of the finding of an identical change in extrahepatic biliary obstruction, it cannot be denied that this change may itself be secondary to prolonged contact of microvilli with static bile constituents.

On the basis of these considerations, the following hypothesis is suggested to explain the morphogenesis of intrahepatic biliary obstruction: The primary cause may be a disturbance of bile formation (e.g., inadequate hydration) leading to faulty drainage of bile from canaliculi. The edema of microvilli may be secondary to prolonged contact with such inspissated bile constituents and may lead in turn to the hydrodynamic disturbances evidenced morphologically by dilatation of some canaliculi. The occlusion of lumens by edematous microvilli may further increase the retention of bile products in the biliary passages. The swollen microvilli may themselves constitute an obstacle to the elimination of bile constituents from cells by producing an increase in the cell-lumen barrier. Thus a vicious circle may be established (Text-fig. 1).

Observation of the reciprocal behavior of parenchymal liver cells on the one hand, and of their microvilli where these project into the lumens of bile canaliculi on the other, shows a remarkable autonomy of behavior of the latter structures. A similar observation was made previ-

ously in the study of microvilli in the space of Disse when these came in contact with soluble immune complexes.[6] It is clear that the microvilli may undergo changes when the main cytoplasmic mass of parenchymal cells remains unaltered.

Our observations of the parenchymal liver cells adjacent to altered bile canaliculi differ from those of others.[1] Schaffner and Popper noted inconstant and irregular alteration of endoplasmic reticulum and normal



TEXT-FIGURE 1. Hypothetical concept of a vicious circle in intrahepatic cholestasis.

mitochondria in intrahepatic cholestasis, and disruption of the normal profiles of the endoplasmic reticulum and increased lipofuscin pigment structures in extrahepatic cholestasis. They stated that the mitochondria were not significantly altered in the latter condition.[1] Although the exact significance of the cytoplasmic alterations of liver cells reported in this paper is at present not apparent, they occur with a sufficient degree of constancy in relation to altered bile canaliculi to suggest some etiologic connection.

Disturbances of cell hydration (e.g., hydropic or vacuolar degeneration) can be anticipated when the outflow of bile from the pertinent

canaliculi is obstructed. It should be noted, however, that the dilatation of the endoplasmic reticulum which we designate as hydropic or vacuolar degeneration is considered by others as not necessarily related to water retention,[8] though an alternative explanation is not offered.

The increase in the number of intracytoplasmic "bodies" of various composition and the pericanalicular cytoplasmic condensation probably of glycoprotein material may be similarly related to the interference with elimination of bile or its constituents. Abnormal liver cell mitochondria have been reported in a case of biliary carcinoma and in a case of prolonged viral hepatitis.[9] The alterations in those instances consisted of the formation of intramitochondrial structures resembling myelin figures. The abnormality reported here is different in that it consists of an elongation and irregular coiling of the cristae mitochondriales. Since mitochondria are known to be highly integrated structures, concerned with many enzymatic functions,[10] it can be anticipated that such altered mitochondria may have a profound effect on the metabolism of the cells.

The use of auxiliary stains, i.e., PTA and SM, and the strict observance of the criteria of good preservation of tissues, outlined earlier in this paper, are essential in the study of bile canaliculi. It was found repeatedly that differentiation between bile canaliculi and sinusoids was impossible without these measures. It is significant that with these precautions abnormal communications could not be demonstrated between these two structures. This finding differs from those of others.[1]

Demonstration of canalicular ruptures or abnormal communications between bile canaliculi and sinusoids are essential prerequisites for the assumption of direct bile leakage into the space of Disse. Since this finding could not be confirmed in this study, evidence was sought for a possible alternate aberrant pathway of bile excretion. A preliminary observation suggests that this could occur via abnormally located microvilli on surfaces of liver cells not normally provided with them. Bile or its constituents may thus reach the connective tissues of the liver and percolate through into vascular channels, e.g., lymphatics, which are not always provided with a basement membrane. This suggestion does not exclude the possibility of bile regurgitation into the space of Disse by a trans-cellular transport mechanism as suggested by the investigations of Hampton.[11]

Summary

Morphologic alterations of bile canaliculi were found to occur in conditions associated with clinical signs of cholestasis as well as in others in the absence of such clinical evidence. It was suggested that

canalicular dilatation, obstruction by edematous microvilli and inspissation of bile observed with the electron microscope should not be considered as evidence of cholestasis or of a specific form of cholestasis unless quantitation and assessment of the reciprocal frequency of these changes could be achieved.

In intrahepatic cholestasis, obstruction of canaliculi by edematous microvilli was seen relatively more frequently than dilatation. It was suggested that the underlying anomaly in intrahepatic cholestasis might be a disturbance of bile formation (e.g., hydration) leading to stasis of bile in canaliculi. The edema of microvilli could then be the result of prolonged contact of these structures with inspissated bile constituents and might in turn lead to hydrodynamic disturbances evidenced morphologically by dilatation of some canaliculi. The swollen microvilli might themselves constitute an obstacle to the efficient elimination of bile constituents from cells.

In extrahepatic cholestasis, similar changes were noted in bile canaliculi though dilatation was seen more frequently than obstruction by edematous microvilli.

