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Morphogenesis of Intrahepatic Bile Ducts of the Human Fetus

Light and Electron Microscopic Study

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Summary. The development of the intrahepatic bile ducts of the human fetus was investigated by light and electron microscopy. Bile canaliculi with microvilli and junctional complexes are already found in the embryo of 7 mm. Some of them are of the intracellular type. At six to seven weeks, large bile canaliculi bounded by four to seven liver cells appear. Subsequently, bile canaliculi are formed predominantly between three to four adjoining liver cells and this arrangement persists throughout later fetal life.

The early intrahepatic bile ducts develop around the portal vein as epithelial cell plates derived from the hepatic duct and the branches sprout from the epithelial cell plates in several different places. The epithelial cell plates are separated from each other by primitive connective tissue and they change into a complex network of bile ducts. Formation of the intrahepatic bile ducts is completed by three months.

Biliary duct cells at the end of the developing bile ducts are thought to transform into liver cells. Therefore, at the ducts of Hering various transitional cells appear between biliary duct cells and liver cells.

The fine structure of the developing liver cells and biliary duct cells is also described.

Key words: Cholangiogenesis — Intrahepatic bile ducts — Liver cells — Embryology — Human fetus.

Introduction

Development of the intrahepatic bile ducts is complicated and there are two main theories on how it occurs. One is that the bile ducts develop along the portal vein as epithelial proliferations derived from the hepatic ducts and the bile canaliculi are differentiated independently from the liver cell cords (Hammer, 1926; Davis, 1963). The other theory holds that all intrahepatic bile ducts are derived from liver cells (Bloom, 1926; Horstmann, 1939; Elias, 1955; Du Bois, 1963 and others). When the liver cells make contact with the connective tissue in the periportal spaces, they are thought to transform into cuboidal ductal epithelium under the influence of the connective tissue and form the lumen of the duct. In the latter theory, there are two hypotheses on the direction of ductal formation. According to Elias and Du Bois, ductal formation progresses in the direction of the hilus. On the other hand, Bloom stated that liver cells develop by branching from the head of the embryonic hepatic duct, and with ingrowth of connective tissue into the liver parenchyma, the cell cords nearest the connective tissue are transformed into ducts.

Since Horstmann (1939) first described the development of the intrahepatic bile ducts of the human fetus and correlated his observations with the findings by Doljanski and Roulet (1934), the latter hepatocytogenic theory of intrahepatic cholangiogenesis has been widely accepted. If the intrahepatic bile ducts originate from the liver cells under the influence of the connective tissue, it is supposed that transitional cells or intermediate[1] cells between liver cells and biliary duct cells should exist at the ducts of Hering, the junctional portions of bile canaliculi and bile ducts. However, electron microscopic observations have failed to demonstrate the existence of transitional cells or intermediate cells in normal and pathological material (Deams, 1961; Schaffner and Popper, 1961; Steiner and Carruthers, 1961, 1962; Du Bois, 1963). Recently, Picardi *et al.* (1968) observed with the electron microscope that cells intermediate between liver cells and biliary duct cells exist in the human fetus. Therefore, in considering cholangiogenesis, the existence of transitional cells or intermediate cells should be confirmed.

The purpose of this study is to present light and electron microscopic observations on the developing intrahepatic bile ducts as well as on the liver cells of human fetuses and to present a new concept of the development of intrahepatic bile ducts in the human fetus.

Materials and Methods

Twenty-five fresh human embryos and fetuses ranging from one to ten months of ovulation age were used for this study. The ovulation age was estimated from the diagram of Kunitomo and Yamaguchi (1937). Large embryos and fetuses were laparotomized and small blocks of the liver tissue were taken. They were first fixed in 4% or 6% glutaraldehyde buffered with Millong's phosphate or s-collidine (pH 7.4) for about 2 hours at O°C and postfixed in 1% osmium tetroxide buffered with phosphate or s-collidine for 2 hours. Other blocks were fixed in Karnovsky's cacodylate buffered formaldehyde glutaraldehyde-fixative followed by 1.33% osmium tetroxide buffered with s-collidine (Karnovsky, 1965). Small embryos were sliced transversely into four to five blocks and fixed with the same fixatives. After dehydration in a series of ice-cold graded acetones, the blocks were embedded in Epon 812 (Luft, 1961).

Thin sections for electron microscopy were stained with lead acetate or with 2% uranyl acetate and lead acetate. Electron micrographs were taken with a JEM 7-A electron microscope. For light microscope observations, thick sections (1–2 μ) were stained with 0.5% toluidine blue.

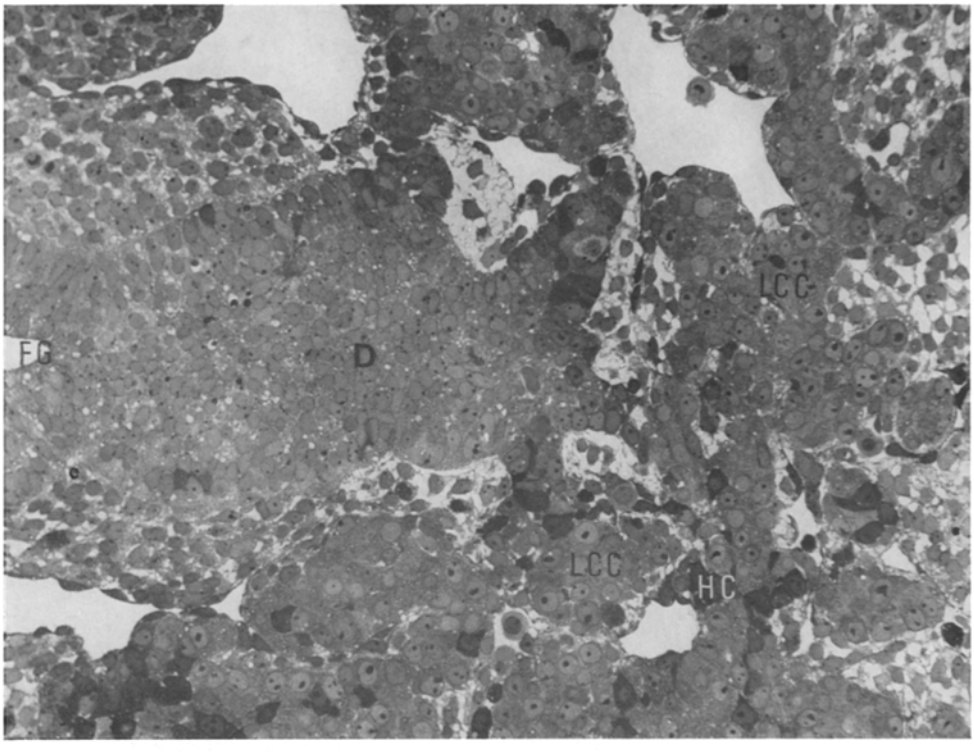
Observations

Light Microscopy

One Month. In an embryo of 5 mm, the endoderm of the foregut proliferates to form the hepatic diverticulum. Epithelial cell cords bud off from the cranial portion of the hepatic diverticulum and protrude into the septum transversum. Epithelial cell cords gradually transform into liver cells and two types of cells, light cells and dark cells, are distinguishable in the expanding liver cell cords. (Fig. 1).

In an embryo of 7 mm, liver cells which are irregular in shape and size and contain a fairly large round nucleus, proliferate around the sinusoids and form cell cords of three to five cells thick. The liver cells in the meshes of the sinusoids are

1 Transitional cell and intermediate cell are synonymously used.



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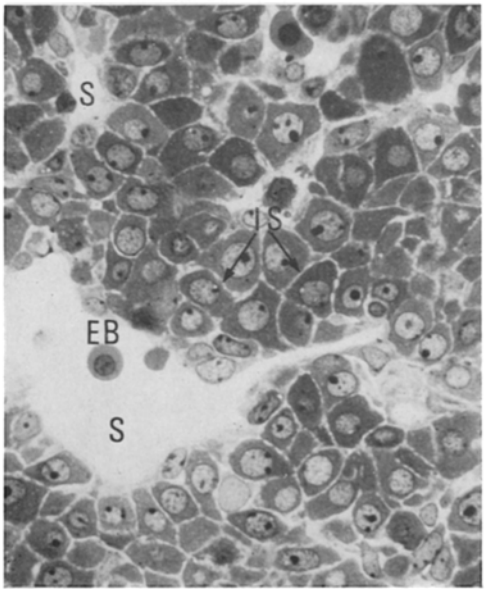

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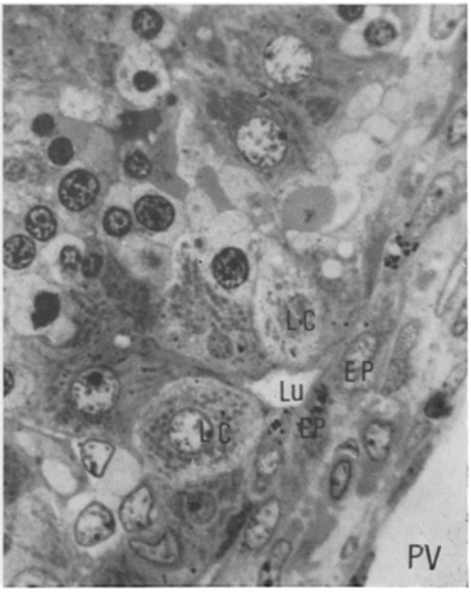

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Figs. 1–3

loosely connected with each other and wide intercellular spaces between the liver cells are conspicuous. Neither bile canaliculi nor bile ducts can be seen at this stage of development (Fig. 2).

Two Months. In the periportal spaces, many biliary vesicles which suggest the ducts of Hering are observed. These vesicles are bounded by liver cells on one side and on the other by biliary duct cells. The duct cells, which are situated on a thin connective tissue layer, are cuboidal or flat in shape, small in size and contain oval nuclei. The liver cells are polygonal and large, and contain round nuclei. Pigment granules are observed more frequently in the duct cells than in the liver cells. At this stage, the end of two months, all of the biliary vesicles are partially surrounded by biliary duct cells and no bile duct appears to be present (Fig. 3).

In the hilus of the liver of an embryo of 16 mm, a hepatic duct which is lined by columnar or cuboidal epithelium runs up to the liver parenchyma and connects with the liver cell cords. The lumen of the hepatic duct seems to be continuous with the bile canaliculus. At the junction between the hepatic duct and the liver cell cords, the columnar or cuboidal epithelial cells of the hepatic duct show a gradual transition to large polygonal liver cells (Fig. 4).

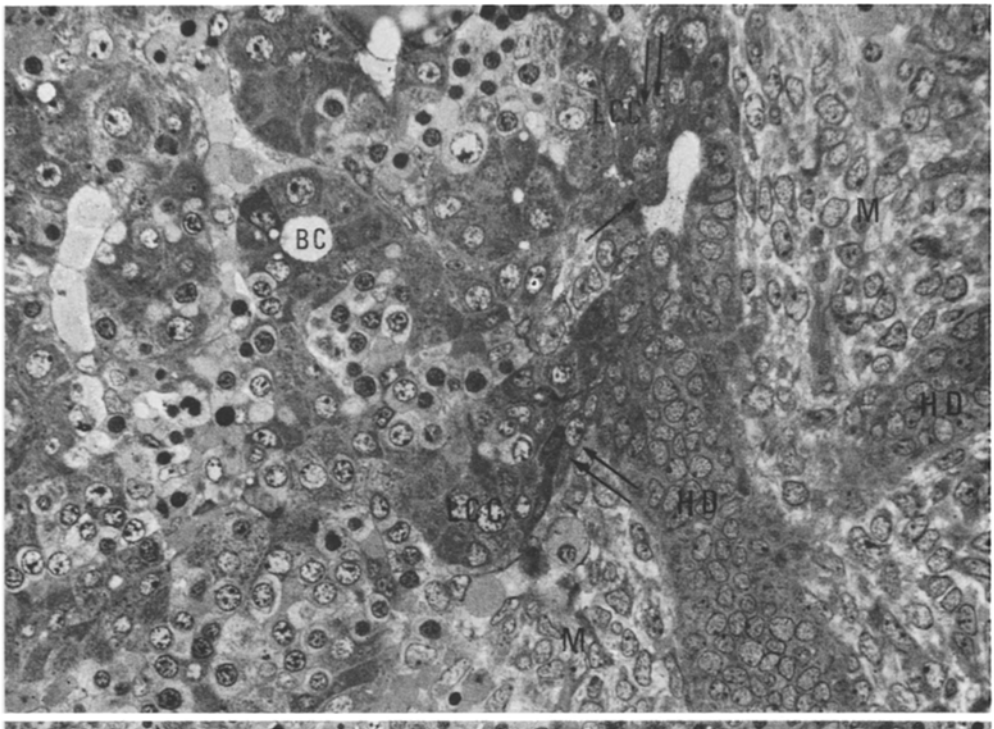
Large bile canaliculi surrounded by four to seven liver cells are frequently observed. All of their lumina are round or oval in shape and their oblique or longitudinal profiles are not observed (Fig. 4).

