THE HISTOLOGY OF THE BOVINE LIVER WITH SPECIAL REFERENCE TO THE FORMATION OF BILE CANALICULI

by

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A THESIS

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INTRODUCTION

The object of this study was to examine and record the normal microscopic structure of the bovine liver and its biliary system.

Early histologists (1, 7, 15, 20) believed from their study of microscopic sections, that the liver parenchyma consisted of radiating cords, each made up of two or more columns of cells. Enclosed in the axis of a cord, the bile capillary appeared as a minute intercellular channel resembling a tubular gland. From this the organ was classified as a compound tubular gland. Some workers (1, 2, 23) observed that the bile canaliculi (capillaries) of vertebrates lay between two cells in man, three cells in the frog, four cells in some lower vertebrates, four to seven cells in the chicken and five cells in the viper and elephant. Such observations implied that in the lower vertebrates, the hepatic cords were constructed with more columns of cells than in the higher vertebrates. The structure of the hepatic cord served as an index of the evolutionary state of the animal.

A recent re-examination (9, 10) of the structure of the parenchyma of the mammalian liver based on serial sections, injection techniques, wax-plate reconstructions, and stereograms rendered the conventional concept of the pseudo-cell-cord theory obsolete. The interpretation was that the parenchyma was made up of plates or laminae, called laminae hepatis. These are one cell thick and are perforated at many places by openings known as stomata. The distance between the stomata and the size of the stomata vary by the width of one to the width of several cells. The laminae enclose spaces, lacunae hepatis, which communicate with each other by the stomata. The community of lacunae of one hepatic lobule form the

labyrinthus hepatis in which the plexus of hepatic sinusoids is suspended. This has led to a new classification of mammalian livers into three types. Speculation on the physiological reasons or the taxonomic significance of the different types of mammalian livers is premature.

A study to examine and evaluate the parenchymal architecture of the bovine liver, in view of these newer types, will be reported in this thesis.

REVIEW OF LITERATURE

No specific reference to the structure of the bovine liver appears in the early textbooks on histology. Most of the references are devoted entirely to the tissues of man, with incidental references to the liver of other vertebrates. Recent books on comparative histology refer only to the goblet cells in the extrahepatic ducts and gall bladder and the presence of mucous and serous glands in the wall of the gall bladder of the bovine.

Various workers (1, 5, 6, 7, 11, 14, 15, 16, 17, 19, 20, 22, 23, 24, 28, 30) examined microscopic sections of liver and referred to the hepatic cells of the parenchyma as being made up of radiating cords, rows, strands, end-pieces, trabeculae or chains.

Greep (12) described the parenchyma as consisting of anastomosing strands which are one cell thick in rat, rabbit, cat, dog, and man. Ham (13) used hepatic cords synonymously with plates which are one cell thick in man. Maximow and Bloom (18) described irregularly arranged radial columns or plates one cell thick in man. Smith (26) in an early edition referred to the anatomical units as radially arranged anastomosing tubules or hepatic cords. In a later edition (27) he argued against the usage of

the term cord and favored the term laminae or plates.

Books on the histology of domestic animals do not devote much attention to the bovine liver. Foust and Getty (11) made no mention of it, while Trautmann and Fiebiger (32) referred in general to the liver of domestic animals and described hepatic laminae.

