Structural and histochemical observations on the hepatic system of Siphonops annulatus (Amphibia - Gymnophiona)

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Running title - Structure of Gymnophiona liver.

Abstract

The hepatic system of eleven specimens of routinely fed Siphonops annulatus was studied using light, and electron microscopy, and histochemistry. Its general structure agrees with previously known information from amphibia. Its hepatocytes, however, present an unexpected scarcity of rough endoplasmic reticulum, few lysosomes and fat droplets, an abundance of glycogen beta particles and an exceptional amount of smooth endoplasmic reticulum. The macrophages, visible in its sinusoid capillaries (Kupffer cells), present morphological and histochemical characteristics that confirm their participation in the phagocytosis and digestion of aged erythrocytes. The abundance of leucocytes and occasional mitotic figures in the sinusoidal lumen suggest a possible intrahepatic hematopoietic activity. Ito's (fat storing) cells and collagen reticular fibers were observed in Disse's space.

Key words - Liver, Gymnophiona, structure, histochemistry.

Introduction

Siphonops annulatus is a Gymnophiona adapted to terrestrial and borrowing life like an earthworm. Found in humid and semiarid areas this species produces eggs with a leathery covering. Its metamorphosis occurs before hatching thus representing a group of Gymnophiona well adapted to terrestrial life. S. annulatus is therefore a suitable animal to study the process of adaptation of an amphibian to an exclusively terrestrial habitat. More informations on the biology and its general anatomy and structure of the initial digestive tract and urinary system of this animal were published (7,2). Because of their limited distribution and the environment they live in, the Gymnophiona are one of the least known groups of terrestrial vertebrates (3). Although the microscopic structure of several organs of Gymnophiona have been studied, the literature regarding their liver is scarce. A detailed analysis of the structure of this organ was to our knowledge only presented for the liver of Ichthyophis kohtaoensis (10). In this paper we present the results obtained by studying this organ with light and electron microscopy, and histochemistry.

Material and Methods

Eleven specimens of routinely fed adult Siphonops annulatus Mikan, 1820, of both sexes, classified according to Taylor (9), were used. They weighed between 23 and 48 g. Their organs were fixed by intraperitoneal injection of 4% paraformaldehyde or 2% glutaraldehyde dissolved in 0.1M phosphate buffered saline respectively for light and electron microscopy. Posfixation in 1% osmic acid and uranyl acetate was used for electron microscopy. Embedding was performed in Leica Historesin and Araldite respectively for light and electron microscopy. One, two and three micrometer thick sections were stained with pararosanilin 0.1% and toluidin blue 0.2% in 1% sodium borate. Thin sections of this material embedded in hydrophilic resin produce figures of higher resolution as compared to paraffin embedded material thus permitting a better study of cells and tissues (5). The distribution of glycogen was studied using the periodic acid schiff (PAS) reaction according to (1). Perl's classical method for the study of iron present in hemosiderin was performed according to the instructions present in (4). Thin sections for electron microscopy were contrasted with uranyl acetate and lead citrate. Further information regarding the use of this material was presented in (7,2).

Results

The liver of S. annulatus presents the following main components: 1. A thick hematopoietic capsule. 2. Hepatocytes. 3. Sinusoid capillaries and their content. 4. Bile ducts and gall bladder. 5. Aggregates of macrophages containing melanin. Items 2 to 4 will be studied in this paper while items 1 and 5 will be dealt with on some other occasion.

Hepatocytes - They present a piramidal form and are aggregated in chords limiting an internal lumen, the bile canaliculi. Their nuclei are large, presenting conspicuous nucleoli surrounded by nucleolar associated chromatin. Their heterochromatin is clearly visible and forms a thin layer beneath the nuclear envelope (Figs.1 and 6). Their cytoplasm contains two main components: the smooth endoplasmic reticulum with its characteristic aspect of a membraneous anostomosing network (Figs.6, 7 and 8) occupying most of the space, and round beta glycogen particles with an average diameter of 15 - 30 nm. No aggregates of these particles (alfa form), present in the mammalian liver, were found in this material. When fixed with paraformaldehyde, the glycogen particles are pushed to one side of the cell forming empty regions in the cytoplasm, which are clearly visible in the preparations stained for mitochondria and glycogen (Figs.1 and 3). When fixed in glutaraldehyde, these particles appear associated with the smooth endoplasmic reticulum and are uniformly distributed in the cell (Fig.8). The amount of glycogen, however, is variable and conspicuous differences can be observed in neighboring cells (Figs.3 and 7) and between different specimens. This high diversity of glycogen content might be related to the feeding cycle for these animals, which are fed at intervals of several days. Round and elongated mitochondria are frequent and present a dense matrix with scarce cristae (Figs.8 and 9). Scarce cisternae of rough endoplasmic reticulum are present, contrasting with what is observed in mammalian material where this organelle is abundant. Typical lysosomes and lipid droplets are present in most hepatocytes, but in a small amount (Figs.2 and 6). Golgi complexes are present, but not conspicuous (Fig.9). Hepatocytes are bound to one another by relatively small desmosomes, present in their lateral membranes. The bile canaliculi present the usual structure observed in vertebrates, and have abundant microvilli in their lumen, and also show conspicuous, tight junctions, adhesion belts and desmosomes (Fig. 9).