In an embryo of 20 mm, development of the hepatic duct progresses and the hepatic duct extends towards the portal vein by the sprouting of branches to the liver parenchyma at several places. These branches are thought to develop into bile ducts. At the branches and the tip of the hepatic duct, darkly stained cuboidal duct cells show a gradual transition to large polygonal liver cells. These transitions and differences between liver cells and duct cells will be described in detail. The liver cells are large and polygonal, and contain round nuclei. Their cytoplasm stains lightly. The duct cells are cuboidal in shape and contain oval nuclei. The cytoplasm of the duct cells is compact and stains darkly with toluidine blue. The cells at the transitional portions are irregularly arranged and are irregular in shape and size. However, they become larger and lighter as they approach the

Fig. 1. Light micrograph of the liver from a 5 mm embryo showing hepatic diverticulum (*D*) and developing liver cell cords (*LCC*). Endoderm of the foregut (*FG*) proliferates to form the hepatic diverticulum. Liver cell cords bud off from the endodermal epithelial cells of the hepatic diverticulum. Two types of cells are recognizable in cell cords of the liver. The light cells are parenchymal and the dark ones are thought to be hemopoietic (*HC*). ×300

Fig. 2. Light micrograph of the liver of a 7 mm embryo showing liver cell cords and sinusoids (*S*). The cell cord is three to five cells thick. Liver cells are irregular in shape and size, and wide intercellular spaces (*IS*) are conspicuous. Sinusoids (*S*) are rather large and contain nucleated red blood cells (*EB*). ×500

Fig. 3. Light micrograph of a 16 mm embryo showing the biliary vesicle at the periportal space. The lumen (*Lu*) is bounded by large polygonal liver cells (*LC*) on one side and flattened biliary duct cells (*EP*) on the other. *PV* Portal vein. ×800



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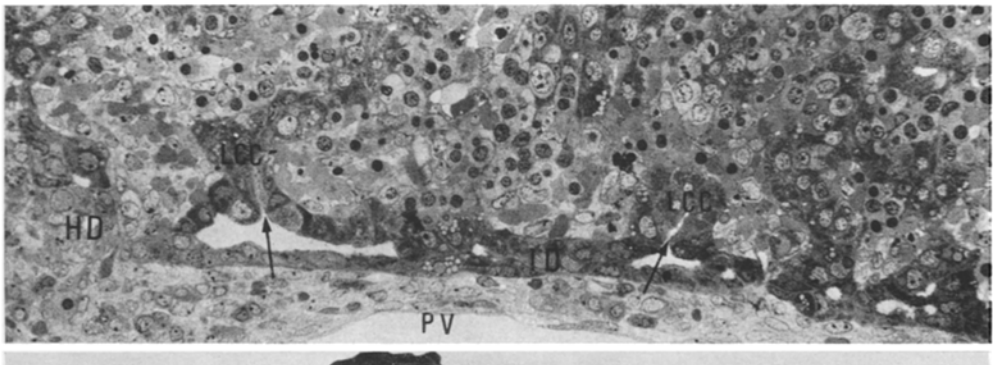

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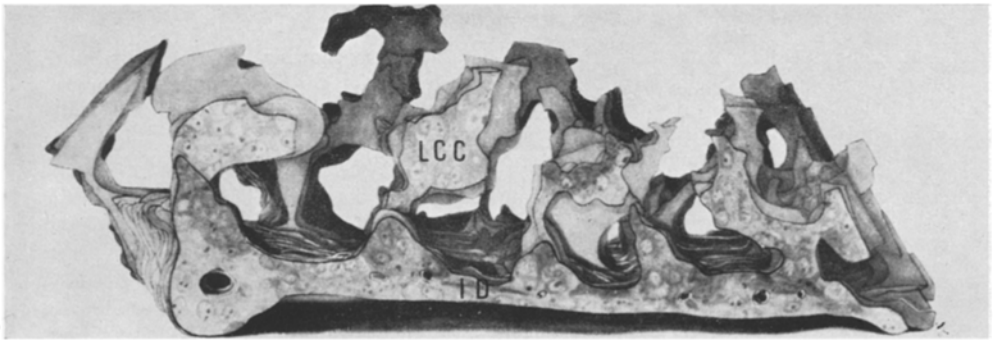

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Figs. 4–6

large polygonal liver cells. Mitotic figures in the hepatic duct cells and in the branches are frequently observed (Fig. 7).

In an embryo of 25 mm, development of the intrahepatic bile ducts is greatly advanced and the first intrahepatic bile duct appears developing around the large portal vein in continuity with the hepatic duct (Fig. 5). For the purpose of clarifying the developmental mode of the early intrahepatic bile ducts, 40 serial sections about 1 μ thick, were cut and photomicrographs were taken. All of the other tissues were cut out from the photographic prints leaving the hepatic duct, intrahepatic bile duct and liver cell cords and reconstruction of the duct was accomplished by superimposing the cut-out prints. From the reconstruction, it becomes clear that the early intrahepatic bile duct develops around the portal vein as an epithelial cell plate and liver cell cords sprout almost perpendicularly at several different places (Fig. 6).

Three Months and Later Stages. In a fetus of the fourth month, bile ducts at various stages of differentiation are observed at the margin of the portal vein branches. Flattened cuboidal epithelial cells which have developed around the portal veins as a continuous epithelial cell plate become discontinuous. Many biliary vesicles which suggest the duct of Hering, appear in the periportal spaces. Bile ducts are formed by cuboidal duct cells. Early bile ducts, developing as epithelial cell plates, change into a complex network of bile ducts around the portal veins as the fetus grows. Bile canaliculi are bordered by three to four liver cells and the canaliculi with longe lumina, often observed at the early stage, are no longer found (Fig. 8).

In the fetuses older than four months, formation of the bile ducts greatly advances and well formed interlobular bile ducts are found in the periportal spaces. Bile ducts are composed of cuboidal biliary duct cells with small oval nuclei. At the duct of Hering, dark flat cells are sometimes interposed between duct cells and liver cells (Fig. 9).

Electron Microscopy

One month. Liver cells of an embryo of 7 mm are arranged randomly and are irregular in size and shape. They show complicated convolutions and have micro-

Fig. 4. Light micrograph of a 16 mm embryo showing the liver parenchyma and the hepatic duct in the mesenchyme (*M*) of the hilus. The hepatic duct (*HD*) is connected with the liver cell cords (*LCC*) and the lumen of the hepatic duct seems to be continuous with a bile canaliculus (arrow). Note the gradual transition of the epithelial cells of the hepatic duct to the liver cells (double arrows) and also note the tubular arrangement of the liver cell cords. A large bile canaliculus (*BC*) is bounded by seven liver cells. $\times 600$

Fig. 5. One of the light micrograph of the serial sections of a 25 mm embryo showing the hepatic duct (*HD*) and the intrahepatic duct (*ID*) developing around the portal vein (*PV*). From the intrahepatic bile duct the liver cell cords (*LCC*) sprout at several different places. The lumen of the intrahepatic bile duct is continuous with that of the liver cell cords (arrows). Note the gradual transitions of the duct cells of the intrahepatic bile ducts to the liver cells. *PV* Portal vein. $\times 360$

Fig. 6. Photograph of reconstructed serial sections of a 25 mm embryo liver showing the developmental process of the intrahepatic bile duct. The intrahepatic bile duct (*ID*) develops as an epithelial cell plate and liver cell cords (*LCC*) bud out from the epithelial cell plate almost perpendicularly.

villi on their surfaces. Intercellular spaces are 0.8 to 1.5 μ wide and numerous microvilli project into these spaces (Fig. 10).

The liver cells contain many round or oval mitochondria with poorly developed cristae and granules. Rough surfaced endoplasmic reticulum is fairly well developed and free ribosomes in rosettes are abundant. The Golgi apparatus is located near the nucleus in lamellar arrays of cisternae and lysosomes are frequently seen in the cytoplasm (Figs. 10, 11).

Bile canaliculi with microvilli and junctional complexes are present. Some of them are of intracellular type. Numerous microvilli 1–2 μ in length protrude into the lumina ranging from less than 1 μ to 5 μ in diameter. Junctional complexes interrupt the free communication between the bile canaliculus and the intercellular spaces. The structural relationship between the bile canaliculus and the junctional complexes is sometimes complicated and requires a reconstruction by serial section to clarify it fully (Figs. 10, 11).

Two months. The hepatic duct of an embryo of 20 mm is composed of cuboidal epithelium lying on a basement membrane. The apical surface of the epithelium has many microvilli and junctional complexes join the epithelial cells at the apical portion. The oval shaped nucleus of the epithelial cell is located in the basal portion of the cell and cell organelles are found mainly in the supranuclear portion. Mitochondria are rod-shaped and not numerous, rough surfaced endoplasmic reticulum is sparse and the Golgi apparatus is poorly developed. Free ribosomes in small clusters are abundant. Aggregations of glycogen granules are conspicuous. Lysosomes are often observed in the cytoplasm (Fig. 12).

At the points where the hepatic ducts branch out into the liver parenchyma, ductal epithelial cells of the branches of the hepatic duct are irregular in shape and small in size. They are surrounded by a basement membrane and lumina are formed in several places. As the ductal epithelial cells approach the liver parenchymal cells, they show gradual transition into the liver cells. Epithelial cells of the transitional portion appear denser than the liver cells and their size is one-half to one-third that of the liver cells. Their cytoplasm is abundant in cell organelles. Mitochondria are numerous and may be slightly smaller than those of the liver cells, but are larger than those of the hepatic duct cells. Rough surfaced endoplasmic reticulum is well developed and occurs in parallel arrays. The Golgi apparatus is also well developed. Glycogen granules are scattered throughout the cytoplasm, instead of aggregating as in the hepatic duct cells. Abundant lysosomes with myelin figures are found and they are often located close to the glycogen granules (Fig. 13).

In the periportal spaces of two month old embryos, ducts of Hering are found. They are bounded by biliary duct cells lying on a basement membrane and by liver parenchymal cells (Figs. 14, 15). The cells composing the ducts of Hering are attached to each other by junctional complexes and the luminal surface of the ducts bears many microvilli. Lateral cell membranes of the biliary duct cells often show infoldings, which are not as complex as those of mature cells. Biliary duct cells of the ducts of Hering are cuboidal in shape and contain a relatively large oval nucleus. Mitochondria of the duct cells are oval or rod-shaped and are smaller than those of the liver cells. The number of the mitochondria in the duct

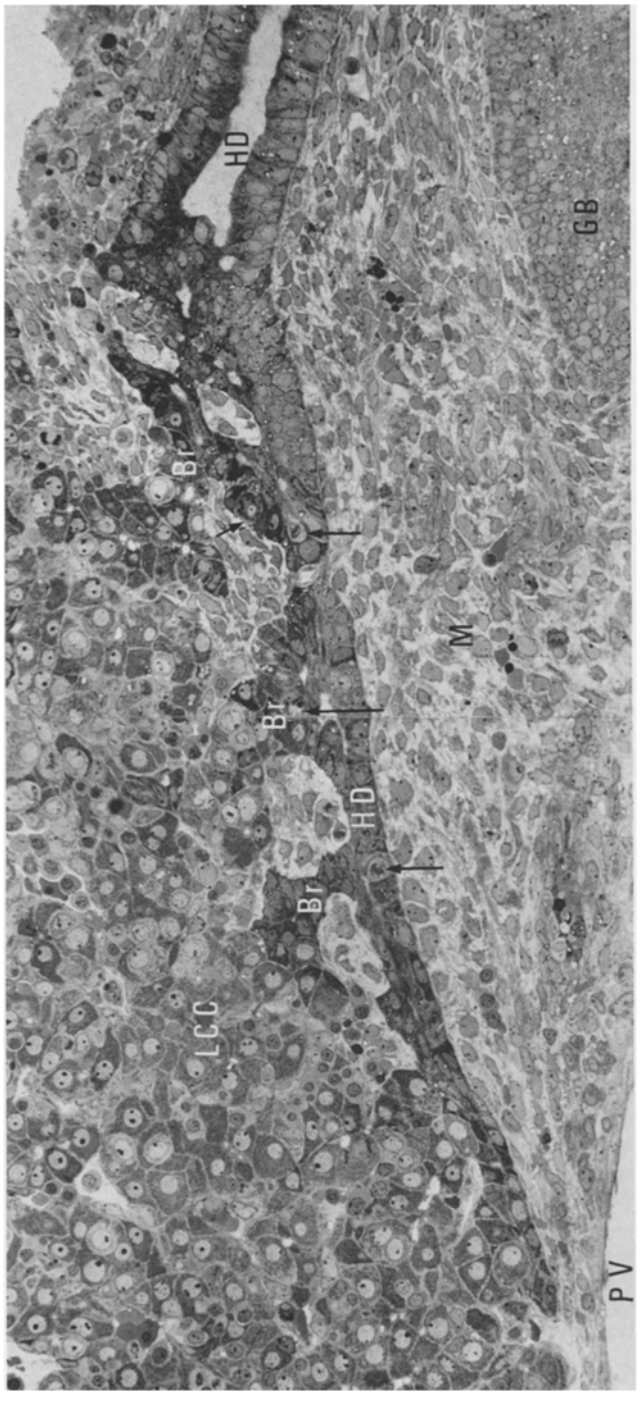
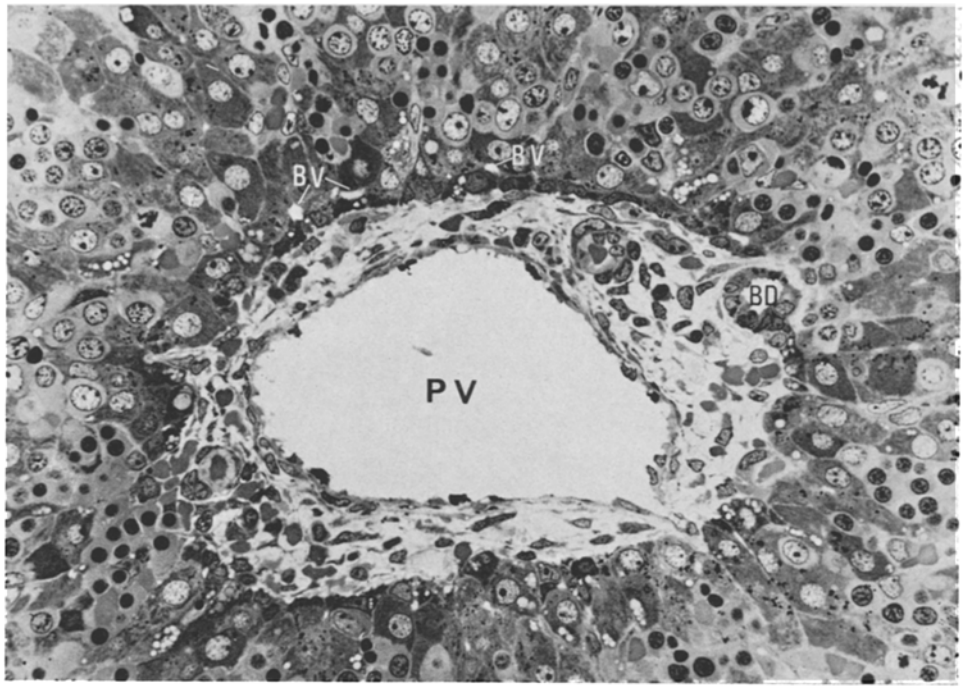