Several early histologists (1, 7, 15, 20) stated that the opposing surfaces of cells were grooved to enclose a minute channel, the bile capillary, comparable to the lumen of a simple tubular gland. Thus, Bohm et al. (1) illustrated four types of bile canaliculi formed in the hepatic cords. He said the cords were comprised of two rows of cells in man, three rows in the frog, four rows in lower vertebrates and five in the viper. Dahlgren and Kepner (8) described the liver of cryptobranchus as a tubular gland and noted bile capillaries with five or six surrounding cells or in some sections only two cells. Calhoun (2) stated that in the chicken, the liver epithelium was arranged in a tubule of four to seven cells about an intralobular bile capillary. This tubular arrangement was well marked in cross-section, but in longitudinal section the tubule looked like a plate or lamina. Miller (20) described bile capillaries in human liver formed between three or four cells. Swift (31) stated that in some animals, two cells made up the bile capillary, in others four. Satterthwaite (25) noted that in the rabbit bile capillaries were formed between two cells and rarely three or more cells, and that in the dog each liver cell was suspended between two or three (rarely four) bile capillaries. Sterling (29) stated that four or five cells bounded the bile capillary in the frog or newt. Cowdry (6) observed that in the lower vertebrates which have tubular organization of the liver, the bile capillary was surrounded by

five or six cells, and in man two or rarely three, cells. Mariappa (17) reported that the bile capillary was formed between five cells in the fetal Indian elephant.

The recent re-examination of liver structure made by Elias (9, 10) used the newer methods of investigation outlined in the introduction. He classified mammalian livers into three types; (a) saccular liver, hepar sacculare, of man and cat, characterized by lacunae hepatis which are wide and irregular in shape, (b) tubular liver, hepar tubulare, of horse and rabbit, which have narrow, tubular and cylindrical lacunae, and (c) transitional, intermediate type, seen in the dog, which has both types of lacunae. It is obvious that the blood storage capacity is greater in the saccular liver than in the tubular liver. According to Elias (10), hepar tubulare has 16.5 percent of the bile canaliculi lined by three cells and 83.5 percent by two hepatic cells. In hepar sacculare, the percentage of bile capillaries lined by three cells is much lower. The bile canaliculi are intercellular, mostly intralaminar, but some are extralaminar. They form a polygonal network, each mesh of which ordinarily includes a hepatic cell. Meshes near the stomata are incomplete and include part of a cell.

Palade (21) by histochemical methods for phosphatase, demonstrated the bile capillaries. Trautmann and Fiebiger (32) and other histologists upholding the cell-plate theory have stated that the bile capillary was formed between two or three cells in mammals. McIndoe (19) illustrated the bile capillaries as being formed between the opposing faces of two hepatic cells in the human liver and placed them in several categories according to their shape.

Wachstein and Zak (33), Sobotta and Piersol (28), Wathen and Pederson

(34), Radasch (24), Carleton (3), Stohr (30), Hill (14), Piette (23), Cowdry (6), and Dahlgren and Kepner (8) have noted intracellular bile channels and knob-like secretion vacuoles which have been proved to be artefacts by Elias (10) and McIndoe (19).

Elias (10) cited several histologists from as early as 1849 who demonstrated that the mammalian bile capillaries are not just glandular lumina, but are minute tubules with a solid, stiff wall of their own. McIndoe (19) denied the existence of a separate membranous wall because of the absence of a double contour of the wall. Elias (10) confirmed the existence of a separate, resistant membranous wall for the bile capillary of the mammal by first destroying hepatic cells by maceration.

MATERIALS AND METHODS

This study includes the livers of three calves and three adult bulls. All were healthy grade dairy animals.

After exsanguination through the jugular vein, the animals were opened by an oblique incision through the abdominal wall extending from the xiphoid region along the right costal arch and lumbar transverse processes to the tuber coxae. This flap was everted. The posterior vena cava in front of the junction with the right renal vein and the portal vein an inch behind the portal fissure were isolated by blunt dissection and ligated. The ansa sigmoidea of the duodenum at both ends, the hepatic artery an inch behind the portal fissure, and the esophagus at the cardia were isolated and ligated in a similar manner. The right thoracic wall was removed with the pectoral limb by cutting the ribs from the sixth rib forward. The right diaphragmatic lobe of the lung was gently lifted to allow ligation of both

the posterior vena cava and the esophagus in front of the diaphragm. All the ligated structures were cut an inch from the ligatures. The attachment of the lesser omentum to the omasum was gently separated and broken close to the omasum. The right kidney was isolated by blunt dissection from its attachments except at the renal impression. Finally the diaphragm was cut along its attachment. The liver with the diaphragm was gently lifted from the body and laid (diaphragm facing downward) on several layers of cheese cloth on a table. Throughout the dissection, great care was taken to see that no traction was exerted on the organ. To avoid injury, its surfaces were not handled, because trauma would be detrimental to successful injection and perfusion.

