THE LIVER

Morphology, Biochemistry, Physiology

VOLUME I

Edited by

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PREFACE

This treatise which is written for morphologists, biochemists, pharmacologists, and clinicians is an attempt to present in comprehensive form the entire field of present knowledge on the liver under normal and pathological conditions, as revealed by morphological and physiological studies. Without neglecting the fundamental experience of the past, emphasis is placed on the recent developments in the study of hepatic structure and function, particularly on the subcellular and molecular level. Thus, the treatise tries to fill the gap between the many valuable textbooks that are unavoidably limited to human pathology and the numerous excellent reviews and monographs that are concerned either with special aspects of the liver or refer to the liver cells only as a convenient example for studies on basic cytology.

The first volume is devoted to embryology, to macroscopic, microscopic, and ultramicroscopic morphology, and to the chemical constitution and biochemical function of the liver and its role in the metabolism of proteins, lipids, and carbohydrates. Volume II will discuss the excretory function of the liver cell, the physiology of the Kupffer cells, and the correlation of the liver with endocrine organs, vitamins, and blood. Volume II will also describe liver function tests and the methods of experimental surgery, and will deal in its final chapters with the general and experimental pathology of the organ and its regeneration, and with the problem of necrotropic substances.

The authors of the individual chapters emphasize the results obtained by animal experimentation supported by evidence based on modern investigation techniques such as electron microscopy, histochemistry and cytochemistry, differential centrifugation, and isotope labeling. The correlation of structure with function is stressed in all instances; the participation of the liver in the function of other metabolic systems and its relation to other organs are pointed out and discussed.

The extensive documentation by carefully compiled references should make the treatise useful for the active worker in the field. The bibliography is based on three categories: basic publications, recent papers, and reviews containing numerous references. It is hoped that the reader interested in additional information will readily be guided to the original communications.

I am deeply grateful to the contributors who, in spite of the numerous duties and tasks with which they are burdened, nevertheless agreed to participate in the elaboration of this treatise. My thanks are also due to Dr. Robert J. Schnitzer for his help during the period of the treatise's preparation and to the staff at Academic Press for the painstaking care in the production of the volumes.

CH. ROUILLER

August, 1963

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CHAPTER 1

THE EMBRYONIC LIVER

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I. Morphology

A. LIVER DEVELOPMENT

1. *The Hepatic Primordia*

The study of embryological liver development which was initiated by von Baer (1828, 1837) has been the object of much research. The results obtained have served as the basis for the "classic" conception of hepatic histogenesis which can be found in the majority of embryological texts (Lewis, 1912; Hamilton *et al.*, 1946; Patten, 1948; Arey, 1954). This concept can briefly be summarized as follows: The liver is formed from two distinct primordia, the hepatic diverticulum of endoblastic origin and the vascular network which develops precociously between the vitelline veins, and to which are added vascular elements of umbilical vein origin. The hepatic diverticulum is differentiated in very young embryos in the form of a thickening in the ventral floor of the foregut corresponding to the future duodenum, near the origin of the yolk stalk. This primordial thickening rapidly forms a double diverticulum (Fig. 1., *H.D.*) which thrusts into the mesenchyme of the septum transversum. The hollow caudal portion gives rise to the gallbladder, cys-

tic duct, and common bile duct (ductus choledochus). Epithelial cords or tubules bud off from the cranial portion and proliferate actively. From the beginning, the hepatic diverticulum lies close to the vitelline veins (Fig. 1., *V.V.*) which flank the gut. Ramifications from these veins form a network into which the proliferating hepatic cords are intricated. The result is an intermingling growth of liver epithelial cords and sinusoidal vessels. The branching of developmental hepatic cords is characteristic and