Fig. 7. Light micrograph of a 20 mm embryo showing the developmental process of the hepatic duct. Hepatic duct (*HD*) extends toward the portal vein (*PV*) as a sprouting of the branches (*Br*) to the liver parenchyma (*LCC*) at several places. At the branches and at the tip of the hepatic duct, darkly stained cuboidal duct cells show a gradual transition into large polygonal liver cells. Note the mitoses frequently observed in the duct cells (arrows). *M* Mesenchyme of the hilus, *GB* Gall bladder. ×360



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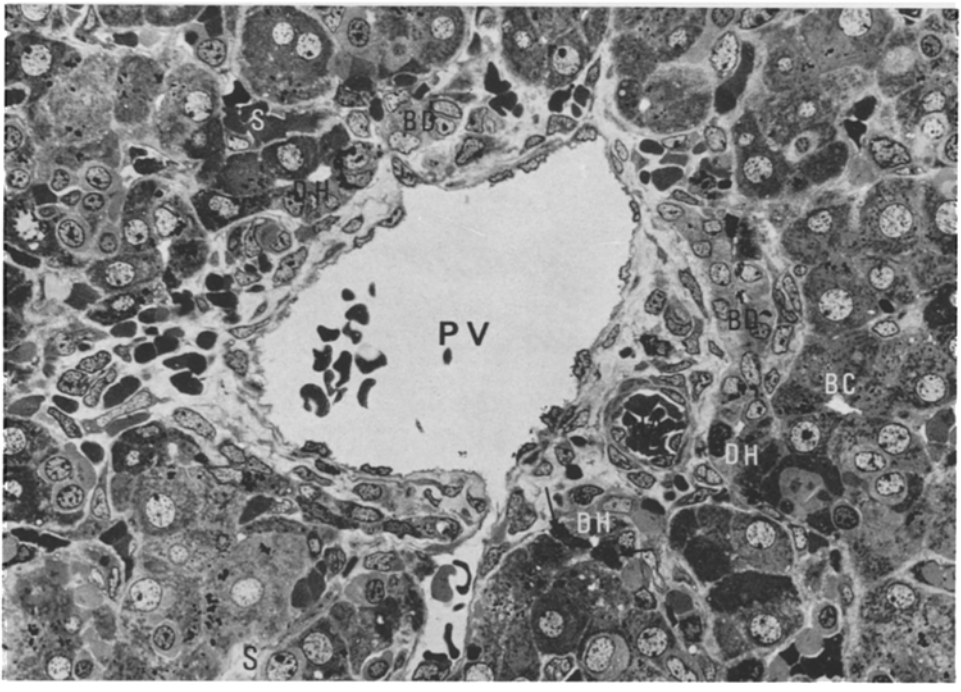

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Figs. 8 and 9

cells is fewer than in the liver cells. Rough surfaced endoplasmic reticulum is poorly developed in general. However, in an embryo of 16 mm, it is fairly well developed and is often organized into compact parallel arrays (Fig. 14). No glycogen granules can be found in the duct cells. Dense bodies, probably lysosomes are frequently present and lipid droplets are occasionally observed in the duct cells (Figs. 14, 15).

At the duct of Hering of an embryo of 16 mm, the liver cell adjacent to the biliary duct cells lies on a basement membrane and takes part in the formation of the duct. The existence of a basement membrane at the outer surface of the liver cell is worthy of special note (Fig. 14).

At the duct of Hering of an embryo of 22 mm, one dividing duct cell is found interposed between biliary duct cells. The stage of mitosis of the cell is considered to be at prophase. The mitotic figure in the biliary duct cells of the duct of Hering is of interest considering the development of the bile duct (Fig. 15).

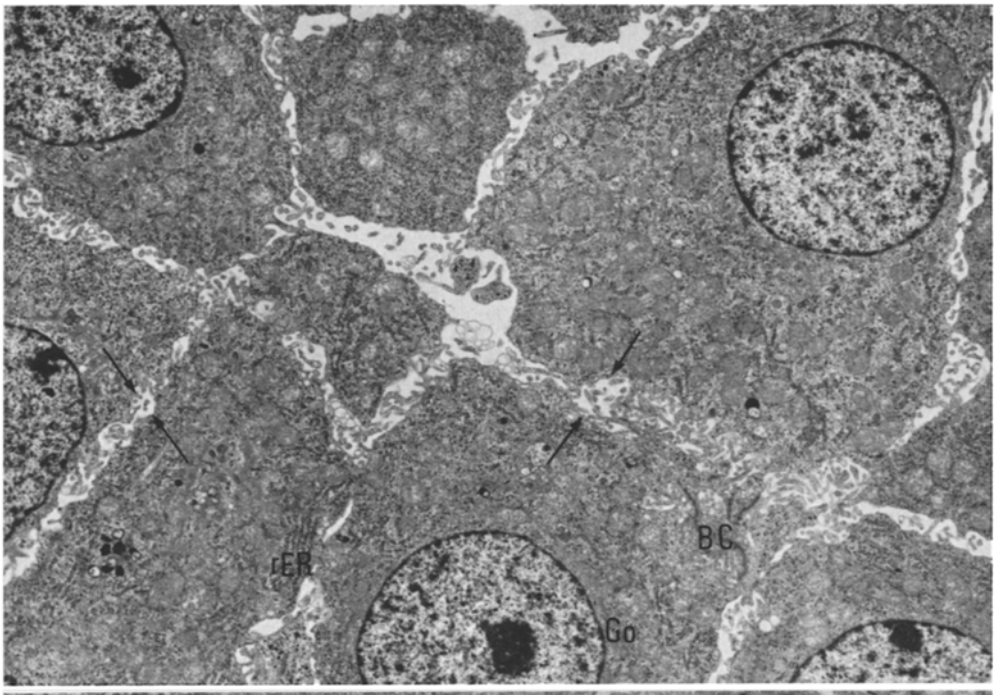
Intercellular bile canaliculi are rarely observed in two month old embryos, but at six to seven weeks large bile canaliculi bounded by four to seven liver cells appear. Microvilli project into the lumen, but their length and distribution are uneven. Although smaller bile canaliculi are sometimes observed around the large bile canaliculus, their functional meaning is obscure (Fig. 16). At seven to eight weeks, typical bile canaliculi appear. They are bounded predominantly by three to four adjoining liver cells and their lumina are sealed by junctional complexes. The luminal surface bears numerous microvilli whose length and distribution are fairly uniform (Fig. 17).

Liver cells contain various organelles at this stage. Mitochondria are oval or rod-shaped and their cristae are well recognizable. The Golgi apparatus in lamellar arrays is often located between the bile canaliculus and the nucleus, and it is not well developed. Dilated vacuoles or sacs in it are not observed. Rough and smooth surfaced endoplasmic reticula are abundant and the latter are occasionally aggregated into clusters. Microbodies are often observed, but the crystalloid is not detected. Although glycogen granules are not present in an embryo of 16 mm, they appear diffusely scattered throughout the cytoplasm at an embryo of 20 mm. Lysosomes are frequently seen (Figs. 14, 17).

Three Months and Later Stages. Interlobular bile ducts are found in the periportal spaces of three month old fetuses. They are composed of cuboidal biliary

Fig. 8. Light micrograph of a 94 mm fetus, about four months old, showing ductal formation in the periportal space. Many biliary vesicles (*BV*) can be seen. They are bounded by cuboidal epithelial cells on one side and by liver cells on the other. An interlobular bile duct (*BD*) is seen in the periportal connective tissue. It is composed of cuboidal biliary duct cells. *PV* portal vein. ×420

Fig. 9. Light micrograph of a 340 mm fetus, about seven months old, showing ductal formation in the periportal space. Interlobular bile ducts (*BD*) and ducts of Hering (*DH*) are seen in the periportal space. Interlobular bile ducts are composed of cuboidal biliary duct cells. Ducts of Hering are composed of liver cells on one side and cuboidal biliary duct cells on the other and dark flat cells are often intercalated between them (arrows). Bile canaliculus (*BC*) is bounded by two to four liver cells. *S* Sinusoid, *HA* Hepatic artery, *PV* Portal vein. ×600



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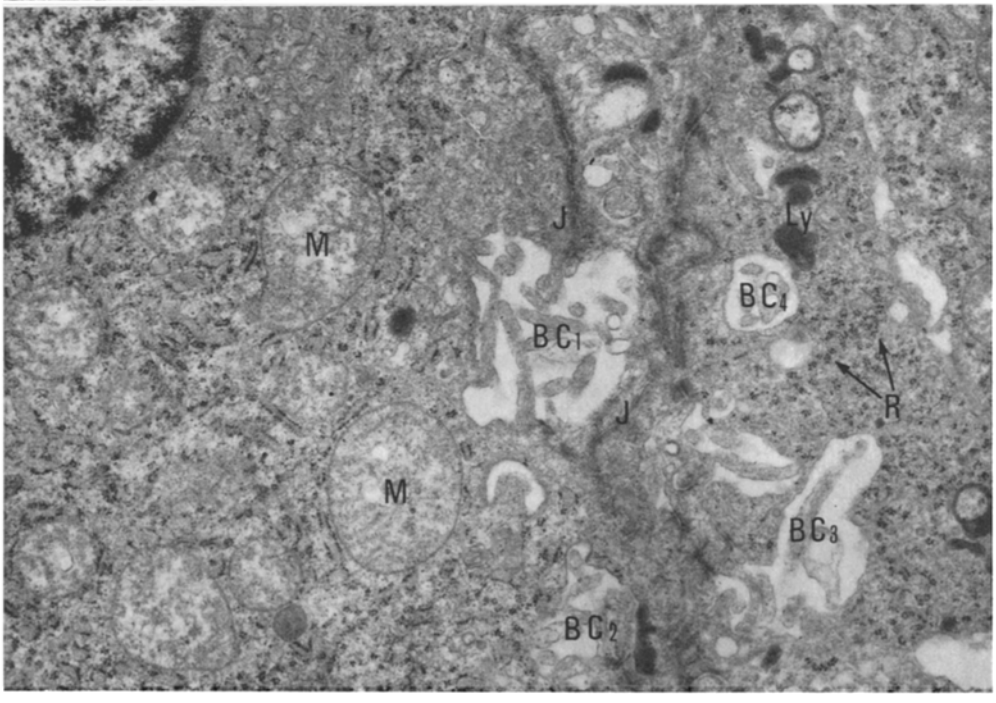

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Figs. 10 and 11

duct cells and the outer surfaces of the ducts are surrounded by a basement membrane. The biliary duct cells are attached to each other at the apical portion by junctional complexes and lateral cell membranes often form infoldings especially at the basal portion. Microvilli protrude into the lumen from the apical surfaces, but are fewer in number than those of the bile canaliculi (Fig. 18).

The fine structure of the biliary duct cells of the fetal interlobular bile ducts will now be described in detail. Biliary duct cells are cuboidal in shape and contain large oval nuclei. They lie on a basement membrane and their apical surface bears microvilli. While various cell organelles are found in the supranuclear portion, they are poorly developed. Mitochondria are oval or rod-shaped, small in size and sparse. Mitochondrial cristae are poorly developed as compared with those of the liver cell. Rough surfaced endoplasmic reticulum is scanty, but free ribosomes are scattered throughout the cytoplasm. Smooth surfaced endoplasmic reticulum is not found. The Golgi apparatus is poorly developed. Fine filamentous structures are found within the cytoplasm. Dense bodies bounded by a single membrane are frequently observed. These granules are thought to be lysosomes (Figs. 18, 19). Lipid droplets are occasionally seen (Fig. 18).