Gall Bladder Injection

After isolation of the liver of one calf, 200 ml. of a saturated solution of indigocarmine (sodium indigodisulfonate) was injected into the biliary system through the gall bladder. This was accomplished by properly securing a needle in the gall bladder and allowing the solution to flow by gravity. After the injection the organ was fixed in absolute alcohol by perfusion through the portal vein. Serial sections of a paraffin block of this liver were prepared by the usual processes. Observations were made after mounting, clearing and covering the sections without further staining.

Intravenous Injection

Another calf was injected with a saturated aqueous solution of indigocarmine intravenously. A dose of 100 ml. was injected into the jugular vein at 30 minute intervals for a total of three injections. This procedure was

based on the findings of Chrzonszczewsky (4) on pigs, dogs, cats, and rabbits. After the second injection the calf micturated blue urine, which indicated excretion of the dye by the kidneys. After the third dose the calf showed marked dyspnea. Half an hour after the third injection the calf was exsanguinated and the liver removed and fixed as outlined above. The organ was deep blue with a gall bladder distended with dark blue bile. Representative pieces were embedded in paraffin the following day. Serial sections six to eight microns thick were cut, mounted, cleared of paraffin and covered without further staining for examination.

No Dye

A third calf liver was removed and fixed as before except 10 percent buffered formalin was substituted for absolute alcohol as a fixative. Sections of the gall bladder, cystic duct, and ductus choledochus were fixed in buffered formalin. Small pieces of liver from three adult bulls were also placed in 10 percent buffered formalin. These specimens were processed by the usual paraffin technique and serial sections cut six microns thick were stained with hematoxylin and eosin.

Silver Impregnation

At the same time, pieces of liver from the calf (No Dye) were saved for silver impregnation. Specimens from the adult animals were fixed in 10 percent formaldehyde for silver impregnation and all were fixed for a minimum of 30 days. Basically the technique used was that of Rio Hortega as given by McIndoe (19). One modification was that when half of the precipitate was dissolved, the supernatant solution was filtered and used

for impregnation. Another change was that the 10 microns thick frozen sections were alternately heated and cooled for only 10 minutes because longer periods gave colors that were too dark.

Maceration

Finally, a fixed (10 percent formalin) piece of liver from an adult animal was placed in water for maceration of the hepatic cells. The piece was approximately one centimeter square by two to three millimeters thick. On the tenth day and each succeeding day a small portion was removed and crushed on a glass slide under a cover glass until only the meshwork of bile capillaries remained.

RESULTS AND DISCUSSION

Gall Bladder Injection

Calf. The dye was found in the intralaminar bile canaliculi and interlobular bile ducts. The deposition of the dye was most evident in the polygonal network of bile canaliculi in the laminae of hepatic lobules just under the capsule (Plate I, Fig. 1). In other lobules, the deposition of the dye was either minute or totally wanting. Perhaps the deposition of the dye was not sufficient to demonstrate the extralaminar bile canaliculi.

This observation confirms the intercellular and intralaminar nature of bile canaliculi reported for five different mammalian livers by Elias (10). Failure to demonstrate the dye in the extralaminar bile canaliculi is no proof of their absence.

Intravenous Injection

Calf. Observations were identical to those mentioned above but the deposition of the dye was greatly decreased. A possible explanation may be that at the dose given the dye was eliminated primarily by the kidney. Higher doses would probably have been toxic.