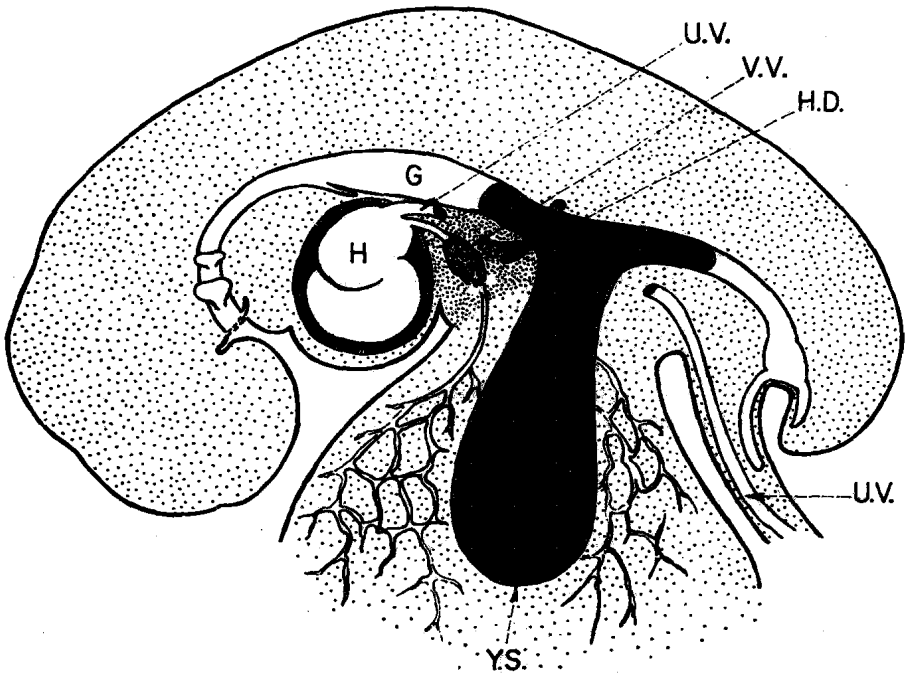


FIG. 1. Human embryo of 4 mm. *G*, gut (sectioned in the intestinal region); *H*, heart; *H.D.*, hepatic diverticulum growing in the septum transversum (finely stippled zone); *U.V.*, umbilical vein; *V.V.*, vitelline vein; *Y.S.*, yolk sac partially open.

establishes the basic architectural pattern of the adult liver. The details of the several schemes proposed to describe these ramifications, which take place with an angular precision and terminate in the realization of the hepatic lobule, will not be set forth in this work. Consideration of these schemes leads to two theories concerning the origin of the epithelial constituents of the liver:

a. The development of the liver takes place like that of an exocrine gland. The proximal portions of the hepatic cords, or rather tubules, directly form the entire system of intrahepatic bile ducts; and the distal

portions are responsible for the hepatoblast framework which is characteristic of the lobule (diagram in Patten, 1948).

b. Only the hepatoblast framework and the hepatic canaliculi are formed from elements of the primitive epithelial sponge work. The intrahepatic bile ducts are secondarily differentiated as the result of new bud growths from the hepatic canal. Their ramifications follow the same course as the branches of the portal vein. This concept implies the formation of connections between the bile canaliculi and bile ductules at the periphery of the perilobular spaces.

These concepts of development of the hepatic primordia, which were considered valid for mammals and even for vertebrates in general, have been reexamined. In 1948, Elias by means of reconstructions from serial sections of the adult liver arrived at the conclusion that the hepatic parenchyma is not constituted of epithelial trabecular systems surrounded by sinusoid networks. On the contrary, the livers of all vertebrates (from cyclostomes to man) have the same basic structure, i.e., a system of connected epithelial plates, or muralium (Elias, 1948, 1949, 1953; Elias and Bengelgsdorf, 1951, 1952; Hickey and Elias, 1954). These laminae hepatis are two cells thick in the lower vertebrates (muralium duplex), but in some birds and mammals they are one cell thick (muralium simplex). The plates are riddled with perforations of varying sizes and form a vast three-dimensional lacunary network, the labyrinthus hepatis. The labyrinth lined by an endothelium molded to the hepatocytes constitutes the sinusoid network (details in Elias, Chapter 2 of this volume).

This structural unity of the adult liver seemingly implicates the uniformity of development of the hepatic primordia. The recent works of Lipp (1952a,b) and Elias (1955, 1957) with 30 species of vertebrates have demonstrated the existence of twelve basically different types of liver development. The budding epithelial cords from the cranial portion of the hepatic diverticulum, which was formerly considered to be a normal process for all hepatic histogenesis, is, in fact, realized only in the pig embryo; the budding of hollow cylinders occurs only in the chick embryo (Kingsbury *et al.*, 1956). Space limitations preclude reviewing the twelve modes of histogenesis cited by Elias; only the development of the human liver will be described in detail.