Ducts of Hering are frequently observed in the periportal spaces in the fetuses later than three months old. As observed in the early embryos, they are bounded by liver cells and biliary duct cells. In the ducts of Hering, cells different from both liver cells and biliary duct cells are often intermediate between the liver cells and biliary duct cells (Figs. 20, 21). They differ from liver cells and biliary duct cells in shape and size of the cell, in cytoplasmic density, in shape of the nucleus, and in cell organelles. These cells are thought to be intermediate cells. They resemble to some extent both liver cells and biliary duct cells, but classification is sometimes difficult. These cells are often larger than duct cells and contain irregular shaped nuclei (Figs. 20, 21). The density of the cells is often greater than that of the liver cells and duct cells (Fig. 20). Cell organelles are fairly abundant. Mitochondria are well developed and are larger than those of the duct cells, but smaller than those of the liver cells. Rough surfaced endoplasmic reticulum is also fairly well developed. The Golgi apparatus is not well developed (Figs. 20, 21). Glycogen granules can not be found even when the adjacent liver cells contain them (Fig. 21).

Bile canaliculi are predominantly bounded by three to four liver cells and the luminal surface bears microvilli whose distribution and length are fairly uniform (Figs. 22, 23). These forms persist throughout later fetal life.

Fig. 10. Electron micrograph of a 7 mm embryo liver. Note the wide intercellular spaces provided with numerous microvilli (arrows) and also note the irregularity in shape and size of the liver cells. A bile canaliculus (*BC*) with microvilli and junctional complexes is seen. *rER* Rough surfaced endoplasmic reticulum, *Go* Golgi apparatus. ×3600

Fig. 11. Electron micrograph of a 7 mm embryo liver showing various bile canaliculi (BC_1, BC_2, BC_3, BC_4). Most of them are of intercellular type. Mitochondria (*M*) are round or oval and their cristae and granules are poorly developed. Free ribosomes (*R*) are abundant and are aggregated into rosettes. *J* Junctional complex, *Ly* Lysosome. ×14300

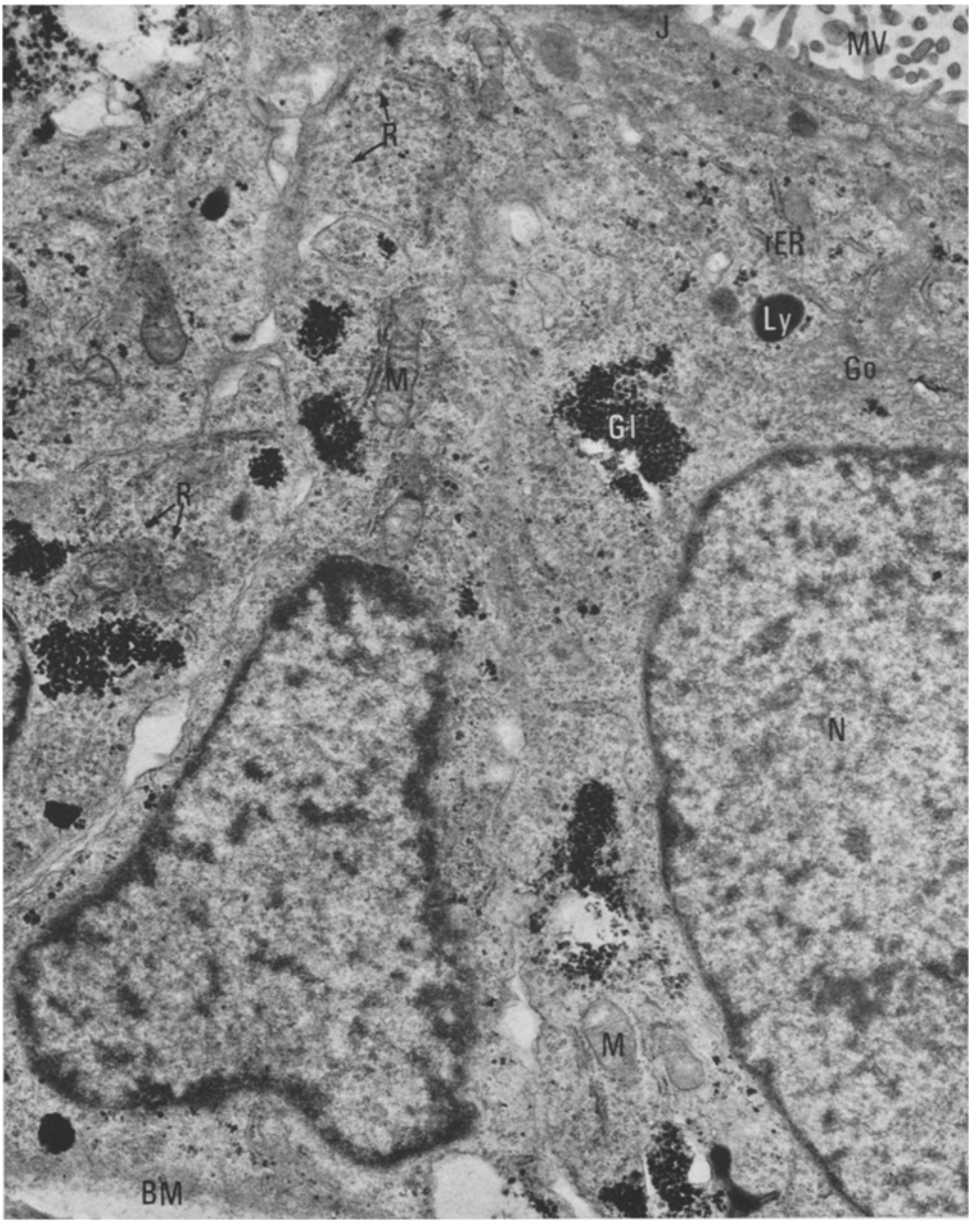

Fig. 12. Electron micrograph of a 20 mm embryo showing the hepatic duct epithelium. Epithelial cells are attached to each other by junctional complexes (*J*) and apical surfaces are provided with microvilli (*MV*). Cell organelles are poorly developed. Free ribosomes (*R*) in rosettes are abundant. Note the aggreations of glycogen granules (*Gl*). *N* Nucleus, *M* Mitochondrion, *Ly* Lysosome, *Go* Golgi apparatus, *BM* Basement membrane. ×14000

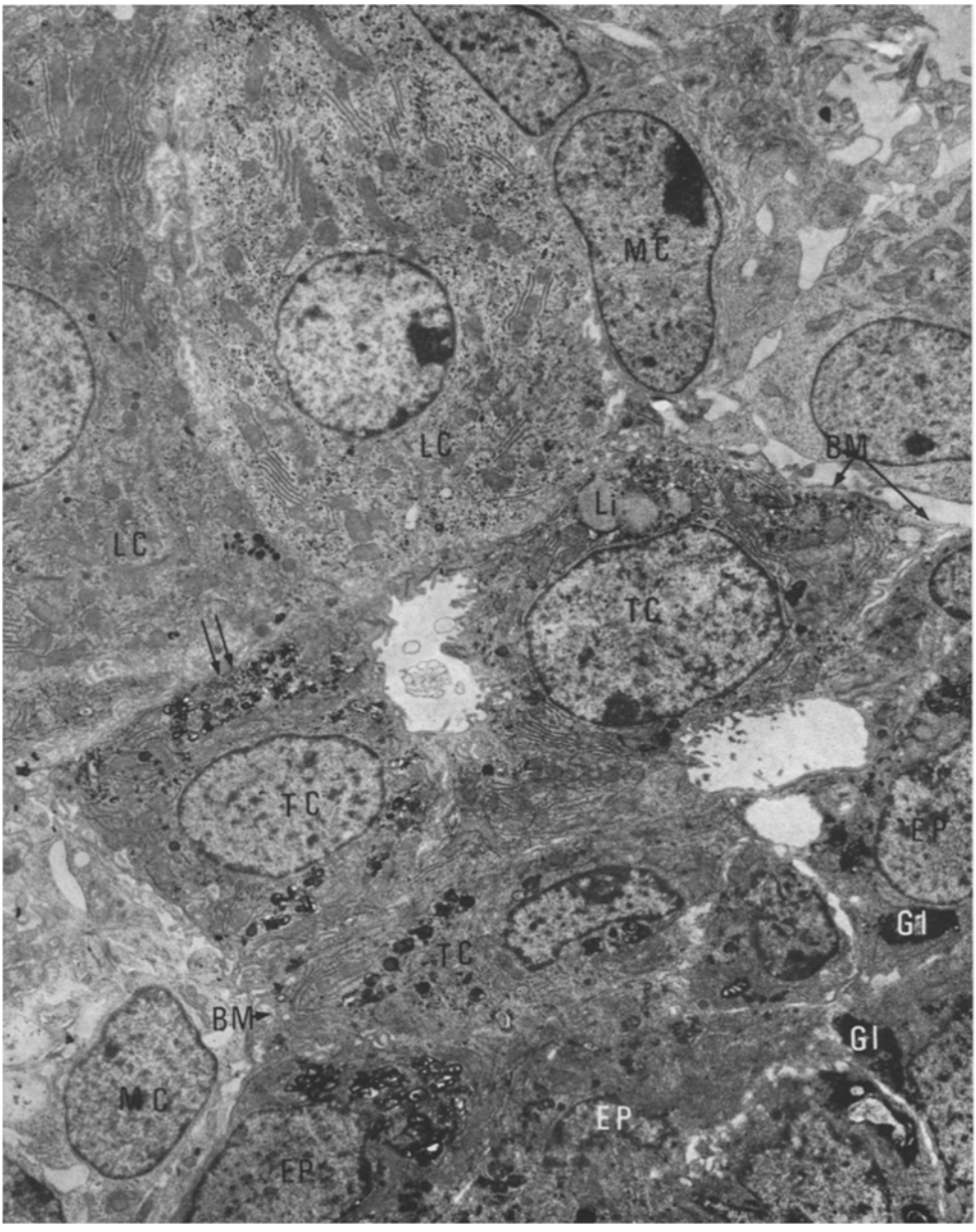

Fig. 13. Electron micrograph of a 20 mm embryo showing a branch of the hepatic duct to the liver parenchyma. Ductal epithelial cells (*EP*) of a branch of the hepatic duct are irregular in shape and small in size. As they approach the liver parenchymal cells (*LC*), they show gradual transition into the liver cells. Epithelial cells of the transitional portion (*TC*) appear denser than liver cells and their size is one-half to one-third that of the liver cells. They are abundant in cell organelles and contain glycogen granules. Lysosomes with myelin figure are located close to the glycogen granules (two arrows). Note the presence of a basement membrane (*BM*) around the branch. *MC* Mesenchymal cell, *Gl* Glycogen granules. ×4300

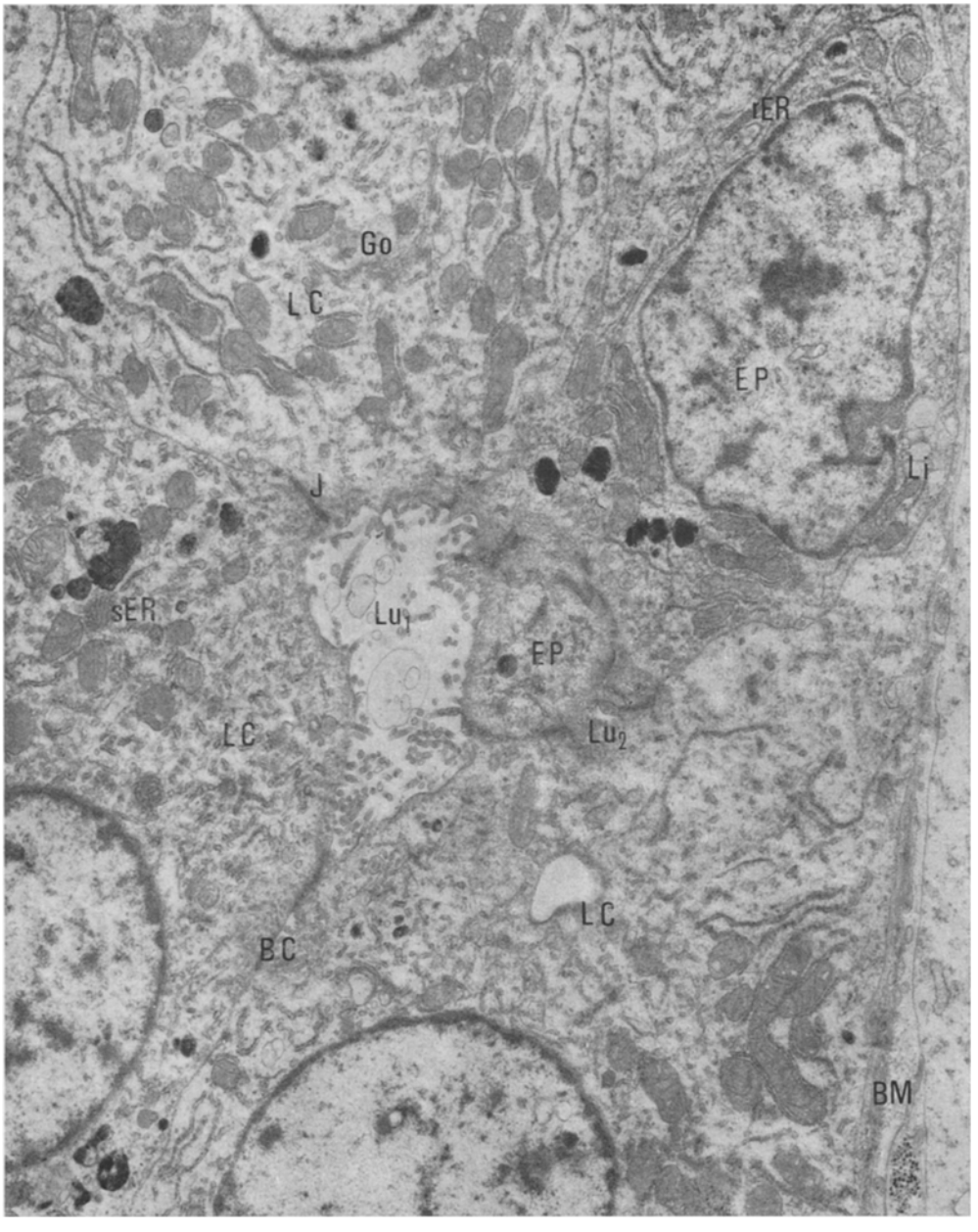

Fig. 14. Electron micrograph of a 16 mm embryo liver showing the duct of Hering in the periportal space. Two lumina are seen. One (Lu_1) is formed by three liver cells (*LC*) and two biliary duct cells (*EP*) and the other (Lu_2) is formed between one liver cell and two biliary duct cells. Luminal surfaces are provided with microvilli and are sealed by junctional complexes (*J*). The Golgi apparatus (*Go*) of the liver cell is located between the nucleus and lumen. Smooth surfaced endplasmic reticulum (*sER*) is found and is often aggregated into clusters. Rod-shaped mitochondria in the biliary duct cells are fairly abundant and are smaller than those of the liver cells. Rough surfaced endoplasmic reticulum (*rER*) of the biliary epithelial cells is also fairly well developed and is often organized in compact parallel arrays. Lipid droplets (*Li*) are seen. Note the extension of a basement membrane (*BM*) along the outer surface of the liver cell. *BC* Bile canaliculus. ×7500