No Dye

Calf. The parenchymal architecture was identical with that described for the five mammalian livers by Elias (9). The hepatic lobules were not clearly defined by the interlobular connective tissue. The lobules were found to vary from 0.3 to 0.5 millimeters in diameter. The polyhedral hepatic cells measured from 12 to 16 microns in diameter. The lacunae hepatis were found to be narrow, tubular and cylindrical both in longitudinal and transverse sections (Plate I, Fig. 2).

The sections of ductus choledochus revealed the presence of numerous goblet cells in the epithelium and mucous and serous tubular glands in their walls (Plate II, Fig. 1). The gall bladder and cystic duct showed very few of these structures. The circular muscle fibers in the wall of the ductus choledochus formed a thick layer to form a sphincter in the pars intestinalis. There was a large amount of diffuse lymphoid tissue in the wall of the cystic duct (Plate II, Fig. 2) and gall bladder (Plate III, Fig. 1), which does not confirm the statement of Trautmann and Fiebeger (32) that nodules of lymphoid tissue occur in these locations.

Adult. Sections from the livers of the adult bulls revealed the usual structure of the hepatic parenchyma. The hepatic lobules measured from 0.6 to 1.4 millimeters and the hepatic cells from 18 to 28 microns in

diameter. The lacunae hepatis were tubular and cylindrical (Plate III, Fig. 2). The lamina limitans (Plate IV.) was extensive and some lobules showed minute intralobular ducts.

The above mentioned observations confirm the findings in recent work on the mammalian liver by Elias (9) and other observations of Trautmann and Fiebiger (32) on the liver of the ox. The tubular and cylindrical nature of the bovine lacunae hepatis would justify the classification as hepar tubulare like those of the horse and rabbit (Plate V.).

That livers of such diverse species can be classified as hepar tubulare denotes that it is of no taxonomic value. Saccular and tubular livers indicate only blood storage capacity - more for the former and less for the latter. The blood storage capacity of liver is related to its functional activity which in turn is directly related to the rate of metabolism. The rate of metabolism is again related to the size of red blood corpuscle. Based on these criteria, the classification of the bovine liver as hepar tubulare with that of the horse is justified since the average size of their red blood corpuscles is the same (5.6 microns). This explains the physiological significance of the types of livers.

Silver Impregnation

Silver impregnation of the bile canaliculi, though light, was evident in the laminae. The intralaminar polygonal network was more pronounced in the liver from the adult bulls than from the calf (Plate VI). The extralaminar bile canaliculi could be demonstrated in a few areas. The bile canaliculi were approximately 0.75 microns in diameter. The intracellular secretory canals and secretory vacuoles were absent. These observations

confirm the findings of Elias (10) in the livers of five mammalian species.

Maceration

The polygonal network of bile capillaries was isolated by 20 days of maceration. After crushing bits of macerated tissue, the canaliculi were demonstrated as distinct networks with only cytoplasmic debris adherent. This showed that the canaliculi have an independent, resistant membranous wall of their own (Plate VII).

This observation confirms the findings of some of the early histologists and Elias (10).

SUMMARY

The cell-cord theory of early histologists, who examined microscopic sections, was based on the interpretation of bicellular interlaminar bridges as transections of hepatic cords. Using newer methods of investigation, Elias (9, 10) proposed a cell-plate theory which explained the formation of bile canaliculi more fully. He also classified the livers of the five mammals he studied into three types.

The architecture of the bovine liver had not been described prior to this study. Three grade calves and three grade adult bulls, all of dairy breeding, were used in these experiments. In the first calf, indigocarmine was injected into the biliary system through the gall bladder after careful isolation of the ligated liver. The organ was fixed by perfusion with absolute alcohol through the portal vein. By this technique, the dye was demonstrated in the intralaminar polygonal network of bile capillaries. This was especially evident just under the capsule. In the second calf, indigocarmine was injected into the general circulation in repeated doses.

