UNUSUAL STRUCTURE OF THE INTRAHEPATIC BILIARY SYSTEM OF THE GRASS CARP (Ctenopharyngodon idella) AND SILVER CARP (Hypophthalmichthys molitrix)

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In nearly all vertebrates so far studied the biliary capillaries are formed by membranes of two or three adjacent hepatocytes [2, 4]. In this zone the hepatocyte membrane is formed by microvilli and by intercellular junctions, tightly joined together, which bound the lumen of the biliary capillary. Only in the goldfish liver have biliary capillaries inside the liver cell been described [9].

In this investigation of the liver of the grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix) we also found an unusual structure of the biliary capillaries. We found no data on the structure of the liver of these fishes in the literature.

EXPERIMENTAL METHOD

Pieces of liver of adult male silver and grass carp, some of them hungry and the rest satiated, were taken for fixation. The material was fixed in a 2.5% solution of glutaraldehyde in phosphate or S-collidine buffer, pH 7.2-7.4, dehydrated in alcohols, and embedded in Epon. Ultrathin sections were stained by Reynolds' method and studied in the JEM-100C electron microscope. Semithin sections 2 μ thick were cut for histological investigation and stained with toluidine blue.

EXPERIMENTAL RESULTS

The liver of the grass carp is identical with that of the silver carp, so that its structure will be described without mention of the species.

A light-optical study of the liver showed that the hepatocytes are arranged in the form of a dense network, separated by a few sinusoids, lined with endothelial cells. Between the hepatocytes in the sinusoids bands of cells or single cells, much smaller than hepatocytes and rather elongated, could also be seen. Large vessels were infrequently seen in the sections and were distributed irregularly. The hepatocytes were polygonal in shape, with a large nucleus and nucleolus. Cells in the satiated fishes were tightly packed together, whereas in the hungry fishes considerable intercellular spaces formed between the hepatocytes.

Electron-microscopic investigation showed that the hepatocyte nucleus had the ordinary structure, but sometimes was goblet-shaped. Mitochondria of the hepatocytes were somewhat lengthened in shape, with matrix of average density and a few cristae. Each organelle was always surrounded by a single long, flat cistern of the rough endoplasmic reticulum (RER). Mitochondria were arranged around the nucleus, in the zone of the biliary capillary, and at the periphery of the cell, mainly in the zone of contact with the sinusoid. The Golgi complex, which differed in the ratio between its component elements in the hepatocytes, was located in the cytoplasm of the hepatocyte around the biliary capillary. The whole cytoplasm, while free from other organelles, was filled with numerous glycogen rosettes and large drops of fat. Inclusions of the third type were granules surrounded by a membrane and containing thin-fibrous material, alternating with granular material of high electron density.

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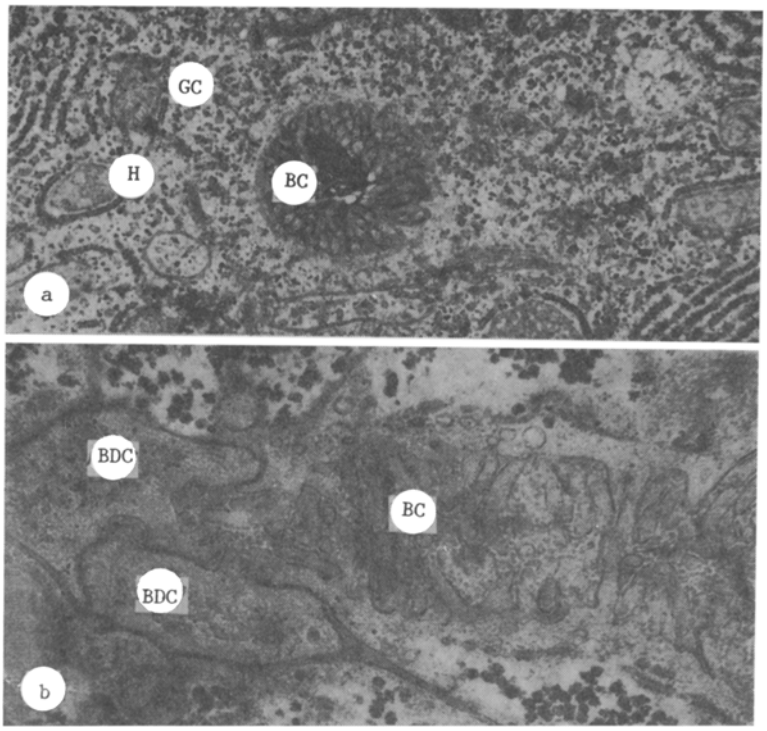

Fig. 1. Ultrastructure of biliary capillary in hepatocyte of silver carp: a) transverse section through biliary capillary containing elements of bile in its lumen (12,500×); b) longitudinal section through biliary capillary (20,750×). Here and in Figs. 2 and 3: H) hepatocyte, GC) Golgi complex, BC) biliary capillary.