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[*Illustrations follow*]

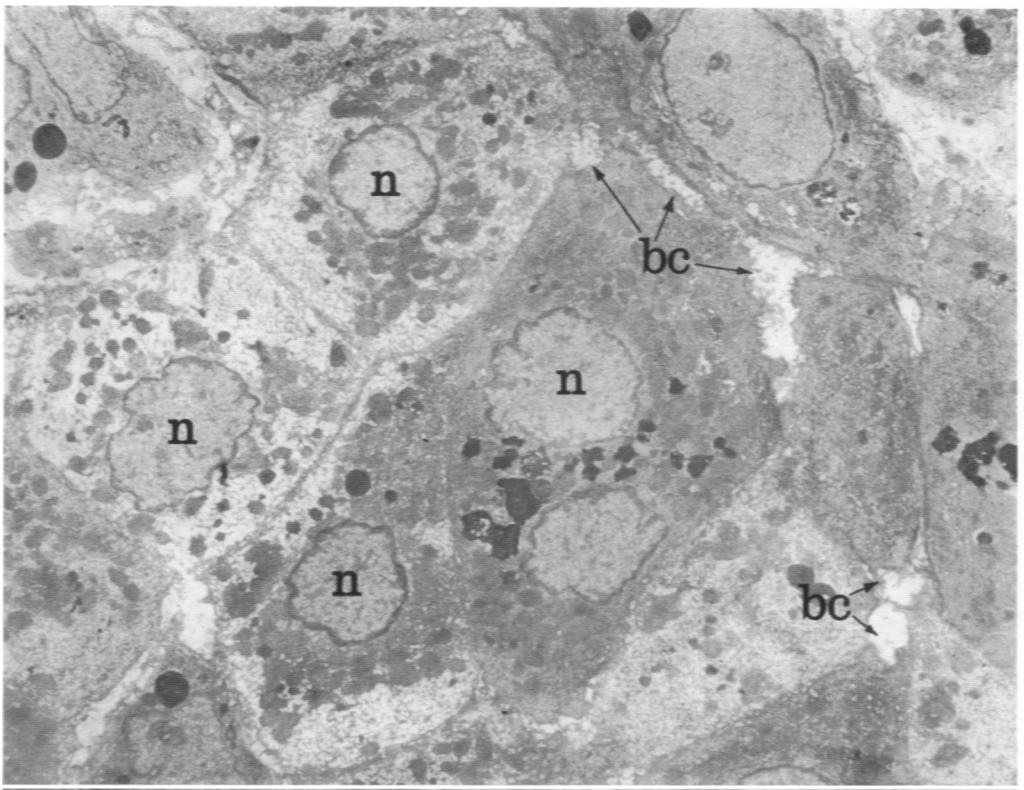
Legends for Figures

Key:

bc, c = bile canaliculus
d = bile ductule
e = bile embolus
f = fibroblastic processes
gl = glycogen
k = Kupffer cell
l = lumen
m = mitochondrion
mb = microbodies (lipid, lipofuscin, "peribiliary," glycoprotein cytoplasmic inclusions)
mv = microvillus
n = nucleus
ncl = nucleolus
pd = bile pre-ductule (duct of Hering)
plc = parenchymal liver cell
pt = portal tract
pv = pinocytic vesicle
rbc = red blood cell
rnp = ribose-nucleoprotein granules
S = sinusoid
V = intracytoplasmic vacuole
ZC = zone of cytoplasmic condensation

FIG. 1. Congenital biliary atresia. Moderately dilated bile canaliculi devoid of content lie between parenchymal cells which contain large irregular lipid deposits. The canalicular dilatation can only be assessed in relation to the size of the neighboring cells. Uranyl acetate impregnation. × 3,920.

FIG. 2. Experimental extrahepatic biliary obstruction (2 days). Widely dilated empty canaliculi show condensation of parenchymal cytoplasm in their margins. Note that some canaliculi are of average size. A perisinusoidal recess reaches to within a short distance of one canaliculus. Silver methenamine stain. × 5,760.



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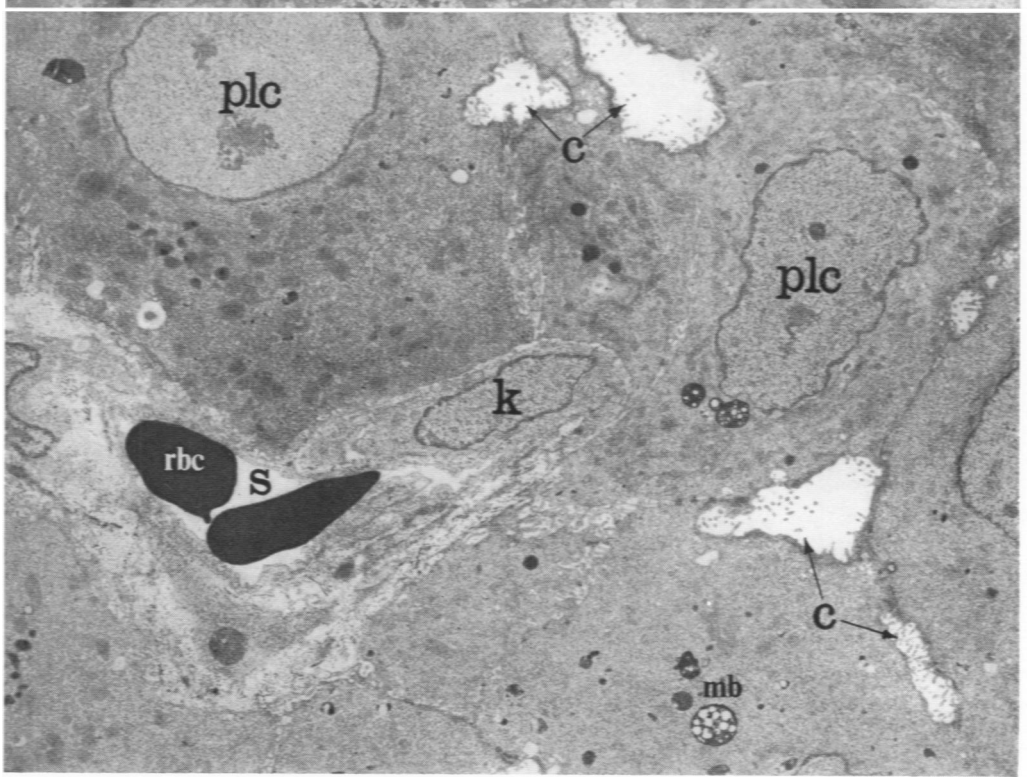