The liver was isolated and fixed as before. Physiological and natural excretion of the dye into the bile capillaries by the hepatic cells was demonstrated, but the amount of the dye was decreased. This was probably due to its primary excretion by the kidneys. The liver of the third calf and the livers of three adult bulls were used for the study of hepatic parenchyma which conformed to the type of hepar tubulare. The sections of gall bladder, cystic duct and ductus choledochus confirmed the findings of Trautmann and Fiebiger (32). However, in the wall of the cystic duct and gall bladder a large amount of diffuse lymphoid tissue was observed in this calf.

Frozen sections of the liver of the third calf and three adult bulls were impregnated with silver carbonate by the method of Rio Hortega, with a few modifications. The network of intralaminar and extralaminar bile capillaries was clearly demonstrated. Intracellular bile canals and secretory vacuoles were not observed. By maceration of hepatic cells, the network of bile capillaries was isolated to demonstrate their resistant membranous wall. These findings confirm those of Elias and other histologists.

The types of liver have no taxonomic value. Their physiological significance is explained in relation to the metabolism of the animal.

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APPENDIX

EXPLANATION OF PLATE I

Figure 1.

Section of liver just under the capsule, indigocarmine injected, X560.

Figure 2.

Section of liver, stained with hematoxylin and eosin, X560.

i. b. - interlaminar bridge.

l. h. - lacunae hepatis.

L. h. - laminae hepatis.

s. - stomata.

PLATE I

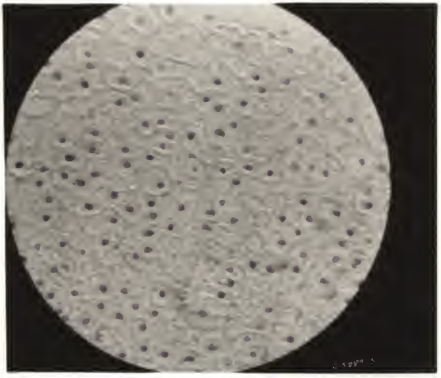


Figure 1

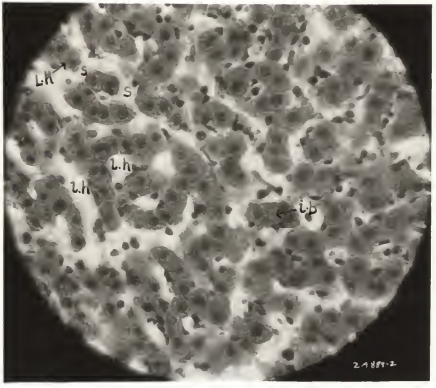


Figure 2

EXPLANATION OF PLATE II

Figure 1.

Section of pars intestinalis of ductus choledochus, hemotoxylin and eosin stain, X140.

g. - tubular glands of the duct.

gl. - glands of mucosa of intestine.

m. - muscularis of duct.

t.m. - intestinal tunica muscularis.

Figure 2.

Section of cystic duct, hematoxylin and eosin stain, X140.

d.l.t. - diffuse lymphoid tissue.