The biliary capillary of the grass and silver carp is formed by one liver cell, due to the fact that the hepatocyte membrane forms a deep invagination which often reaches as far as the middle of the cell, and it is covered over the whole of its extent by microvilli. As a result, depending on how the section passed through the cell, the biliary capillary can be seen to be either lying in the cytoplasm of the hepatocyte and not in contact with the cell membrane (Fig. 1a) or running from the middle of the cell to the surface of the cell membrane (Fig. 1b). This capillary is in contact, not with a hepatocyte, as in nearly all animals studied, but with one or 2-3 cells of the bile duct epithelium, which join together to form small tubules, resembling the cholangioles described in mammals [5]. However, they differ from typical cholangioles in the fact that they are not surrounded by a basement membrane. They are located in spaces between the hepatocytes. Sometimes the same cell forms a primary drainage channel for bile from two hepatocytes, and in that case it makes contact with them with different parts of its membrane. If, in a section, 2-3 cells of a bile duct, which have already formed a small space between them, are in contact with a biliary capillary, this means that bile can flow along the course of the preformed primary bile duct (Figs. 2 and 3).

The study of transverse sections cut through small bile ducts showed that the whole drainage system is a complex network of small branching and interconnected tubules, each of which receives bile from several hepatocytes, but at different levels and on different parts of their surface. Passing between the hepatocytes, they then run along the course of the sinusoids, and only as they approach and enter the portal zone do they become converted into bile ducts, consisting of 12 or 13 cells.

Incidentally, the basement membrane around these bile ducts does not appear until these become surrounded by connective-tissue cells. An irregularly shaped nucleus with heterochromatin and a small nucleolus located juxtamurally are found in the cytoplasm of the cells of the small bile ducts. The nucleo-cytoplasmic ratios are high. Free ribosomes, solitary small mitochondria and very short cisterns of the RER, small vesicles, single glycogen granules, and tonofilaments are distributed in the cytoplasm around the lumen of the bile duct. On the apical surface of these cells the cell membrane forms a few microvilli, and on the lateral surfaces desmosomes are located by the lumen of the duct. Cells of the large bile ducts in the portal zone are trapezoidal in shape. In the basal part the membrane of these cells forms a festooned border. On the lateral surfaces the cell membranes form digitiform outgrowths in order to join them together, but between these interlocking structures quite wide intercellular spaces can be seen. On the apical surface of these cells microvilli are distributed, and closing laminae and desmosomes are formed on the boundary between the cells in the apical region. A lobate nucleus with deep invaginations lies in the cytoplasm

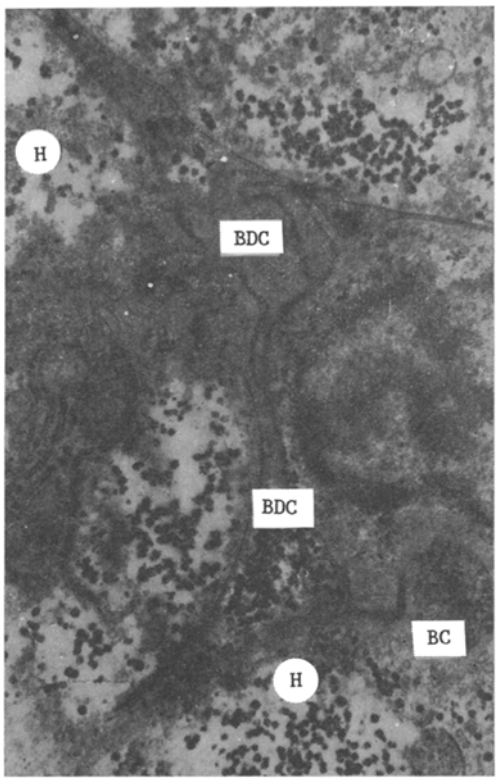

Fig. 2. Bile duct cells in contact with biliary capillaries of two hepatocytes (25,000×). Here and in Fig. 1 and 3: BDC) bile duct cells.