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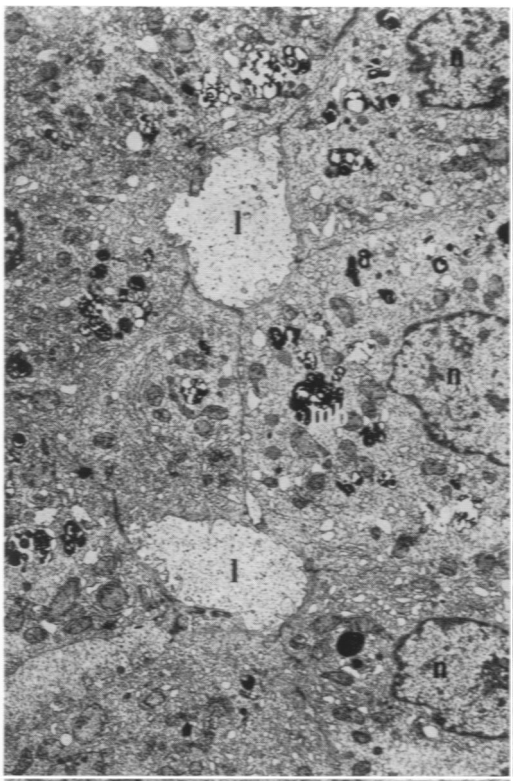
FIG. 3. Intrahepatic biliary obstruction. Two adjacent dilated bile canaliculi with a marked reduction of their microvilli. The lumens contain a granular argentophilic material. Note the complex structure and increased number of peribiliary lipid bodies. Protargol stain. × 5,600.

FIG. 4. Intrahepatic biliary obstruction. The microvilli which project into the dilated lumen of the bile canaliculus are stunted and distorted. The inspissated bile is seen as a faintly diffuse argentophilic cloudlike substance with a scattering of electron-dense particles. Protargol stain. × 9,800.

FIG. 5. Biliary cirrhosis. A dilated bile canaliculus containing a granular, faintly electron-dense substance in addition to an embolus of marked but variable argentophilia. The microvilli of the canaliculus are reduced in number, stunted and often distorted. Protargol stain. × 10,000.

FIG. 6. Congenital biliary atresia. The lumen of a bile ductule with reduced and stunted microvilli is seen. The biliary epithelium around the lumen contains several lipid bodies. One of these appears to have been extruded into the lumen where several small lipid emboli are present. Protargol stain. × 20,000.



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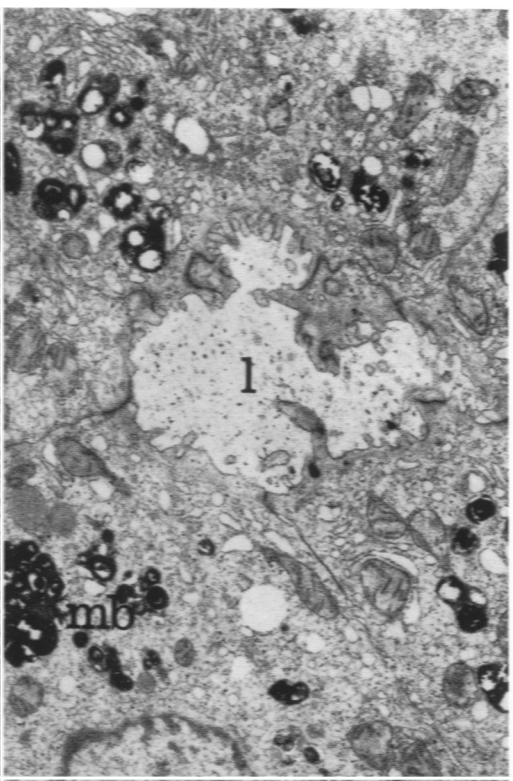

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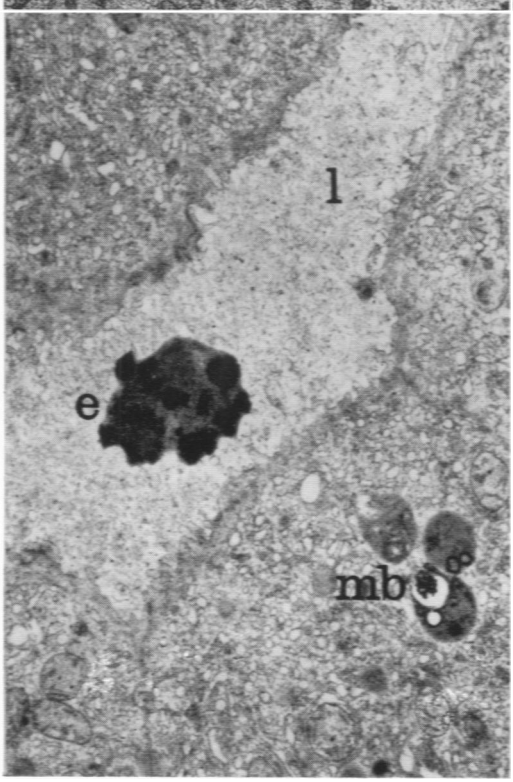