The first sign of the formation of the hepatic diverticulum is a thickening of the endoblastic epithelium which appears in the 7-somite embryo (2.5 mm., 18th day). In the 19-somite embryo (3 mm., 22nd day), the diverticulum is formed. Its wall is thick, and the cellular limits are not distinguishable. Hepatic cell plates or ridges of irregular outgrowths inweave the mesenchyme. In the 25-somite embryo (3.6 mm., 28th day),

these masses have invaded the ventral portion of the septum transversum and grow laterally (Fig. 4) in the direction of the vitelline veins. Figures 2 and 3 clearly illustrate that there are not well-delimited cords that compress the mesenchyme cells, but irregular masses in which the

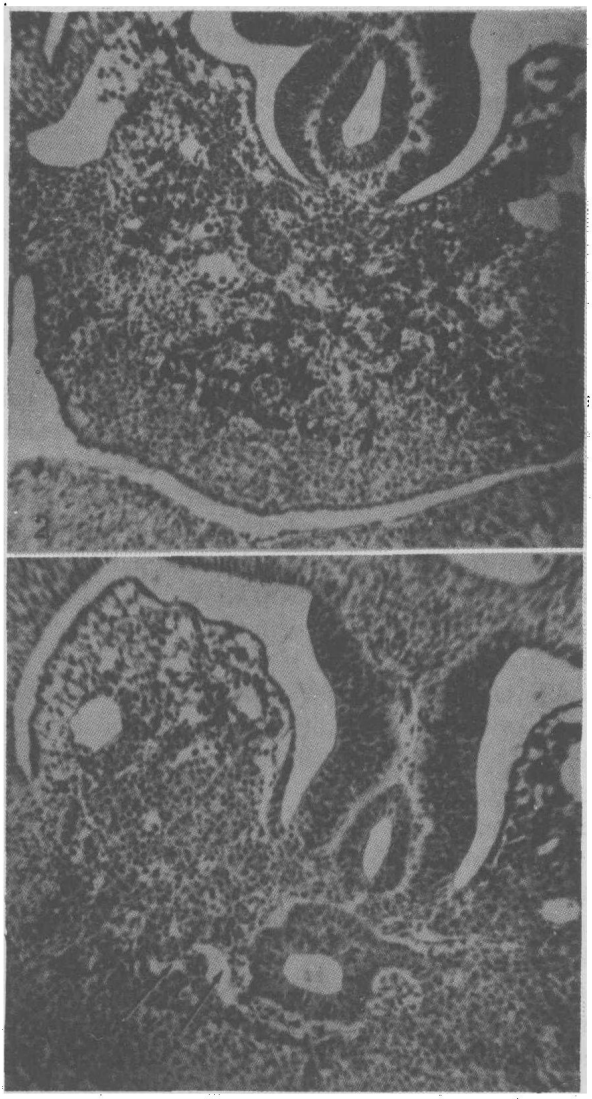


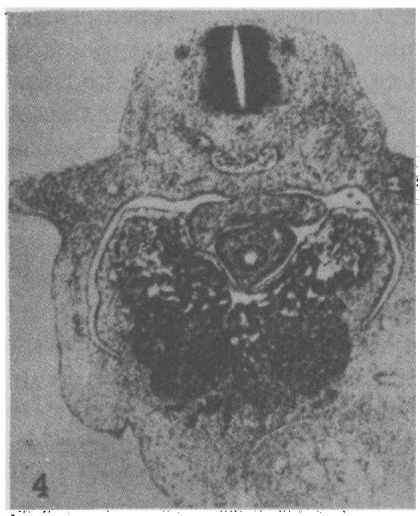
FIG. 2. Human embryo of 3.6 mm., 30th day, cross section (Institute of Anatomy, Basle). Liver topography showing the proliferation of the hepatoblasts into the septum transversum. Magnification: × 105.

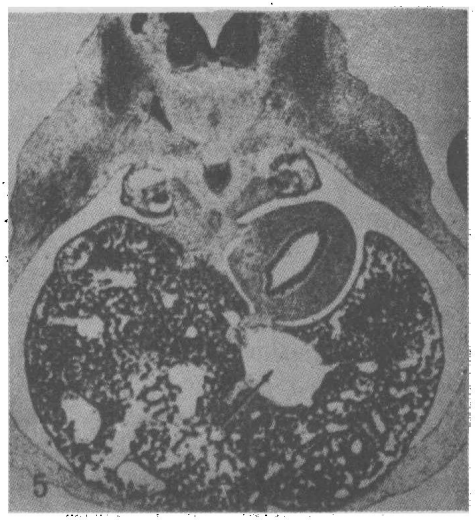
FIG. 3. Same embryo as that shown in Fig. 2. Arrows indicate two mesenchymal sinusoids. Magnification: × 105.