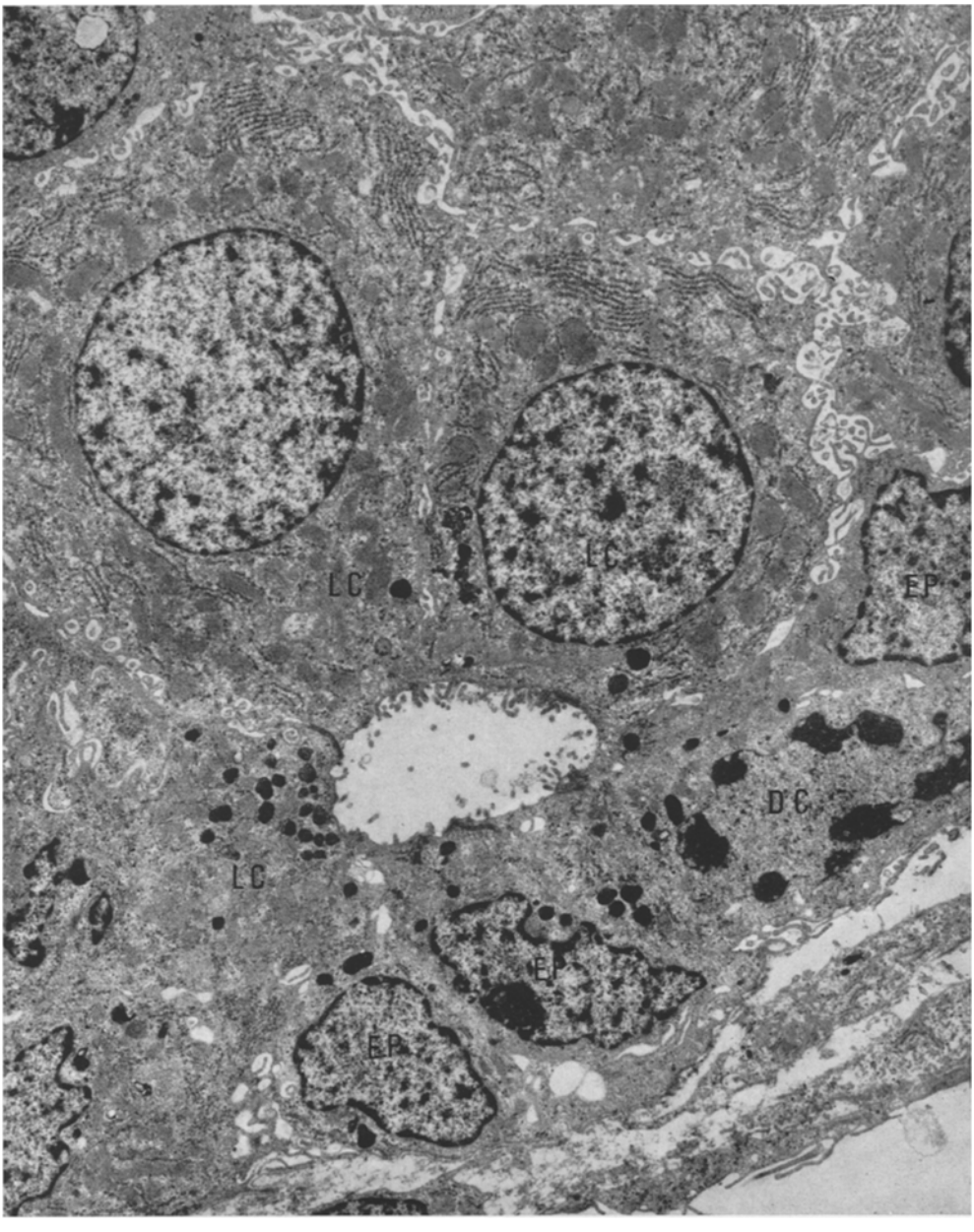

Fig. 15. Electron micrograph of a 22 mm embryo liver showing the duct of Hering in the periportal space. The duct of Hering is bounded by three liver cells (*LC*), three biliary duct cells (*EP*) and one dividing duct cell (*DC*). In the duct cells judged to be under going cell division, chromatin is condensed at the nuclear membrane and the stage of mitosis of the cell is considered to be at prophase. ×6100

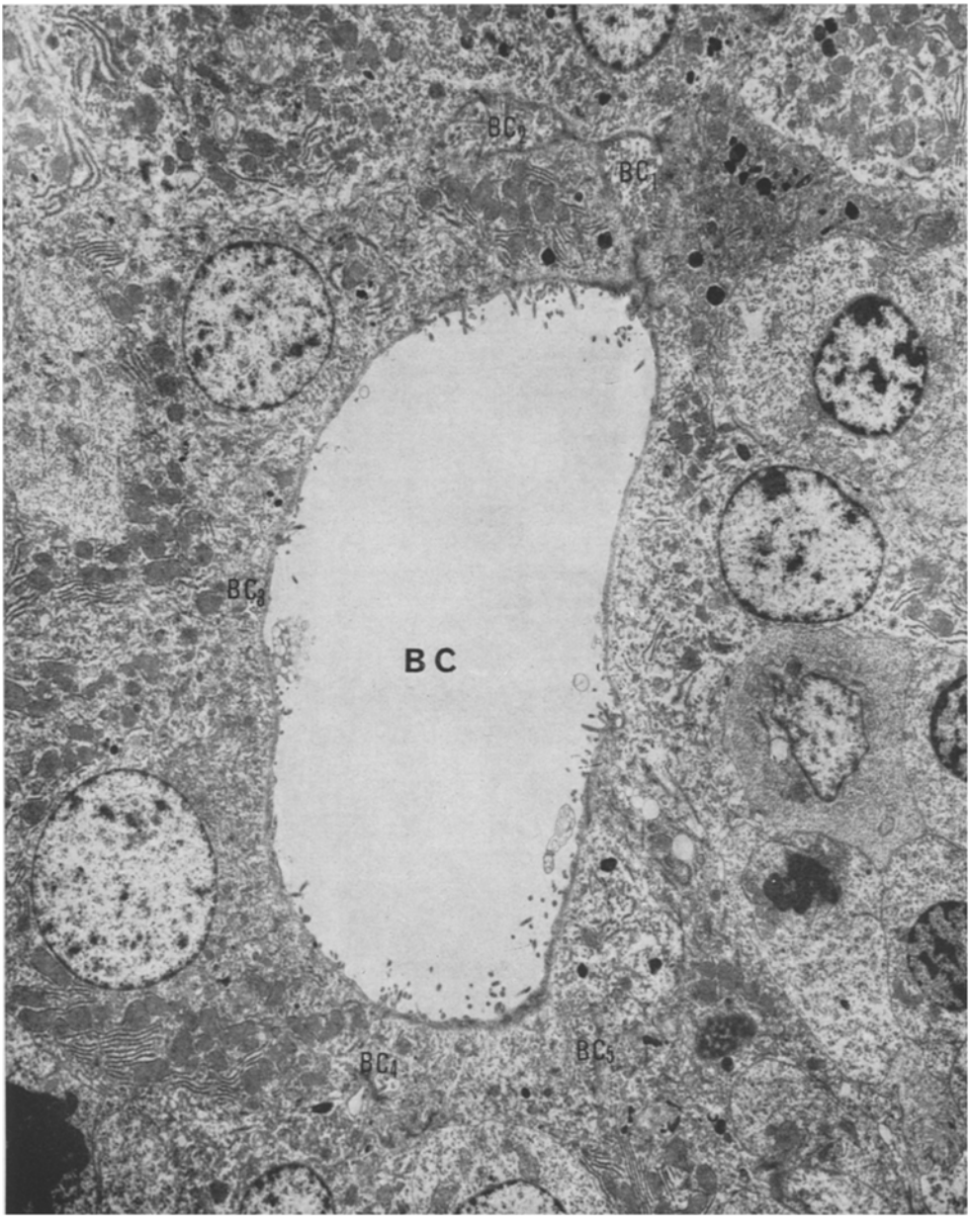

Fig. 16. Electron micrograph of a 16 mm embryo liver showing bile canaliculi and liver cells. A large canaliculus (*BC*) is bounded by six liver cells and its luminal surface is provided with microvilli, but their length and distribution are uneven. Note the presence of smaller bile canaliculi (BC_1, BC_2, BC_3, BC_4, BC_5) around the large bile canaliculus. ×3000

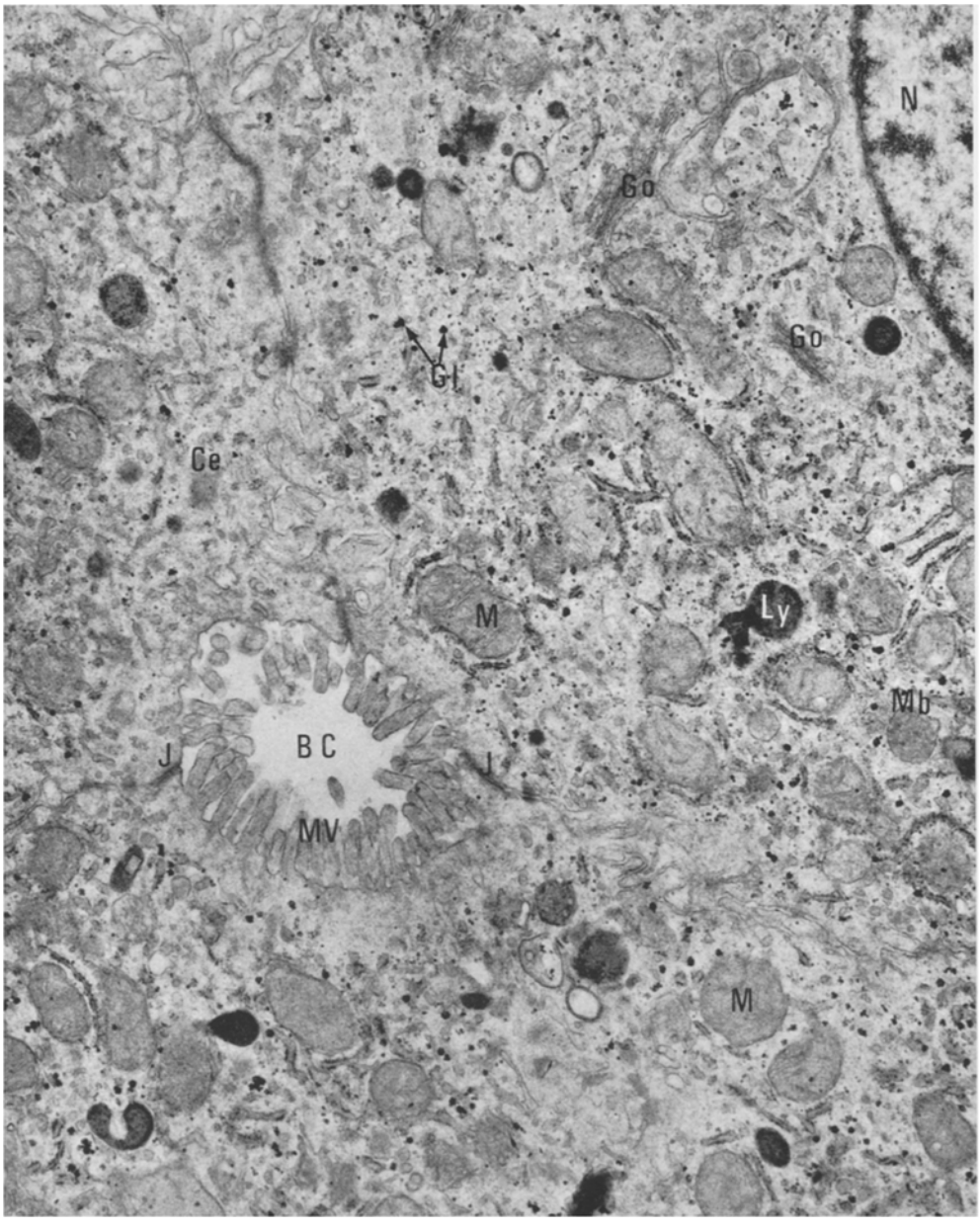

Fig. 17. Electron micrograph of a 20 mm embryo liver showing liver cells and a bile canaliculus. The bile canaliculus (*BC*) is bounded by three adjoining liver cells and the luminal surface is provided with numerous microvilli (*MV*) whose length and distribution are fairly uniform. Glycogen granules (*Gl*) are scattered throughout the cytoplasm. The Golgi apparatus is located near the nucleus. Microbodies (*Mb*) are found, but the crystalloid is not detected. *Ly* Lysosome, *Ce* Centriole, *J* Junctional complex, *N* Nucleus, *M* Mitochondrion. ×15600