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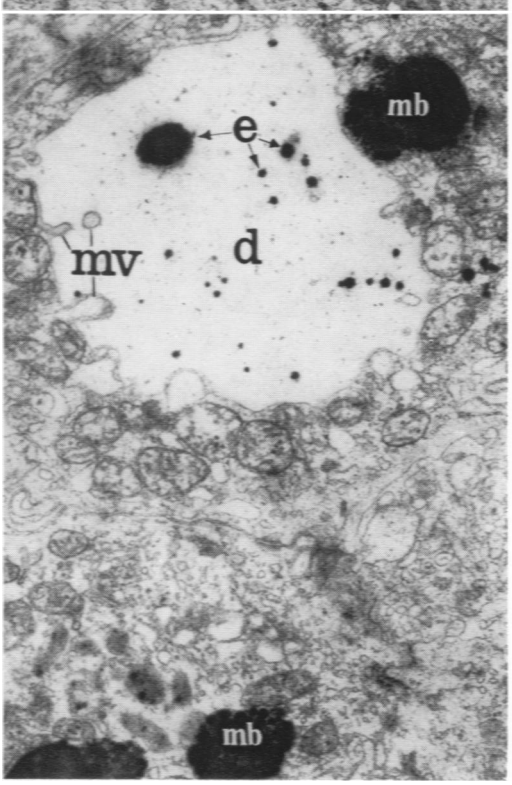

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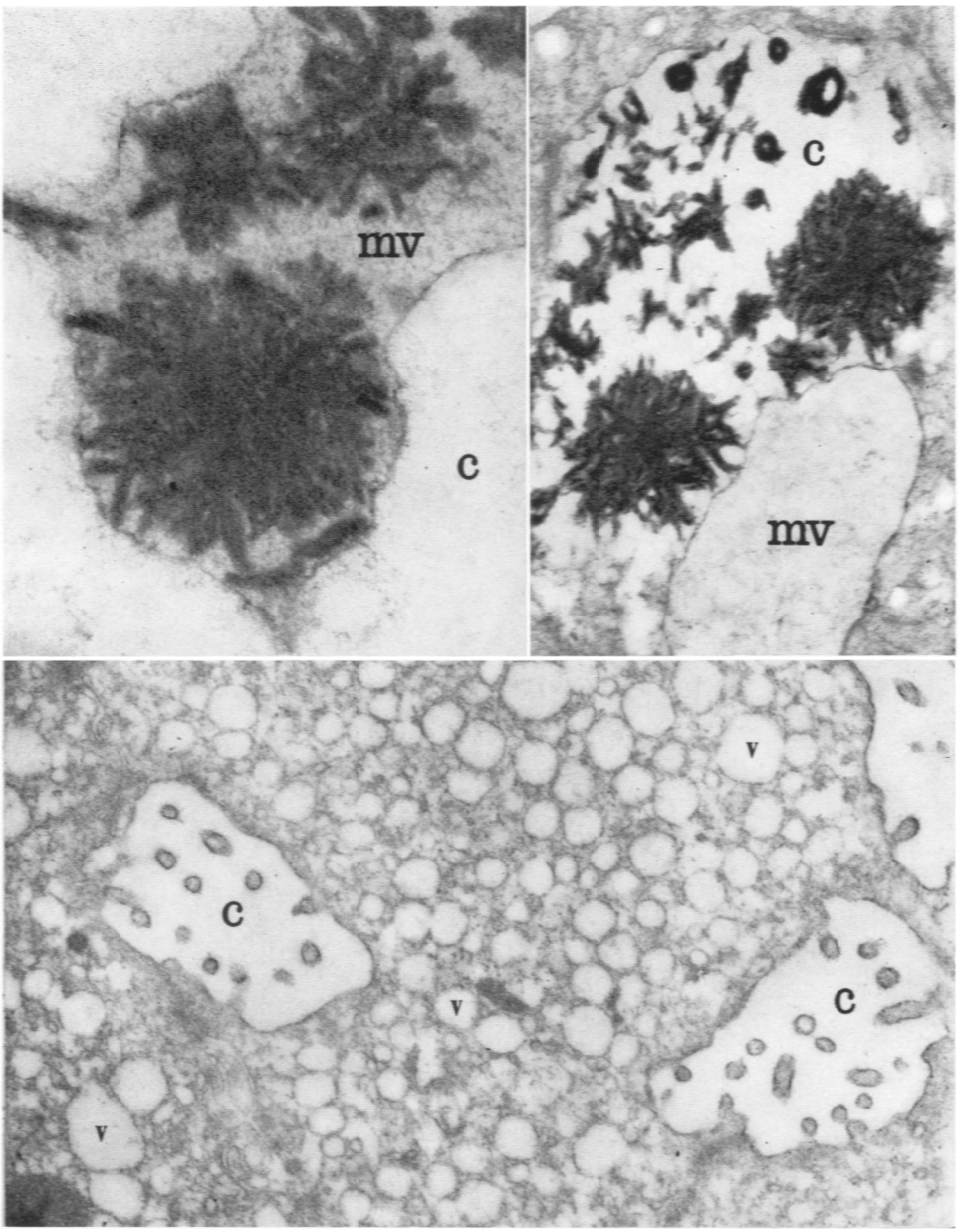

FIG. 7. Congenital biliary atresia. Needle-shaped crystals within a bile canalicular microvillus. These are interpreted as representing cholesterol. Phosphotungstic acid stain. × 59,200.

FIG. 8. Intrahepatic biliary obstruction. Needle-shaped crystals and ring forms of these in the lumen of a bile canaliculus containing also a large edematous microvillus. The material is interpreted as representing cholesterol. Phosphotungstic acid stain. × 29,600.