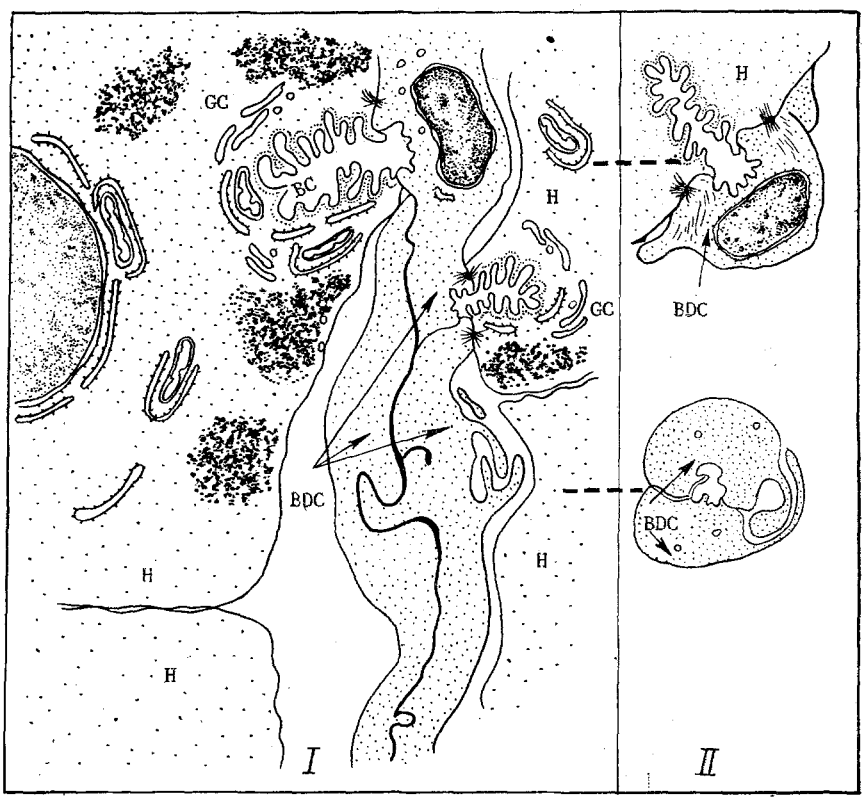

Fig. 3. Longitudinal (I) and transverse (II) sections through cells of small bile ducts.

of the cells, with heterochromatin arranged juxtamurally. The cytoplasm contains free ribosomes, a few cisterns of the RER, and small mitochondria. Closer to the apical surface there are many vesicles and vacuoles of various sizes, some of them probably elements of the Golgi complex. Cells of the bile duct lie on the basement membrane, beyond which are found connective-tissue cells and blood vessels arranged loosely in layers. The ultrastructure of the bile duct cells described above suggests that reabsorption of fluid from the bile takes place in them, followed by its transport through the cell and intercellular spaces into the loose connective tissue and adjacent vessels.

Sinusoids were seen infrequently. The surface of the hepatocytes, usually smooth, formed microvilli in the zone of contact with the sinusoid, but likewise not always. Sometimes the membrane was completely folded and vesicles could be seen in the hepatocyte cytoplasm, evidence of pinocytosis. A loose, interrupted fibrous basal layer was present in the Disse's spaces. The sinusoids were lined with a very thin layer of endothelial cells. In cases when the section went through the nucleus of an endothelial cell it could be seen to have an irregular contour with projections and invaginations, and with juxtamural heterochromatin. Single mitochondria, cisterns of the RER, free ribosomes, and granules of phagosome types were located in the scanty cytoplasm. Spaces were present between cells of the endothelium, evidence that the endothelial cells do not form a continuous layer.

Summarizing the description of the biliary system in the liver of the grass and silver carp, it can be concluded that it is similar to that found in the goldfish liver [9]: biliary capillaries are formed by the membrane of one liver cell, and the whole biliary system consists of special cells of the bile duct, differing sharply in their morphology from hepatocytes.