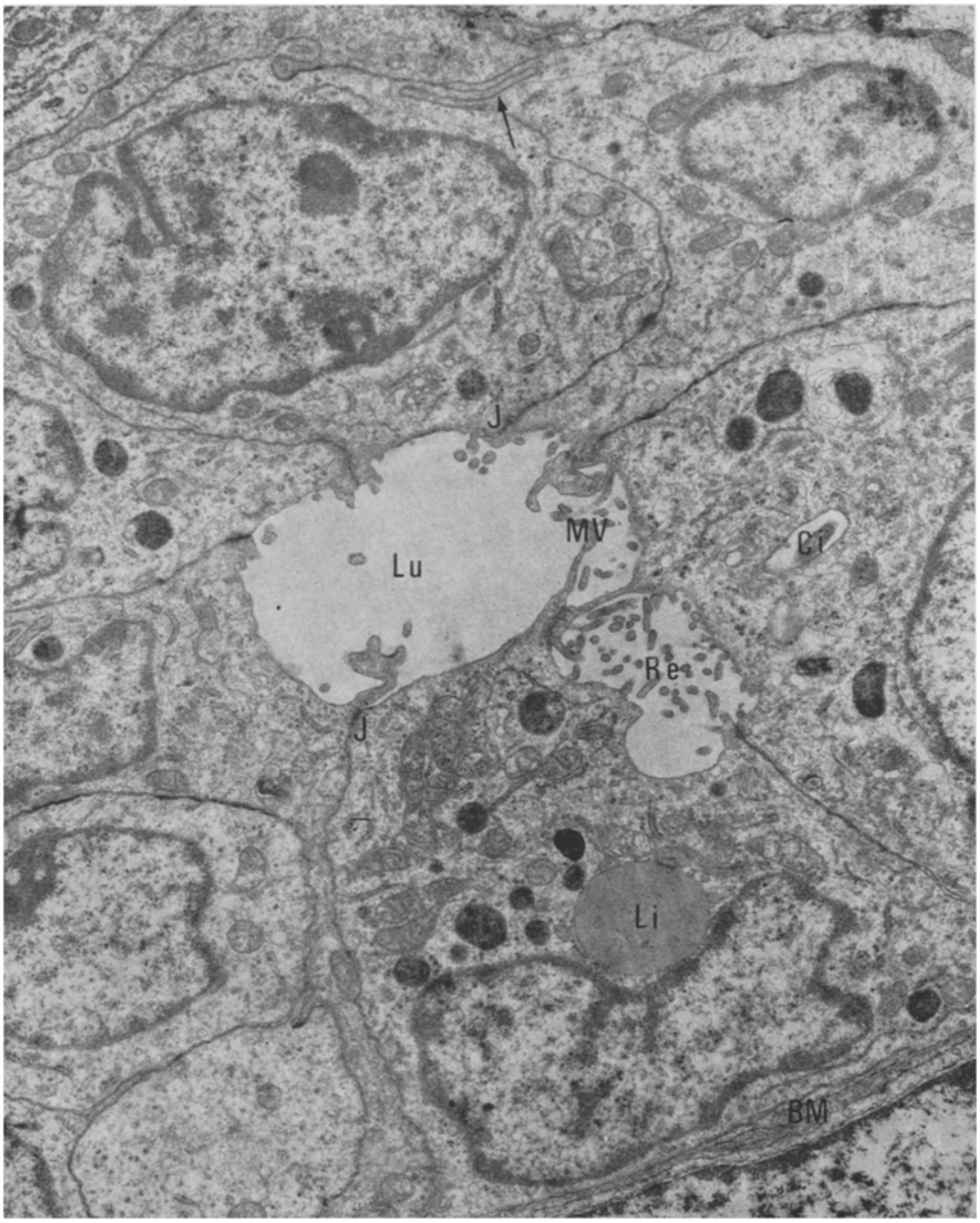

Fig. 18. Electron micrograph of a 47 mm fetus, about three months old, showing the interlobular bile duct. The outer surface of the epithelium is surrounded by a basement membrane (*BM*). Epithelial cells are attached to each other by junctional complexes (*J*) and the luminal surface is provided with microvilli (*MV*), but the number of the microvilli is sparse. Beside the main channel (*Lu*), the smaller channel (*Re*) is formed between two adjacent epithelial cells. The lateral cell membranes of the epithelial cells show infoldings especially at the basal part (arrow). *Ci* Cilium, *Li* Lipid droplet. ×10200

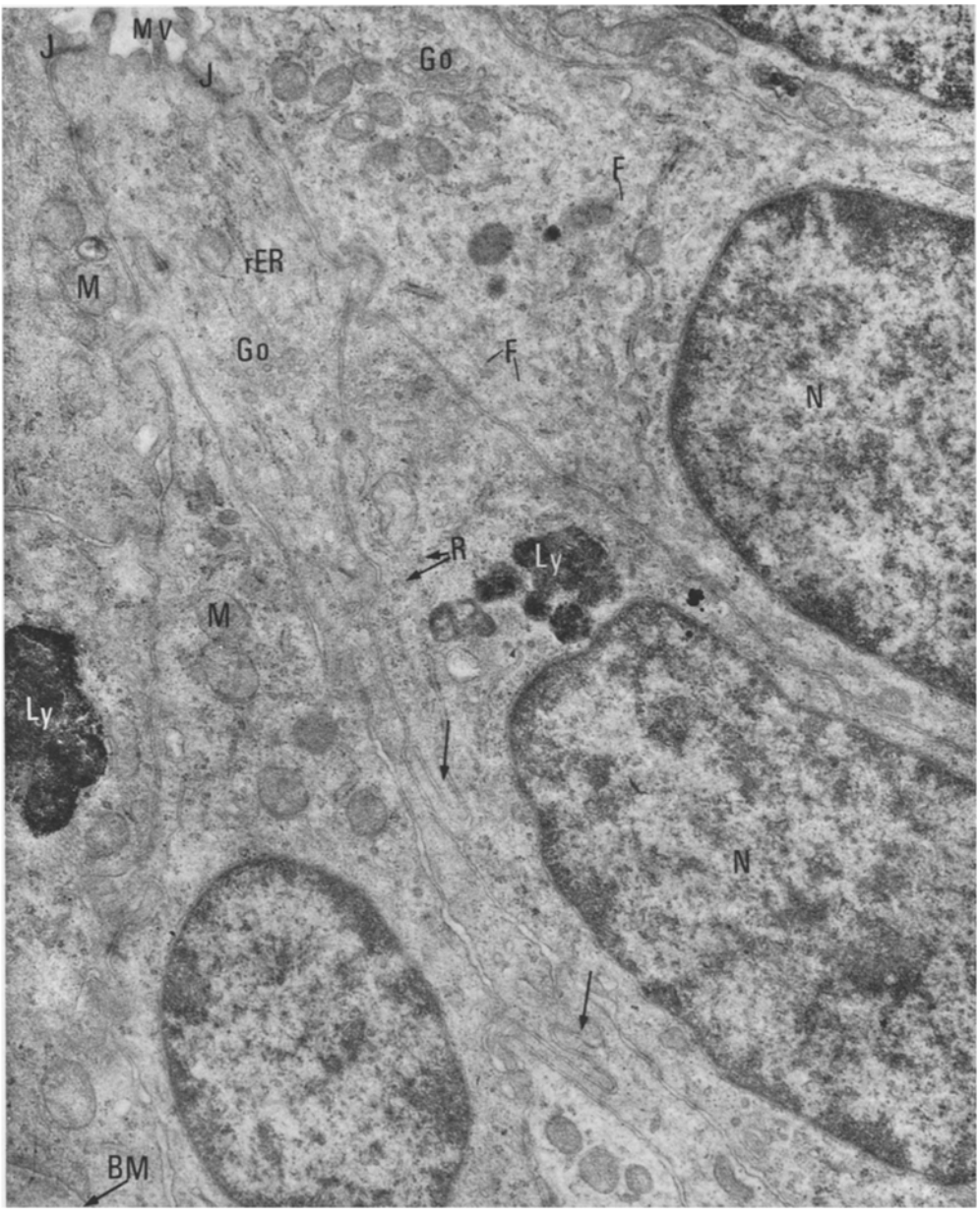

Fig. 19. Electron micrograph of a 320 mm fetus, about seven months old, showing the interlobular bile duct epithelium. Duct cells are attached to each other by junctional complexes (*J*) at the apical portions of the cells and apical surfaces bear microvilli (*MV*). The lateral cell membranes often show infoldings (arrow). Various cell organelles are found in supranuclear portion, but they are poorly developed. Free ribosomes in rosettes (*R*) are abundant. Fine filamentous structures (*F*) cross the cytoplasm. *N* Nucleus, *Go* Golgi apparatus, *rER* Rough surfaced endoplasmic reticulum, *M* Mitochondrion, *Ly* Lysosome, *BM* Basement membrane. ×21800

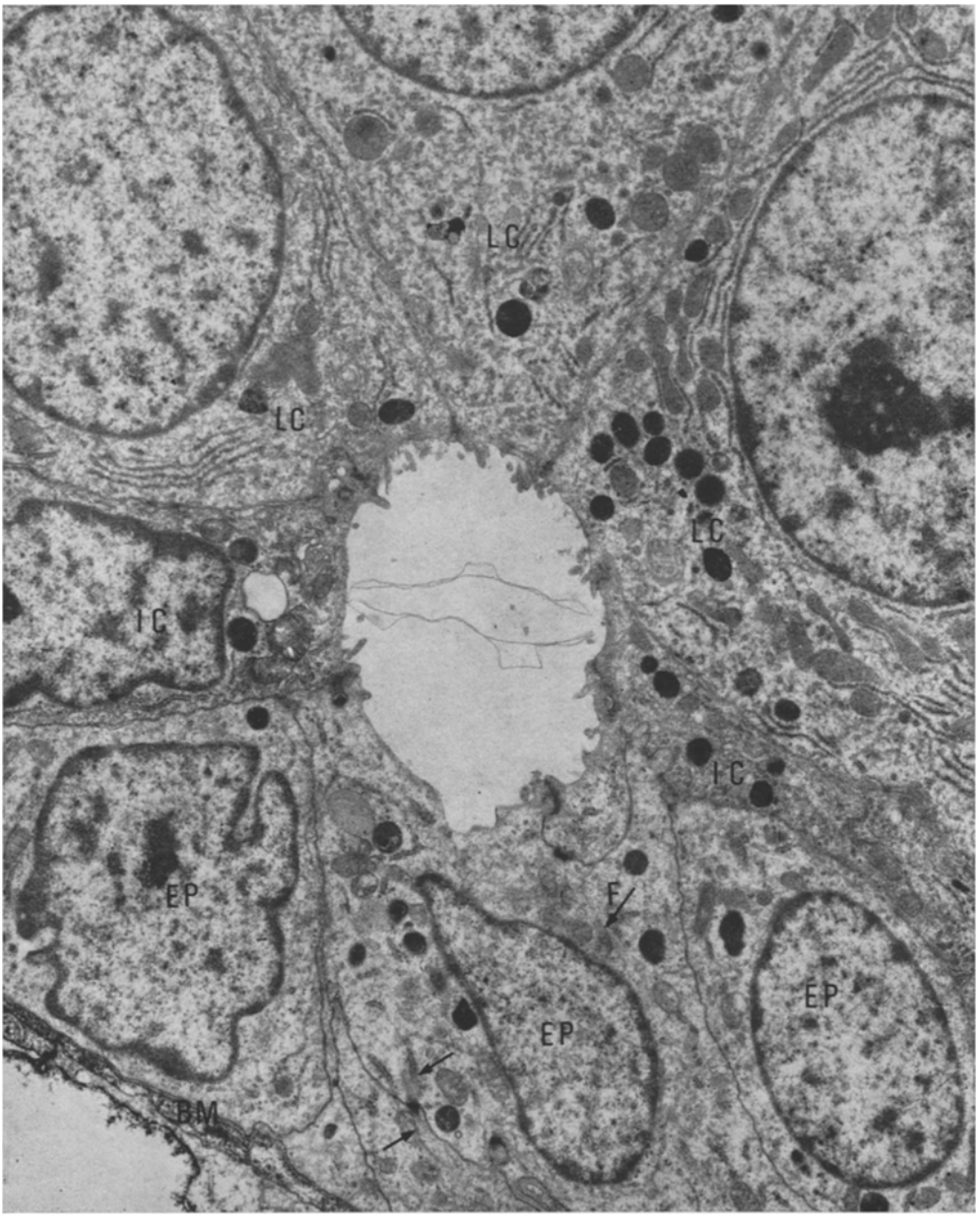

Fig. 20. Electron micrograph of a 47 mm fetus showing the duct of Hering in the periportal space. Two cells (*IC*) different from liver cells (*LC*) and biliary duct cells (*EP*) are intermediate between them. These cells differ from liver cells and biliary duct cells in shape and size of the cell, in cytoplasmic density, in shape of the nucleus and in cell organelles. In the duct cells filamentous structures (*F*) are characteristically found and some of them attach to the desmosome (two arrows). *BM* Basement membrane. ×4200