FIG. 9. Experimental immunologic liver injury (72 hours following the injection of soluble immune aggregates into the portal vein). Diffuse hydropic (vacuolar) degeneration is manifest in parenchymal cells. The two bile canaliculi show no changes in their microvilli. Phosphotungstic acid stain. × 24,640.

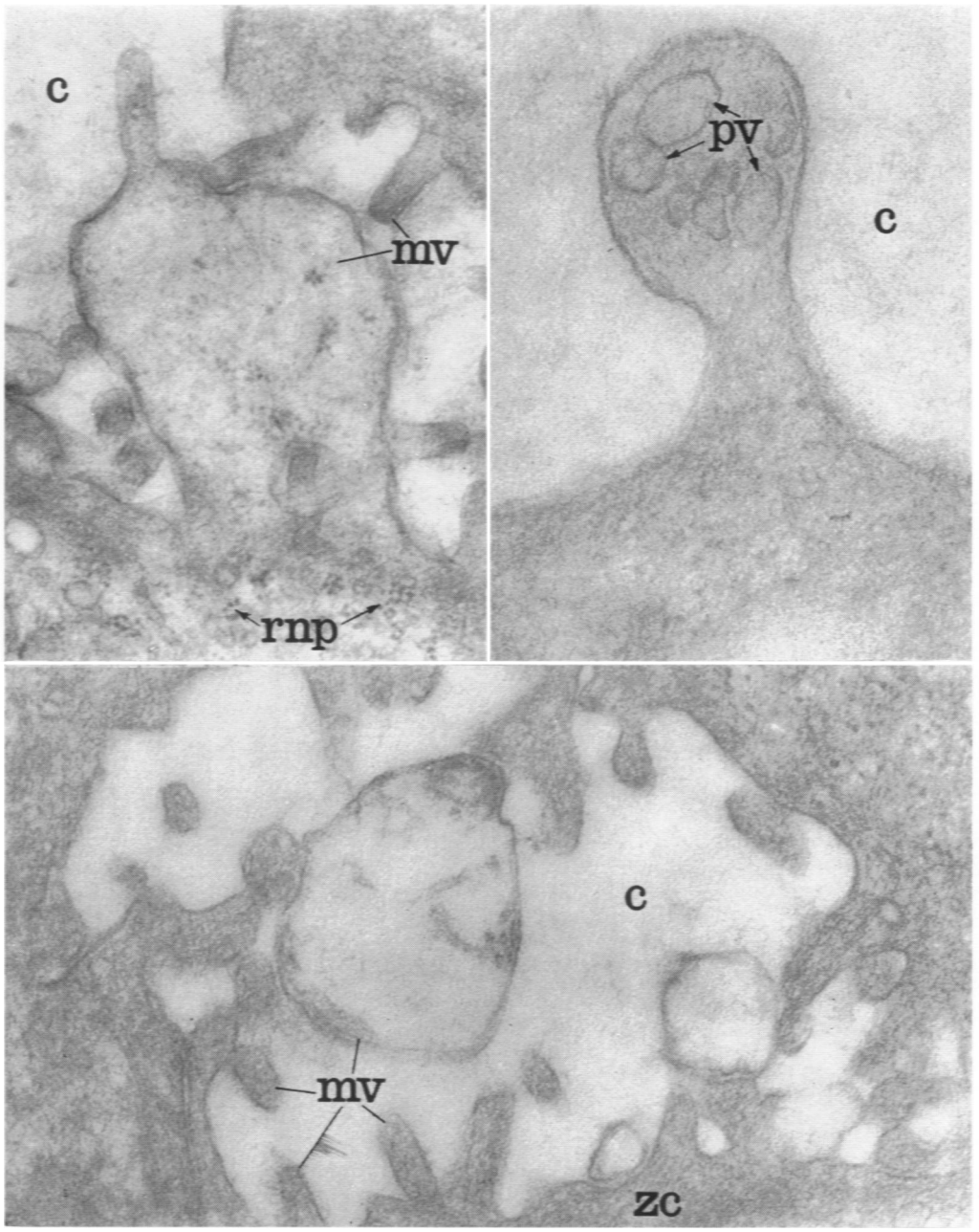

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FIG. 10. Experimental extrahepatic biliary obstruction (2 days). Edema of a microvillus commencing at its base and as yet not involving the extremity. Neighboring microvilli are of average size. Lead hydroxide stain. × 47,260.

FIG. 11. Experimental extrahepatic biliary obstruction (2 days). Early edema of the extremity of a microvillus with a relatively normal waist. Note the presence of smooth-surfaced vesicles in the matrix of the microvillus. Lead hydroxide stain. × 69,500.

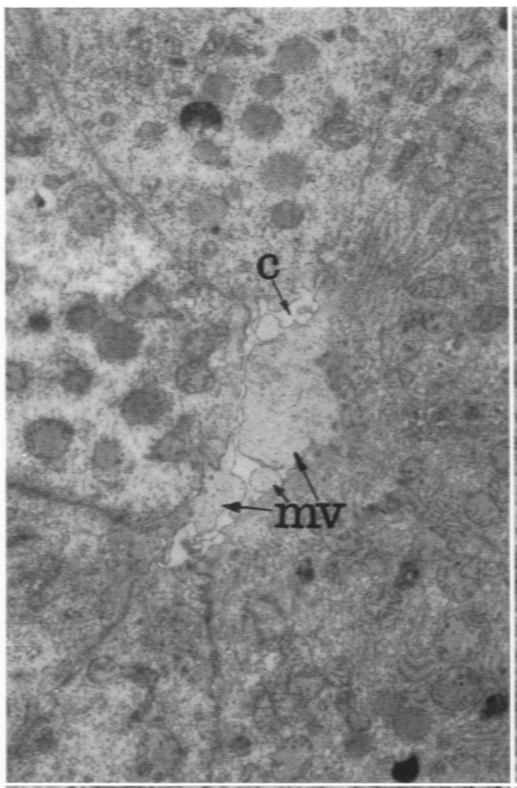
FIG. 12. Experimental extrahepatic biliary obstruction (3 days). Ballooned microvilli are cross-sectioned in the lumen of an apparently empty canaliculus with other intact microvilli in other parts of the lining. Note the zone of condensed cytoplasm adjacent to the canaliculus. Lead hydroxide stain. × 58,380.