cells are often loosely connected, or even completely detached. The first sinusoids are differentiated in the ventral portion of the septum transversum in the form of closed independent vesicles (Fig. 3) around which the hepatic cells are molded (Lipp, 1952a). These primitive sinusoids do not contain blood. They unite progressively in the form of a network which attaches later to that formed laterally by the vitelline veins. Lipp never observed the process of intercrescence which was evoked by Minot (1900) and Lewis (1904) to explain the formation of the hepatic sinuses. (Intercrescence is a particular form of splitting of a large blood vessel, e.g., vitelline vein, in which parenchyma cords enter the vessel pushing before them the endothelium.) Each of the vitelline veins in their passage across the septum transversum branches to form a plexus. The hepatoblasts invade the newly formed intervascular spaces. The double sinusoid network derived from the vitelline veins connects with those sinuses formed independently in the ventral mesenchyme. The primitive labyrinth is rapidly completed by the addition of sinusoid elements formed superficially to the detriment of the umbilical veins (Figs. 4, 5, and 6).

Elias (1955) considers that for the human embryo, the hepatoblasts are not all of endoblastic origin. He observed that the coelomic epithelium in the proximity of the vitelline vein (4-week embryo) proliferates actively and thickens. From here, cells morphologically comparable to the hepatoblasts detach and occupy the intervascular spaces in the dorsolateral region of the septum transversum. These hepatoblasts of mesoblastic origin intermix with those of endoblastic origin, and then it is impossble to distinguish the two types of cells. According to this concept, the epithelial portion of the anterior part of the adult liver is of endoblastic origin, the median portion of endoblastic plus mesoblastic, and the posterior part purely of mesoblastic origin.

The mesoblastic origin of a portion of the hepatic parenchyma seems to be quite generalized since Elias observed this evolution in many mammalian species (guinea pig excepted), certain birds, reptiles, and selachians. However, it must be acknowledged that the microphotographs published by Elias are not convincing. The fact that this was not observed by Lipp (1952b) in his very complete work on the human hepatic embryogenesis leaves a possible doubt concerning the contribution of the mesoblast to the liver parenchyma. In the human embryos of 3½ to 4½ weeks that we have studied, the thickening of the coelomic epithelium as indicated by Elias was observed, but not the subsequent epithelial proliferation. The possibility remains that the latter stage takes place very rapidly and that we have not had at our disposition an embryo with precisely this form of proliferation.





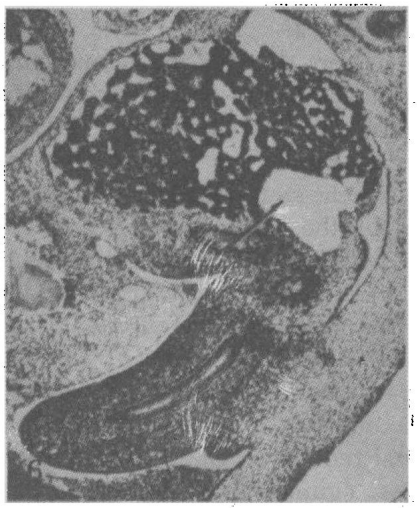


FIG. 4. Human embryo of 4.5 mm., 33rd day, cross section (Institute of Anatomy, Basle). The ventral mesenchyme of the septum transversum is already invaded by the hepatoblast plates. Laterally, the migration of hepatoblasts in the direction of the vitelline vein is less advanced. Magnification: × 35.

FIG. 5. Human embryo of 9 mm., 38th to 39th day, cross section (Institute of Anatomy, Basle). The liver topography is partially realized; arrow indicates the ductus venosus. Magnification: × 25.

FIG. 6. Human embryo of 7–8 mm., 35th day, longitudinal section (Institute of Anatomy, Basle). Liver topography showing ductus venosus (arrow). Under the liver, the gallbladder and a section of the common bile duct; end of the epithelial plug stage. Magnification: × 42.