It is interesting to compare the structure of the biliary tree of mammals and the biliary system of fishes. The first stage of bile drainage in the liver of the grass and silver carp (the liver cell and bile duct cell) is similar in cellular composition to Hering's canal in the mammalian liver [1, 8]. The difference lies in the number of hepatocytes forming Hering's canal (usually several hepatocytes and several bile duct epithelial cells are present there), and also in the fact that cells of the bile duct epithelium, which are components of Hering's canal, are always located on the basement membrane, and the canal itself is always at the periphery of the hepatic lobule. The next stage of bile drainage in the liver of these two species of fishes consisted of a very branched network of tubules, consisting of two, three, or five epithelial cells of the bile ducts, and resembling cholangioles in the mammalian liver. They gradually join together to form a larger bile duct in the portal zone, formed of 12 or 13 cells. However, cholangioles in the mammalian liver are located at the periphery of the hepatic lobule, and not throughout its thickness, as in the fishes described above, and they always lie on a basement membrane, which in the silver and grass carp is characteristic only of the large bile ducts, surrounded by connective tissue in the portal tract.

Thus all elements of the biliary tree are present in the liver of the silver carp, but unlike the mammalian liver, they are located in the substance of the parenchyma of the hepatic lobule and not brought out to its periphery. This evidently also explains the absence of a basement membrane around the small bile ducts, which appears around them as soon as they begin to make contact with connective-tissue cells. This type of structure of the biliary tree, in our opinion, is indirect evidence that epithelial cells of the bile ducts do not originate from hepatocytes, as some workers believe [3, 6, 7, 10], but are independent in their origin.

The structure of the biliary system described above is apparently more complex than that observed in the majority of vertebrates, but its existence in the liver of these two species of carp is justified on the grounds that, when the fish is hungry and the cells are sharply reduced in size and, consequently, the intercellular spaces between them are enlarged, the structure of the biliary passages is not impaired, because it is autonomous.

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MORPHOLOGICAL CHANGES IN THE KIDNEYS IN ACUTE RENAL FAILURE INDUCED BY EXPERIMENTAL PANCREATITIS

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Clinical data indicate a high frequency (from 37 to 60%) of kidney involvement associated with acute pancreatitis [1, 3]. However, there is no unanimity regarding the explanation of the morphological nature of the lesion. Most investigators have relied on autopsy data, which do not rule out the possibility of obscuring of the morphological picture by postmortem changes. Moreover, the sequence of the morphological changes in the kidneys in acute pancreatitis has not been studied.

To determine the precise character of the kidney damage in acute pancreatitis, the writers have studied the time course of the morphological changes as revealed by punch biopsy of the kidneys.

EXPERIMENTAL METHOD

Experiments were carried out on 42 dogs with acute hemorrhagic pancreatitis. An enzymic-hypertensive model of pancreatitis, induced by injection of duodenal contents under pressure into the system of pancreatic ducts, followed by ligation of the ducts, was used [2]. Open renal biopsy was performed with a needle (I-118, technical specification 64-1-2702-73) from the All-Union Research Institute of Instruments every hour for 8 h. The kidneys removed immediately after sacrifice or death of the animal were subjected to histological investigation. The following staining methods were used: hematoxylin and eosin, Van Giesons' PAS reaction, Weigert's, and Mallory's method for fibrin. An electron-microscopic investigation was also undertaken.

EXPERIMENTAL RESULTS

Light microscopy of biopsy material taken 60 min after the beginning of the disease revealed moderate congestion of the glomerular vessels. Electron microscopy revealed thickening and coarsening of the material of the basement membrane of the glomeruli, increased density of the material of the podocytes, the appearance of a fibrous structure in them, and proliferation of mesangial cells (Fig. 1).

By the 2nd hour of the experiment the congestion appeared more severe under the light microscope. Electron microscopy revealed a fibrous structure of the basement membrane and a picture of lysis of the podocyte nuclei; a fibrous structure also was observed in the feet of the podocytes. The picture described above, 3 h after the beginning of the experiment, was supplemented by stasis in the capillaries, and solitary thrombi in the glomerular capillaries. Filling of the glomeruli with exudate and proliferation of mesangial cells without any significant increase in size of the mesangial matrix were observed on the electron micrograph. After 4 h, besides the changes mentioned above, a mild degree of focal segmental glomerulitis was observed under the light microscope in single glomeruli: segmental focal mesenangial proliferation without enlargement of the mesangial matrix (Fig. 2). By the end of the 4th hour isolated regions of splitting of the basement membrane of the glomerulus could be seen on the electron micrograph.

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