FIG. 13. Portal cirrhosis. Edematous microvilli almost totally occlude the lumen of a bile canaliculus; others are stunted. The adjacent cells show no evidence of hydropic (vacuolar) degeneration. Protargol stain. × 7,840.

FIG. 14. Intrahepatic biliary obstruction. The canaliculus in the center of the electron micrograph is almost totally occluded by a massively edematous microvillus and several others less severely affected. Note the absence of hydropic (vacuolar) degeneration in adjacent parenchymal cells. Protargol stain. × 8,400.

FIG. 15. Biliary cirrhosis. A partially occluded bile canaliculus. The microvilli are partly edematous and many are markedly distorted. Note the densely argentophilic granules representing glycogen in the cytoplasm. They are not present in the mitochondria. A few are located within the lumen. Periodic acid-silver methenamine stain. × 13,440.

FIG. 16. Intrahepatic biliary obstruction. Markedly edematous microvilli project into a lumen which contains a diffuse, faintly electron-opaque substance. Note the particles of glycogen in the cells and in the lumen. Periodic acid-silver methenamine stain. × 16,800.



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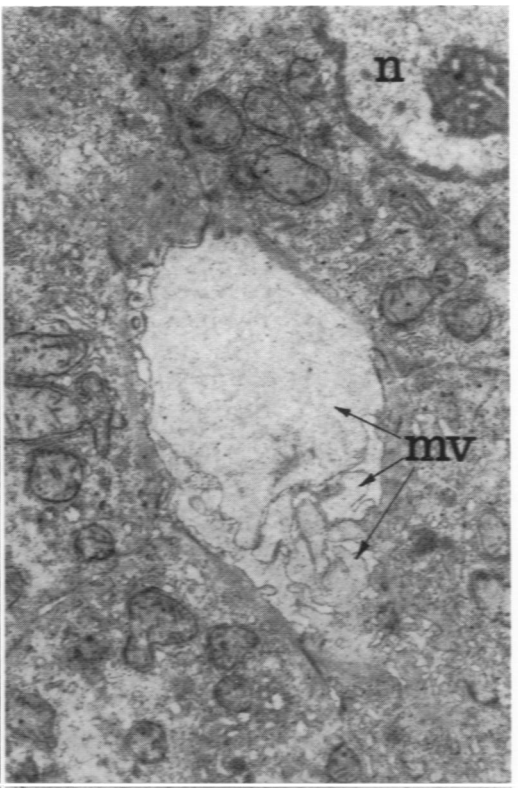

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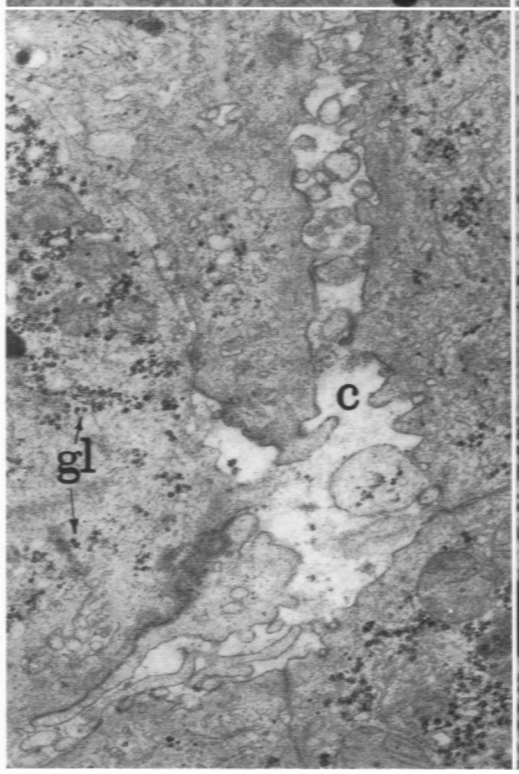

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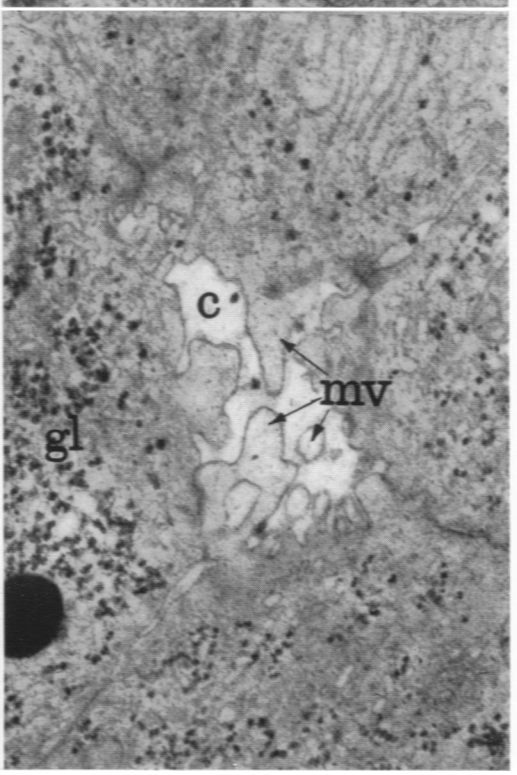