Concerning the formation of the muralium, it can be noted that the liver plates are originally 3–5 cells thick and their transformation into plates one cell in thickness occurs more or less rapidly. Lipp (1952b) assumes that the splitting of the thick plates is the result of the penetration of sprouting sinusoids. Elias (1955) observed in an 8-mm. embryo, plates three cells thick; and in a 4½-mm. embryo, plates one cell thick. He assumes that in this early stage the liver plates may be very plastic. Under conditions of great distension of the sinusoid, the liver plates are one cell thick; they may, however, slide back into their original positions, forming plates several cells thick when the sinusoids collapse. The definitive formation of one-cell-thick plates is progressively realized by the further branching of the sinusoids during the liver's growth. According to Morgan and Hartroft (1961), the majority of the hepatic plates are still two or three cells thick in the newborn. It is not until the fifth year that the typical form of the muralium simplex is achieved throughout the liver.

2. *Cholangiogenesis*

The development of the gallbladder, extra-, and intra-hepatic biliary ducts in the human embryo has been studied by many authors. Extensive bibliography can be found in works of Lewis (1912), Bloom (1926), and Horstmann (1939).

a. Extrahepatic Ducts

The gallbladder and common bile duct (ductus choledochus) develop from the caudal portion or pars cystica of the hepatic diverticulum. The pars cystica, which is closely associated with ventral pancreatic bud, takes its origin from the anterior side of the duodenum. Following the rotation to the right of the duodenum, which occurs at about the fifth week, the attachment of the common bile duct is displaced to its definitive position on the dorsal duodenal side.

The originally hollow pars cystica (Fig. 1, *H.D.*) rapidly elongates and its lumen is obliterated by epithelial proliferation. This takes place in accordance with a process comparable to that which forms the epithelial plug of the gut. In the 6–7 mm. embryo, the future gallbladder and common bile duct are thus represented by a solid epithelial cord of uniform diameter in the septum transversum directly beneath the liver. Several vacuoles appear in the epithelial mass of the proximal region of the cord. From their confluence arises the lumen of the common bile duct, which is first seen in the 7–8 mm. embryo. The vacuolarization progresses in the direction of the cystic portion. This part, on acquiring its lumen, expands; from this time on the gallbladder is distinctly rec-

ognizable. In the embryo of 14–18 mm. (7th week), however, the fundus of the gallbladder is still partially obstructed by the remains of the epithelial plug (Figs. 6 and 7), which disappears at the beginning of the third month. From this moment the cavity is entirely lined by simple columnar epithelium. Development beyond the third month until birth consists essentially of growth processes (details in Lee and Halpert, 1932; Schwegler and Boyden, 1937a,b,c). The first muscle fibers of the canals and gallbladder appear during the third month. Bile secretion starts at the beginning of the fourth month (Streeter, 1948), and

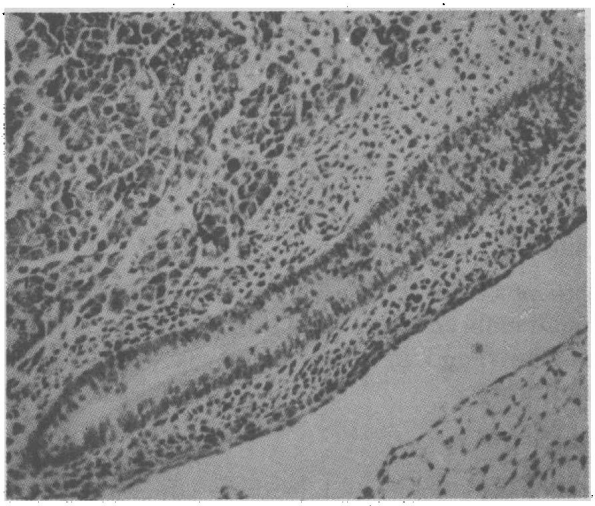


FIG. 7. Human embryo of 10 mm., 39th day, longitudinal section. Gallbladder and common bile duct. Epithelial plug stage of the gallbladder; the common bile duct is already perforated. Magnification: × 190.

from this moment the gallbladder constantly contains bile which is secreted into the intestine and colors the meconium. The characteristic folds of the gallbladder are formed at the end of gestation and are moderately developed in the newborn. The crypts or biliary glands are only slightly distinguishable at birth.

b. Intrahepatic Ducts

The first bile canaliculi are revealed in the form of small vesicles between the hepatic cells of the 10 mm., 6th week embryo (Popper and Schaffner, 1957). They appear long before the bile secretion, which does not begin until the fourth month. Recently, Karrer (1961) showed with the electron microscope that completely formed bile canaliculi, including their characteristic microvilli, exist in the 6-day chick embryo.