PLATE II

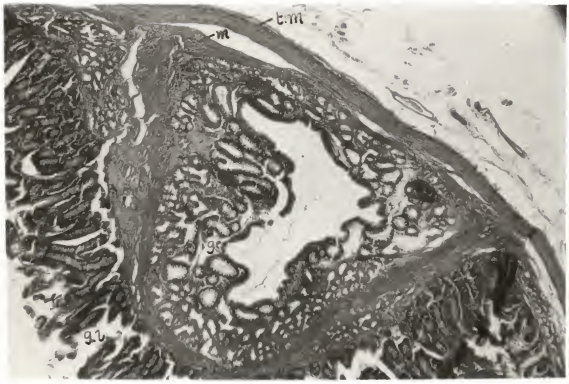


Figure 1

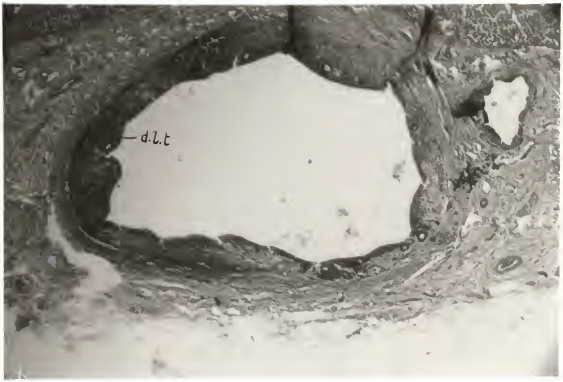


Figure 2

EXPLANATION OF PLATE III

Figure 1.

Section of gall bladder, hematoxylin and eosin, X280.

d.l.t. - diffuse lymphoid tissue.

Figure 2.

Section of liver, hematoxylin and eosin, X560.

c.v. - central vein.

l.h. - laminae hepatis.

L.h. - lacunae hepatis.

PLATE III

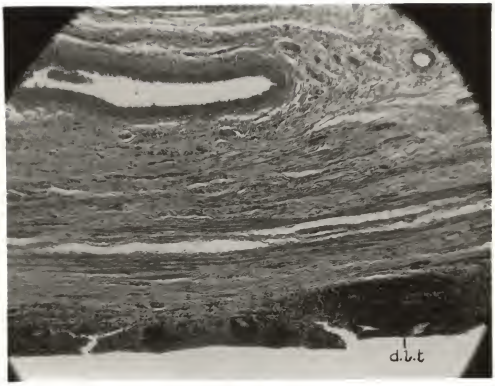


Figure 1

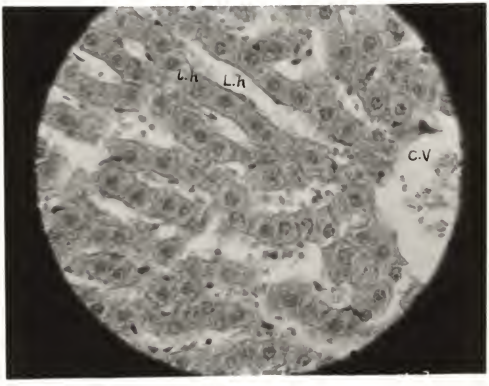


Figure 2

EXPLANATION OF PLATE IV

Section of liver through portal space, hematoxylin and eosin stain, X560.

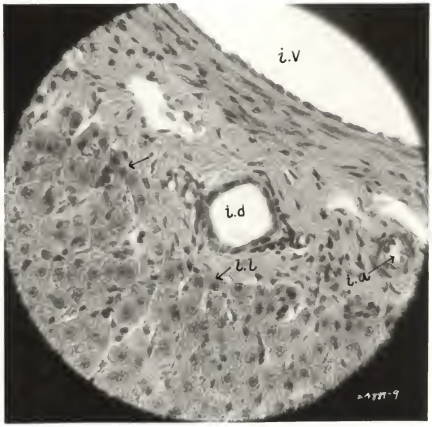
i.a. - interlobular artery.

i.d. - interlobular duct.

i.v. - interlobular vein.

l.l. - lamina limitans.

PLATE IV



EXPLANATION OF PLATE V

Stereograms

A Lamina hepatis and stomata.

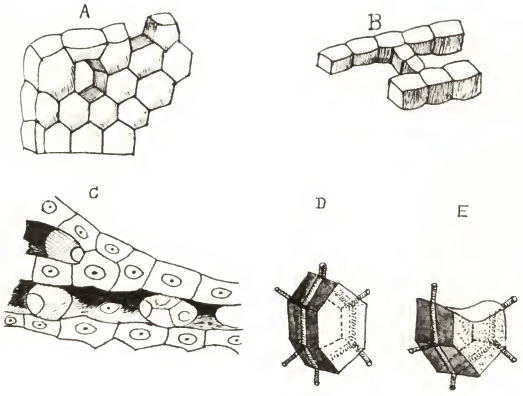
B Interlaminar bridge between laminae hepatis.