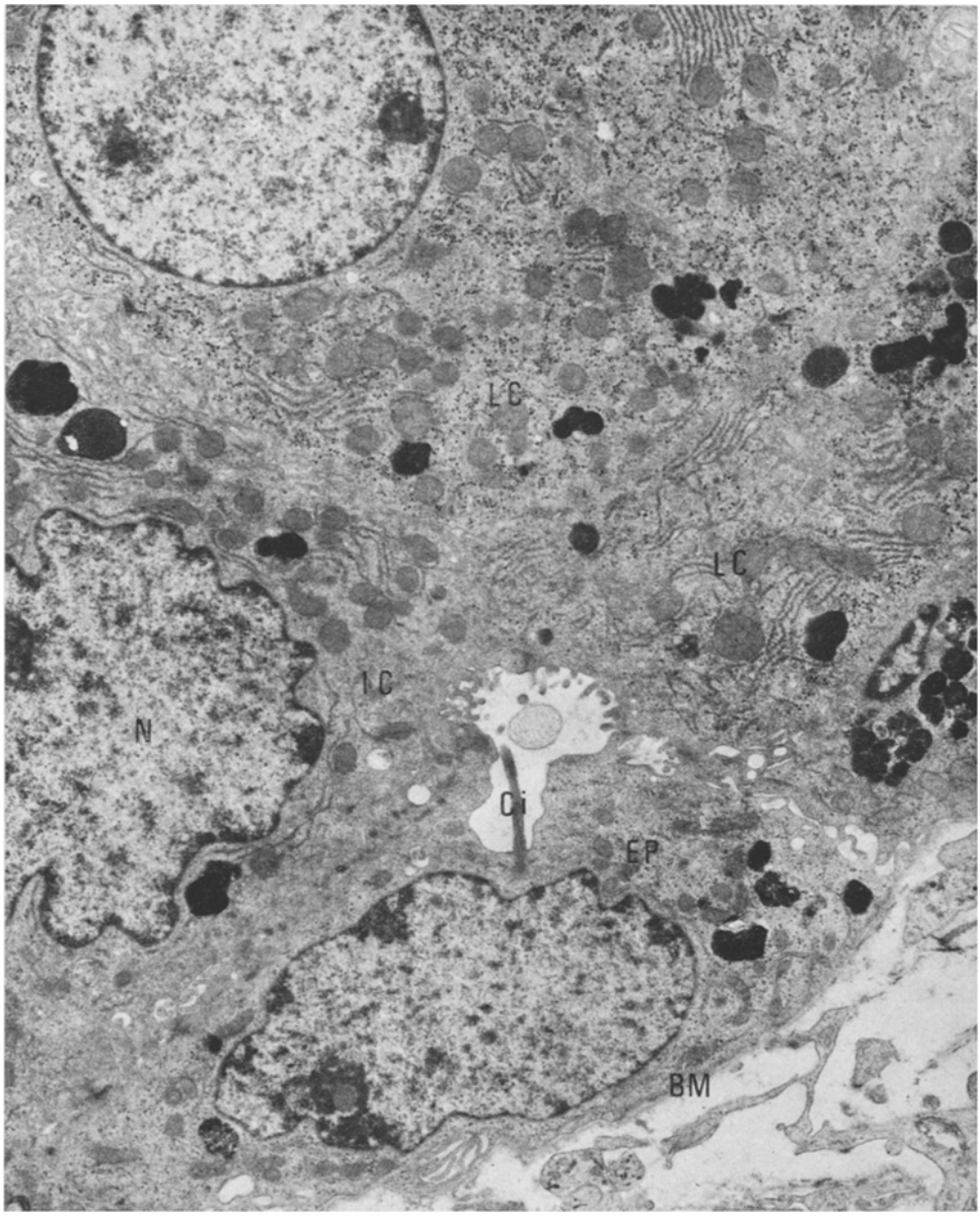

Fig. 21. Electron micrograph of a 320 mm fetus, about seven months old, showing the duct of Hering in the periportal spaces. Possible intermediate cell (*IC*) is intermediate between liver cell (*LC*) and biliary duct cell (*EP*). Its nucleus (*N*) is larger than that of the biliary duct cell and is irregular in shape. Note the absence of glycogen granules in the intermediate cell. A cilium (*Ci*) protrudes into the lumen from the biliary duct cell. *BM* Basement membrane. ×5700

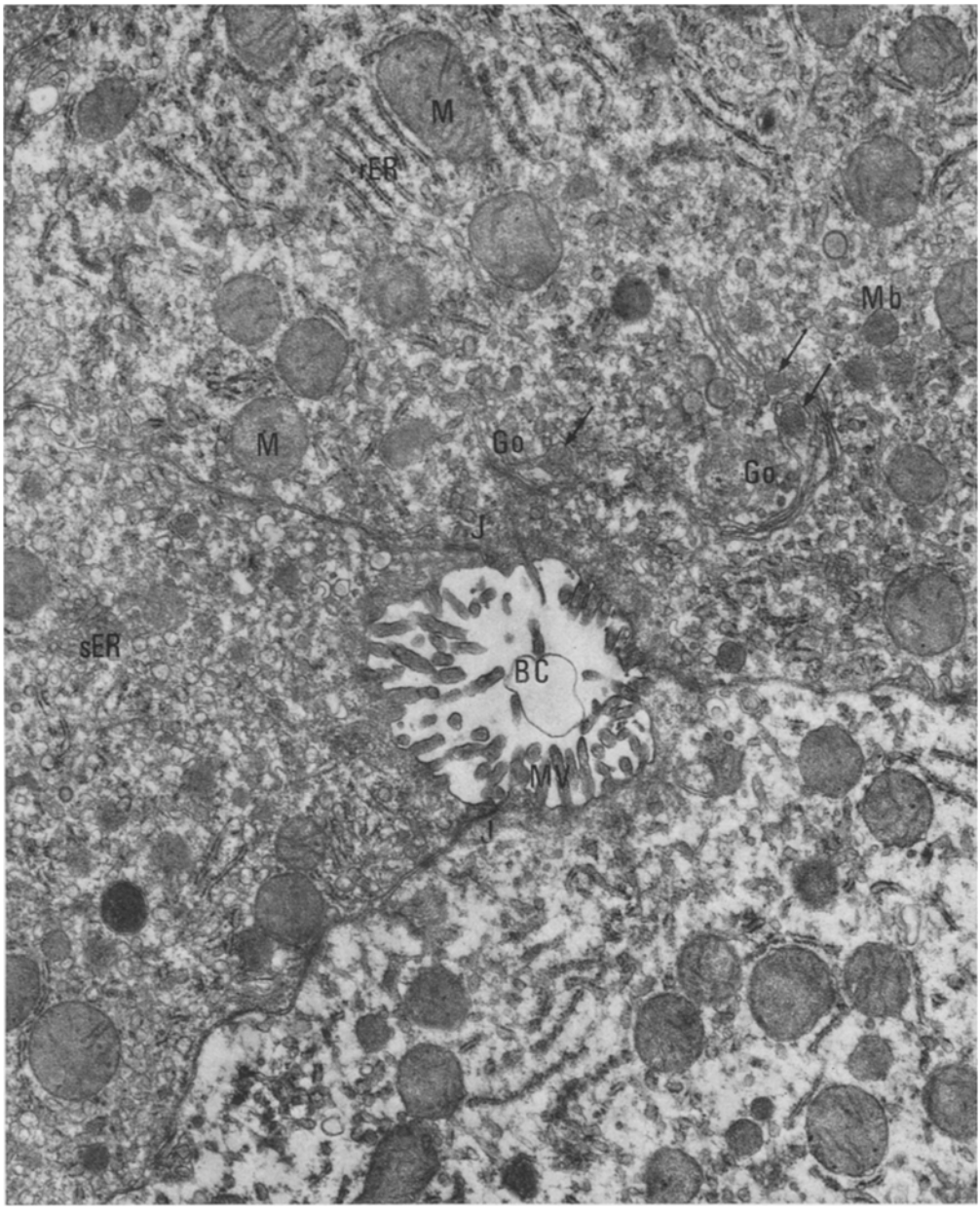

Fig. 22. Electron micrograph of 47 mm fetus, about three months old, showing liver cells and a bile canaliculus. The bile canaliculus (*BC*) is formed by three adjoining liver cells and the luminal surface bears numerous microvilli (*MV*). The liver cells have abundant cell organelles. The Golgi apparatus (*Go*) is located near the bile canaliculus. Note the slightly dense material accumulated in the Golgi vacuoles (arrows). *M* Mitochondria, *rER* Rough surfaced endoplasmic reticulum, *sER* Smooth surfaced endoplasmic reticulum, *Mb* Microbody, *J* Junctional complex. ×13600

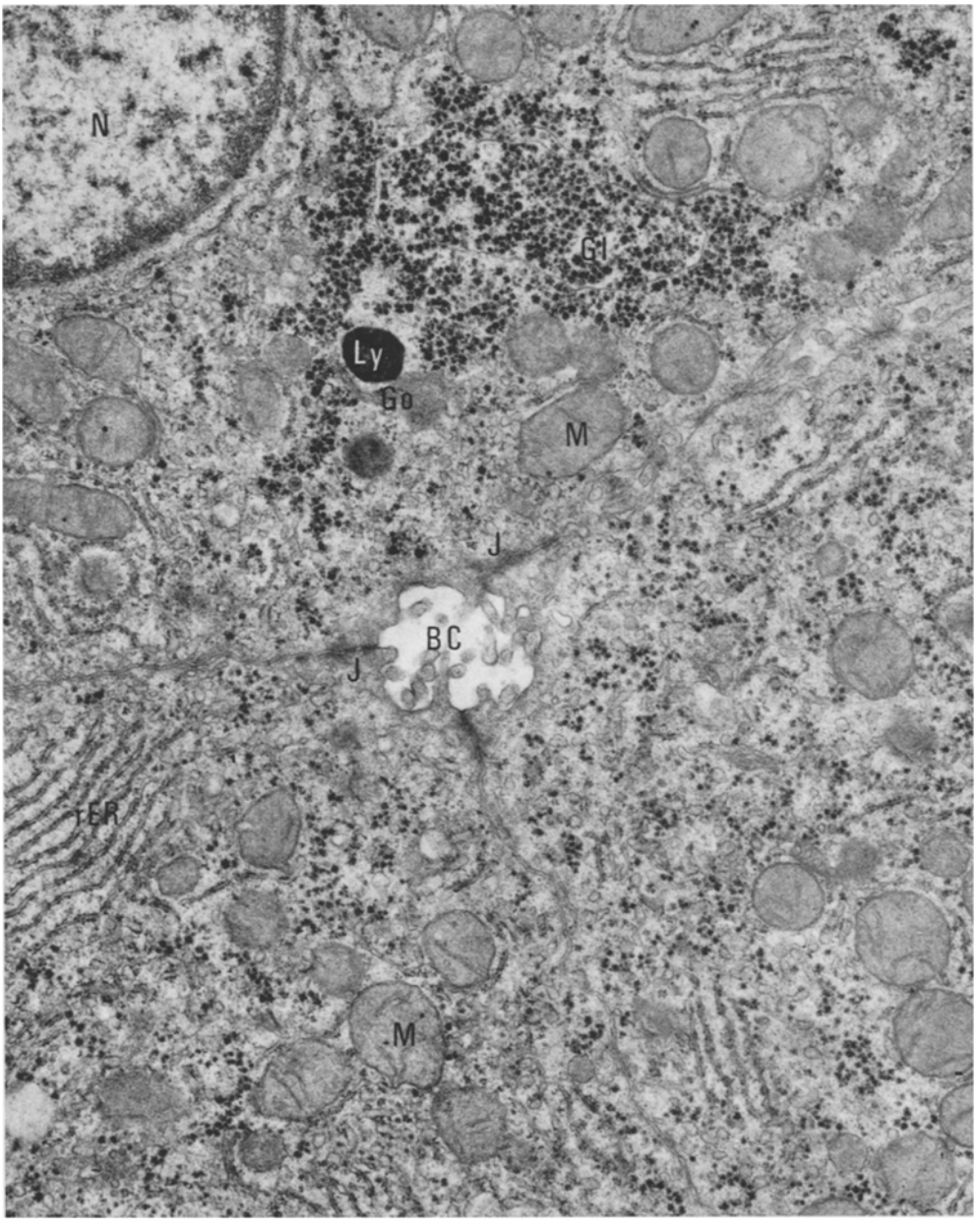

Fig. 23. Electron micrograph of a liver from a 360 mm fetus, about eight months old, showing liver cells and a bile canaliculus. The bile canaliculus (*BC*) is bounded by three adjoining liver cells. The liver cells are rich in cell organelles and cell inclusions. *N* Nucleus, *M* Mitochondria, *rER* Rough surfaced endoplasmic reticulum, *Go* Golgi apparatus, *Gl* Glycogen granules, *Ly* Lysosome, *J* Junctional complex. ×16000

Liver cells contain many cell organelles at this stage. Mitochondria with cristae and granules are well developed and are oval or round in shape. The Golgi apparatus appears in lamellar arrays near the bile canaliculus. The ends of the Golgi cisternae often dilate to form vacuoles in which slightly dense material is observed. There are a number of membrane-bounded granules in the periphery of the Golgi complex (Fig. 22). Rough surfaced endoplasmic reticulum is often arranged in parallel arrays. Smooth surfaced endoplasmic reticulum is also well developed and is often aggregated into clusters. Microbodies are also present. Glycogen granules are diffusely distributed throughout the cytoplasm. Lysosomes are frequently observed (Figs. 22, 23).