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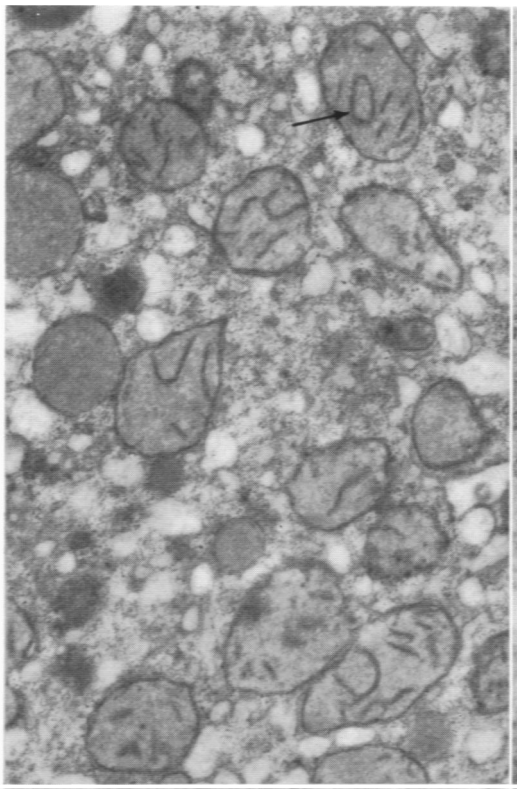
FIG. 17. Congenital biliary atresia. Mitochondria of a parenchymal liver cell show distortion of over-all shape, elongation of some cristae mitochondriales and a "ring" form in the mitochondrion in the right upper corner (arrow). Protargol stain. × 20,800.

FIG. 18. Portal cirrhosis. A mitochondrion in a cell in the vicinity of an altered bile canaliculus. Note the irregular enlargement of the dense body in the mitochondrial matrix. Lead hydroxide stain. × 73,600.

FIG. 19. Intrahepatic biliary obstruction. An abnormal mitochondrion shows a ring-shaped crista and others which are elongated and abnormally oriented. Phosphotungstic acid stain. × 68,200.

FIG. 20. Biliary cirrhosis. An abnormal mitochondrion with elongated tortuous cristae mitochondriales. The fine black precipitate is an artifact caused by staining. Lead hydroxide stain. × 59,400.



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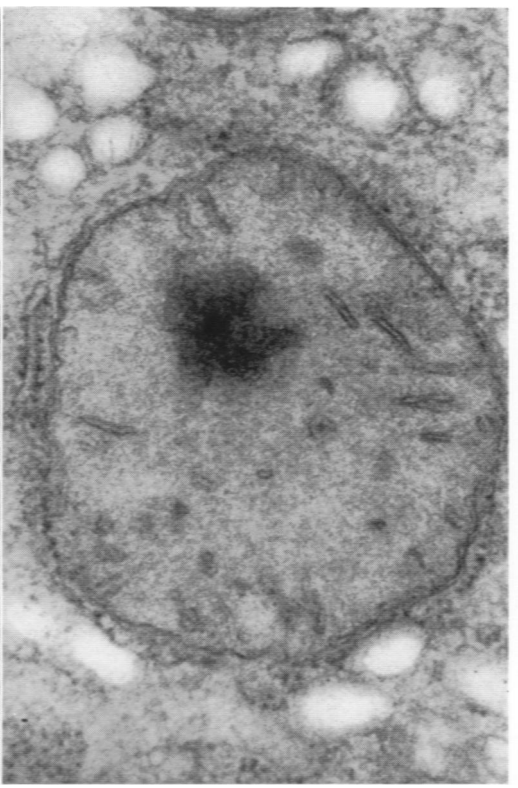

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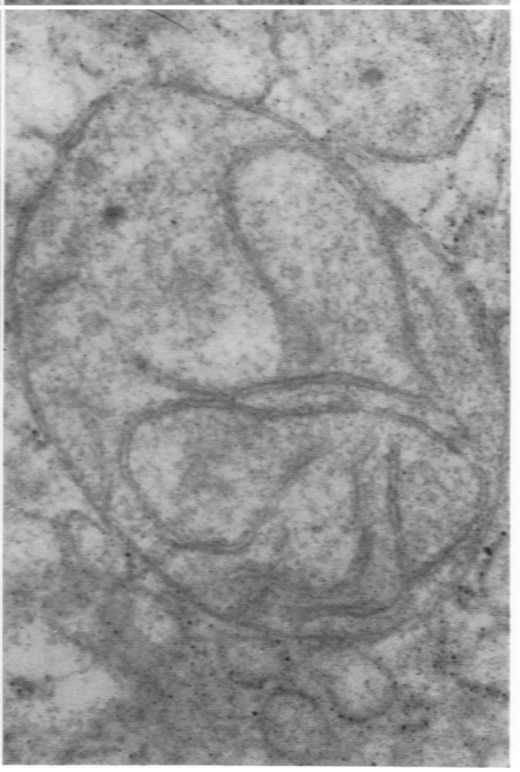