C Laminae hepatis, lacunae hepatis, and stomata.

D Meshwork of bile canaliculi surrounding an hepatic cell, contact surfaces shaded.

E Incomplete meshwork of bile canaliculi with portion of an hepatic cell at stomata, contact surfaces shaded.

PLATE V



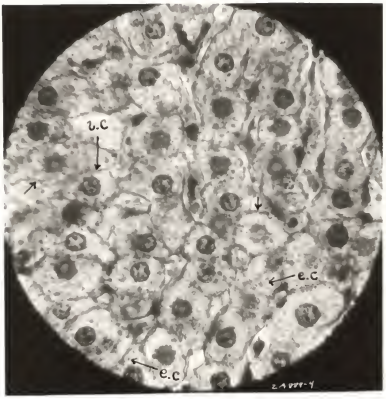
EXPLANATION OF PLATE VI

Section of liver, silver impregnation - Rio Hortega method, X970.

e.c. - extralaminar canaliculus.

i.c. - intralaminar canaliculi (arrows).

PLATE VI



EXPLANATION OF PLATE VII

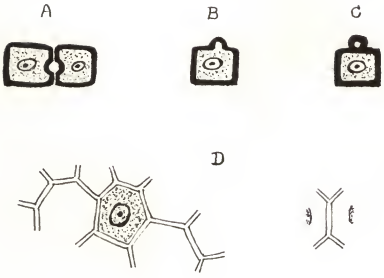
Drawings

A. Transection of an intercellular and intralaminar canaliculus, exoplasm shaded.

B. and C. Formation of extralaminar canaliculus, exoplasm shaded.

D. Meshwork of canaliculi isolated in crush preparation after maceration.

PLATE VII



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The object of the study was to examine and evaluate the parenchymal architecture of the bovine liver and its relation to the biliary system. The available literature did not contain a detailed study.

The livers of three calves and three adult bulls were utilized for the study. Some of the newer methods of investigation were employed to examine the structure in view of the latest cell-plate theory of Elias. The hepatic parenchyma consisted of laminae hepatis of one cell thickness, which radiated from the central vein to the periphery of the lobule. They were separated by lacunae hepatis which were tubular. At the periphery of the lobule the laminae and lacunae hepatis became continuous with those of the adjacent lobules to form a continuous sheet, lamina limitans around the portal space. The laminae were cribriform which permitted the lacunae to communicate with each other. The community of lacunae of a hepatic lobule formed labyrinthus hepatis which contained the plexus of hepatic sinusoids similar to the osseous labyrinth containing the membranous labyrinth of the internal ear. Due to the tubular nature of the lacunae hepatis the bovine liver was classified as hepar tubulare which has definite physiological significance related to metabolism but has no taxonomic value. The hepatic lobules had a diameter of 0.3 to 0.5 millimeters in the calf and 0.6 to 1.4 millimeters in the adult. The hepatic cells were 12 to 16 microns in diameter in the calf and 18 to 28 microns in the adult.

The bile canaliculi were intercellular, mostly intralaminar but occasionally extralaminar, forming a polygonal network, holding a hepatic cell in a mesh. The bile canaliculi in the adult were 0.75 microns in diameter and possessed a resistant membranous wall. There were no intracellular bile channels and secretory vacuoles.

Numerous goblet cells were observed in the epithelium of the ductus choledochus and also there were numerous mucous and serous tubular glands in the wall. The circular muscular layer of the ductus choledochus was thickened to form a sphincter in the pars intestinalis. A large amount of diffuse lymphoid tissue was noted in the wall of the cystic duct and gall bladder of the calf.