Discussion

The first bile canaliculi appear long before the bile secretion begins (Karrer, 1961; Wood, 1965; Stephens and Bils, 1967). In the present study, bile canaliculi with microvilli and junctional complexes are demonstrated with the electron microscope in the 7 mm, about fourth week embryo and some of them appear as intracellular bile canaliculi. Intracellular bile canaliculi have been reported in the goldfish (David, 1961; Yamamoto, 1965), in the fetal liver of the rat (Wood, 1965) and in pathological livers (Steiner, 1962; Abe, 1967) and these intracellular bile canaliculi at a very early embryonal stage are interesting from the point of ontogeny.

At six to seven weeks, large bile canaliculi bounded by four to seven liver cells appear. Liver cells seem to be arranged as tubules, but only the cross sectioned profiles of bile canaliculi are seen. Similar findings were described by many investigators (Bloom, 1926; Elias, 1955; Wood, 1965). Elias thought that such tubules do not occur at any time in the human embryo and that such a figure shows a section through a muralium of two cells thick. Wood, on the other hand, stated that the so-called tubules, in fact, appear to be vesicles or saccules and that there may be continuity between saccules, but that connecting portions are too narrow to be recognizable. Some investigators have suggested that those tubules are forerunners of the intrahepatic bile ducts (Horstmann, 1939; Wilson *et al.*, 1963; Wood, 1965). However, the present observation demonstrates that these tubules can be recognized not only near the portal vein and hilus, but also throughout the liver parenchyma, and that there is no basement membrane around them. Therefore, it is more conceivable that these tubules appear as a result of changes in arrangement in liver cell cords, and that they are transient and special modifications of the bile canaliculi.

At seven to eight weeks, large bile canaliculi are hardly even found and typical bile canaliculi bounded by three or four liver cells appear. This arrangement persists throughout later fetal life.

Many vesicles which are bordered by liver cells on one side and on the other by cuboidal epithelial cells are observed in the periportal spaces even when no fully formed bile ducts appear to be present. These vesicles are named biliary vesicles (Du Bois, 1963) or biliary spaces (Wood, 1965). They are morphologically identical to the duct of Hering when they are examined by electron microscopy and they are formed by liver cells and cuboidal biliary duct cells lying on a basement

membrane, but they are entirely different from the duct of Hering by its definition, for the term "duct of Hering" is used to describe the junction between the bile canaliculus and the interlobular bile duct. Du Bois and Wood explained that duct cells at the vesicles or spaces originate from the transformation of the liver cells under the influence of connective tissue. However, from the present data, the following interpretation of the duct cells at the vesicles is also possible. As shown in this study, the early intrahepatic bile ducts develop as epithelial cell plates. The invading epithelial cell plates become a simple layer at the periphery of the plates and adjoin the liver cells at the periportal spaces to form the vesicles. Such an interpretation is thought to enable biliary vesicle and duct of Hering to be classified into the same category.

Since Horstmann (1939) described the development of the intrahepatic bile ducts of the human fetus and correlated his observation with the findings by Doljanski and Roulet (1934), the hepatocytogenic theory of intrahepatic cholangiogenesis has been widely accepted (Elias, 1955; Du Bois, 1963; Wilson *et al.*, 1963; Wood, 1965; Picardi *et al.*, 1968). The basis of the hepatocytogenic theory of the intrahepatic cholangiogenesis depends largely on the experiments by Doljanski and Roulet who used mixed tissue cultures of liver cells and connective tissue. If the influence of the connective tissue is the only factor for the development of the intrahepatic bile ducts, it would be possible for the liver cells adjacent not only to the interlobular connective tissue, but also to the subcapsular connective tissue to transform into the biliary duct cells. Recently, functions of the connective tissue have been actively investigated, but they are still not clear. Therefore, it may be hasty to conclude that intrahepatic bile ducts are formed from the liver cells under the influence of the connective tissue, based on the classical tissue culture experiment alone, which is regarded as an unusual condition rather than normal. However, it may be significant to re-examine mixed cell cultures of liver cells and mesenchyme of the early embryo with modern cell culture techniques.

In view of previous papers, development of the bile duct in embryos of about two months seems to be very important. However, most of the investigators who have advocated the hepatocytogenic theory of intrahepatic cholangiogenesis have observed only the ductal formation around the peripheral portal vein of relatively large embryos and fetuses. Most of their papers are lacking in illustrations from early embryos and there are few pictures showing the hepatic duct, the bile duct and the liver cell cords developing in continuity. From observations of the ductal formations around the periportal spaces, cuboidal ductal epithelial cells which border the periportal spaces should be considered not as transformed liver cells, but as invading epithelial cells. The connective tissue seems to be concerned only in dissociation of the epithelial cell plates into a complex network of bile ducts. Hammer (1926) has already described intrahepatic bile ducts that grow out of the ends of the hepatic ducts and proliferate along the portal vein. The present study has confirmed his hypothesis, but Hammer did not report that the epithelial cells at the ends of the ducts have the capacity to transform into liver cells. Bloom (1926) observed a developmental process of the intrahepatic bile ducts similar to those of the present study, but his interpretation differs. It is difficult to agree with the hypotheses of Elias (1955) and by Du Bois (1963) from the present data.

Existence of transitional cells has been repeatedly denied by many investigators (Schaffner and Popper, 1961; Deams, 1961; Steiner and Carruthers, 1961; 1962; Du Bois, 1963), but Picardi *et al.* (1968) reported that in the human fetus, intermediate cells exist between the liver cells and biliary duct cells. The present study has demonstrated that various types of cells different from liver parenchymal cells and biliary duct cells are present at the ducts of Hering and at the branches of the hepatic ducts near the liver parenchyma. With respect to their morphological characteristics these transitional cells resemble both liver cells and biliary duct cells. Some transitional cells are difficult to classify into either category. Existence of these transitional cells also could support the concept that the liver cells transform into biliary duct cells under the influence of the connective tissue. However, for the reasons above mentioned, it seems more conceivable that biliary duct cells have the capacity to transform into liver cells and that these transitional cells result from the transformation of the biliary duct cells.

In the present study the characteristic alterations in the developing liver cells are also described. In the embryo of four weeks, free ribosomes in rosettes are abundant and rough surfaced endoplasmic reticulum is relatively well developed. It is known that immature cells are rich in free ribosomes and sparse in rough surfaced endoplasmic reticulum, and hence, liver cells in early stages seem to indicate that strong proliferation occurs. At six weeks when liver cells become polygonal cells, free ribosomes disappear from the cytoplasm and smooth surfaced endoplasmic reticulum appears. Glycogen granules appear at about eight weeks. It is interesting to note in view of glycogenesis or glycogenolysis that smooth surfaced endoplasmic reticulum appears earlier than glycogen granules (Porter and Bruni, 1959). The Golgi apparatus of very early embryos is located near the nucleus as compact lamellar arrays of cisternae and it moves toward the bile canaliculi as the embryo grows old. At three months when bile secretion is said to begin, the Golgi apparatus is located near the bile canaliculi and the peripheral ends of cisternae dilate to form the vacuoles in which slightly dense material is observed. The Golgi apparatus in the liver cells is thought to participate in bile secretion from the changes in its location and in its morphology during the development. There are spherical or ovoid organelles that are smaller than mitochondria and surrounded by a single membrane. They are thought to be microbodies and they are already found in the liver cells of 20 mm embryo. The crystalloid is not detected, however. In addition, there are many dense bodies in the liver cells throughout the fetal stages. They are polymorphic and surrounded by a single membrane. Some of them are sometimes found near the bile canaliculi and called peribiliary dense bodies. They are considered to be lysosomes or derivates of them (Essner and Novikoff, 1960).

The fine structure of the interlobular bile duct epithelium of the human fetus is almost the same as that of the human adult and of various other animals. Hepatic duct epithelium of the 20 mm embryo differs greatly from the bile duct epithelium. Epithelial cells of the hepatic duct contain glycogen granules. However, biliary duct cells in the duct of Hering of the 16 mm embryo and those of the bile ducts in the later stages are different from those of the hepatic duct, and contain no glycogen granules. Although the functional meaning of such difference between hepatic duct epithelium and bile duct epithelium is obscure, this difference may hold the key to the solution of the origin of the biliary duct cells.

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References

Abe, R.: Some observations on the fine structure of the human liver in congenital biliary atresia. Arch. histol. jap. **28**, 503–524 (1967).

Bloom, W.: The embryogenesis of human bile capillaries and ducts. Amer. J. Anat. **36**, 451–465 (1926).

David, H.: Zur submikroskopischen Morphologie intrazellulärer Gallenkapillaren. Acta anat. (Basel) **47**, 216–224 (1961).

Davies, J.: Development of the gastrointestinal tract. In: Human developmental anatomy p. 144–163. New York: Ronald Press Co. 1963.

Deams, W. Th.: The micro-anatomy of the smallest biliary pathways in mouse liver tissue. Acta anat. (Basel) **46**, 1–24 (1961).

Doljanski, L., Roulet, Fr.: Über die gestaltende Wechselwirkung zwischen dem Epithel und dem Mesenchym, zugleich ein Beitrag zur Histogenese der sogenannten „Gallengangswucherungen". Virchows Arch. path. Anat. **292**, 256–267 (1934).

Du Bois, A. M.: The embryonic liver. In: The liver, ed. by Ch. Rouiller, vol. 1, p. 1–39. New York: Academic Press 1963.

Elias, H.: Origin and early development of the liver in various vertebrates. Acta hepat. (Hamburg) **3**, 1–56 (1955).

Essner, E., Novikoff, A. B.: Hepatocellular pigments and lysosomes. J. Ultrastruct. Res., **3**, 374–389 (1960).

Hammer, J. A.: Über die erste Entstehung der nicht kapillären intrahepatischen Gallengänge beim Menschen. Z. mikro.-anat. Forsch. **5**, 59–89 (1926).

Horstmann, E.: Entwicklung und Entwicklungsbedingungen des intraheptischen Gallengangsystems, Arch. Entwickl.-Mech. Org. **139**, 363–392 (1939).

Karnovsky, J. J.: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. **27**, 137A–138A (1965).

Karrer, H.: Electron-mocroscipe observations on the chick embryo livers. Glycogen, bile canaliculi, inclusion bodies and hematopoiesis. J. Ultrastruct. Res. **5**, 116–141 (1961).

Kunitomo, K., Yamaguchi, M.: Quantitative Untersuchungen der kaldianlen Organe der japanischen Embryonen (Jap. with Germ. abstr.) Nagasaki med. J. **15**, 1979–2012 (1937).

Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961).

Picardi, R., Gardiol, D., Gautier, A.: Etude de la cholangiogénèse chez le foetus humain. I. Aspects ultrastructuraux et classification des divers types cellulaires épithéliaux recontrés dens les rêgions périportales. Z. Zellforsch. **84**, 311–318 (1968).

— — — Etude de la cholangiogénèse chez le foetus humain. II. Aspects histologiques et ultrastructuraux de la génèse des canaux biliaires intrahepatiques. Z. Zellforsch. **84**, 319–327 (1968).

Porter, K.R., Bruni, C.: An electron microscopic study of the early effect of 3-Me-DAB on rat liver cells. Cancer Res. **19**, 997–1009 (1959).

Schaffner, F., Popper, H.: Electron microscopic studies of normal and proliferated bile ductules. Amer. J. Path. **38**, 393–410 (1961).

Steiner, J. W., Carruthers, J. S.: Studies on the fine structure of the terminal branches of the biliary tree. 1. The morphology of normal bile canaliculi, bile preductules (ducts of Hering) and bile ductules. Amer. J. Path. **38**, 639–661 (1961).

— — Experimental extrahepatic biliary obstruction. Some aspects of the fine structural changes of bile ductules and pre-ductules (ducts of Hering). Amer. J. Path. **40**, 253–270 (1962).

Stephens, R. J., Bils, R. F.: Ultrastructural changes in the developing chick liver. J. Ultrastruct. Res., **18**, 456–474 (1967).

Wilson, J. W., Groat, C. S., Leduc, E. H.: Histogenesis of the liver. Ann. N.Y. Acad. Sci. **111**, 8–24 (1963).

Wood, R. L.: An electron microscope study of developing bile canaliculi in the rat. Anat. Rec. **151**, 507–530 (1965).

Yamamoto, T.: Some observations on the fine structure of the intrahepatic biliary passages in goldfish. Z. Zellforsch. **65**, 319–330 (1965).

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