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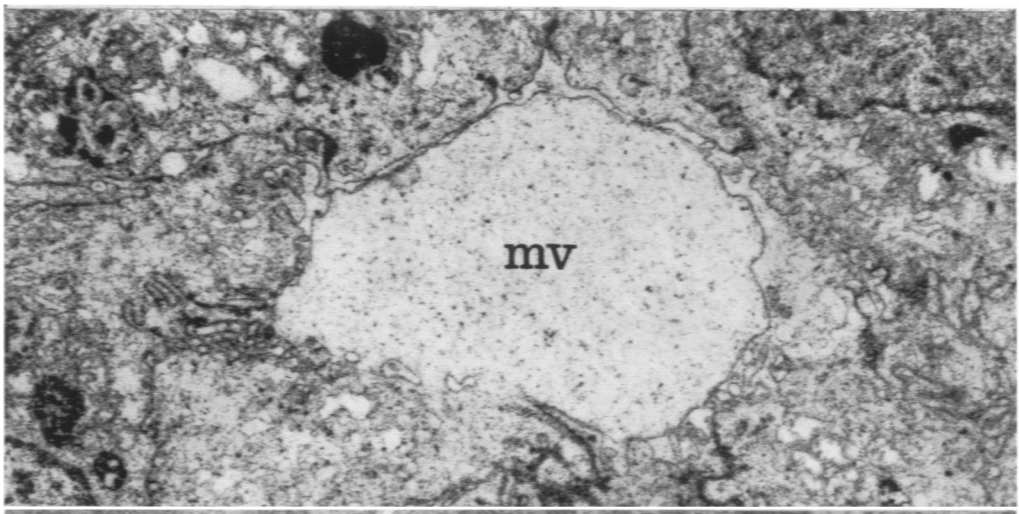
FIG. 21. Congenital biliary atresia. A large edematous microvillus is obstructing the lumen of a bile ductule. Very few apparently normal microvilli can still be recognized. Protargol stain. × 10,800.

FIG. 22. Biliary cirrhosis. A portion of the orifice of a dilated bile pre-ductule is seen on the right. The pre-ductule can be recognized since part of its lining is formed by a biliary epithelial cell. Note the different mitochondria in the latter epithelium (arrows) as compared to parenchymal cells. A liver cell adjoins a portal tract in which cytoplasmic processes of fibroblasts can be recognized. Note that the parenchymal liver cell is provided with microvilli on all its surfaces, including that which faces the portal tract. These are thought to constitute a possible pathway of aberrant bile secretion. Protargol stain. × 9,600.



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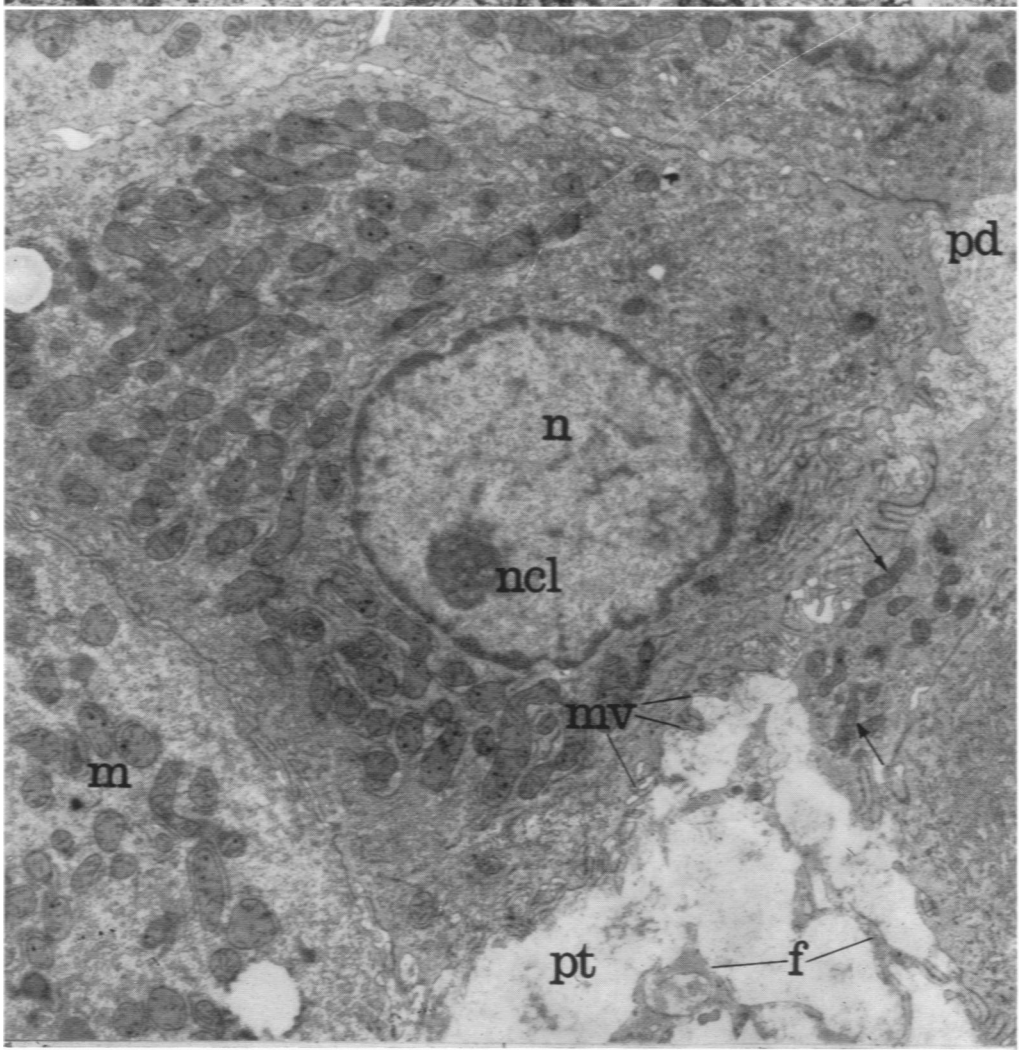

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