The sinusoid capillaries - These structures are limited by a discontinuous layer of thin fenestrated endothelial cells, permitting, therefore, an intimate contact of the blood with the hepatocytes. A very thin and discontinuous basal lamina can be observed in the space between the endothelium and the hepatocytes (Disse's space). This space presents abundant hepatocyte microvilli and contains thin collagen fibrils that can aggregate in bundles forming the reticular fibers, clearly present in our material and initially described in the liver of mammals. These fibrils present an average diameter of 48.3 nm and are thinner than the 70.2 nm diameter present in the fibrils of this animal's skin collagen fibers. Occasionally small cells with scarcely branched cytoplasm containing lipid droplets, probably Ito's (or fat storing) cells, are observed in this space. The lumen of the sinusoids contains mainly erythrocytes, leucocytes and macrophages. Frequently accumulations of leucocytes are observed in this region (Fig. 4), suggesting that they might be collections of the same cells. The fact that occasionally mitoses are locally present suggest a possible intrahepatic hematopoietic activity. Frequently large cells with a variable amount of cytoplasmatic particles of diverse aspect are present, resting on the luminal surface of the sinusoid endothelium. These cells have the characteristic of cells of the mononuclear phagocyte system and are known as Kupffer cells. (Fig. 2). The amount of Kupffer cells is variable from specimen to specimen. In some of them they are scarce and present few intracytoplasmic granules, the opposite occurs in other specimens. The liver and spleen of the animals with few of these cells do not present the aggregates of pigment containing cells, (the subject of a following paper). The study of Kupffer cells shows aspects such as cell to cell adhesion to erythrocytes and very intense Perl's reaction in large intracytoplasmic granules (Fig. 10) strongly suggesting erythrocyte phagocytosis and digestion.

The ductal system and gall bladder - This system begins in the bile canaliculi. These are followed by the bile ductules constituted by small cuboidal cells with clear cytoplasm (Fig.11). Ductules abut on the bile ducts which are also formed by cubic cells surrounded by scarce connective tissue (Fig. 11). These structures, gradually increasing in size, are surrounded by an increasing amount of connective tissue and present a lining of prismatic epithelial cells (Fig.12). They converge and end in the gall bladder. The common bile duct that binds the gall bladder to the intestine presents a thin, prismatic, mucous secreting epithelium that resembles the lining of the stomach. Occasionally typical mucous secreting goblet cells are observed together with this epithelium (Fig.5). The presence of goblet cells in a continuous mucous epithelium is a rare situation in vertebrate epithelia. Under the lamina propria, a thin muscularis mucosae is visible and a thick layer of smooth muscle cells is present in an inner circular and outer longitudinal disposition. The gall bladder presents a very thin wall with a simple prismatic epithelial cell layer and loose connective tissue interspersed with thin, smooth muscle cells. (Fig.13).

Discussion

Our results are in agreement with most of the observations reported in I. kohtaoensis (10). Thus we obtained similar findings regarding the structure of the liver ultrastruc-

ture of the hepatocytes, sinusoid capillaries and Kupffer cells. We present a more detailed study of the glycogen particles, desmosome distribution, and present evidence that the cells they described as fibroblasts in Disse's space are probably Ito's (fat storing) cells. The conspicuous basophilic bodies (ergastoplasm) present in the cytoplasm of mammalian hepatocytes are not present in our material. This coincides with the scarcity of cysternae of the rough endoplasmic reticulum. This situation called our attention this cellular component characteristically abundant in mammalian hepatocytes, known to be the site of synthesis of most of their plasma proteins (8). The exceptionally great quantity of smooth endoplasmic reticulum and glycogen also differ from what is observed in mammalian hepatocytes. These observations suggest that these cells have preferential metabolic pathways with different characteristics from what is conventionally known from studies in mammals. The fact that the fibrils of the reticular fibers of the liver of S. annulatus present a smaller diameter than the fibrils of its skin collagen fibers confirms, in this species, the results we obtained comparing these fibrils in fishes, amphibia, reptilia and mammalia. It is known that reticular fibers are constituted mainly by type III collagen, while collagen fibers contain type I collagen (6). To our knowledge this is the first time that results regarding the distribution of collagen types in Gymnophiona are presented. The occurance of erythrocyte phagocytosis and a positive reaction with Perl's method for hemosiderin iron proves that also in S. annulatus Kupffer cells are involved in the removal and digestion of aged erythrocytes. In S. annulatus the morphological aspects of this process appear more clear-cut, due to the large size of the cells in this species.

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All light micrographs when not specified are stained by pararosanilin followed by toluidin blue.

Fig.1. Section showing the disposition of the hepatocytes limiting a biliary canaliculus. Observe mitochondria stained with Heidenheins hematoxylin. X 950

Fig 2. On top, the border of an aggregate of cells containing pigment. Four Kupffer cells in the sinusoid characterized by red stained granules (lysosomes) of diverse size and quantity. Also in the sinusoid an erythrocyte, lynphocytes and neutrophiles. The pink granules in the hepatocytes are mitochondriae while the scarce dark ones are lysosomes. X 380

Fig.3. Liver parenchyma showing a diversity of glycogen content and its displacement to a cellular pole, an artefact produced by paraformaldehyde fixation. A blood vessel in the center and an aggregate of leucocytes at left. PAS and toluidin stain. X 50

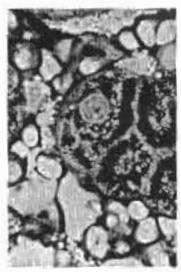

Fig.4. Showing a region with many leucocytes and erythrocytes and Kupffer cells in a sinusoid. X 380

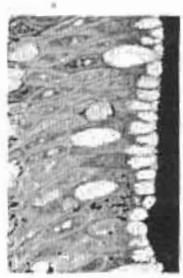

Fig.5. Section of the common bile duct with its lining of mucous secreting cells associated with goblet cells. PAS and toluidin stain. X 50

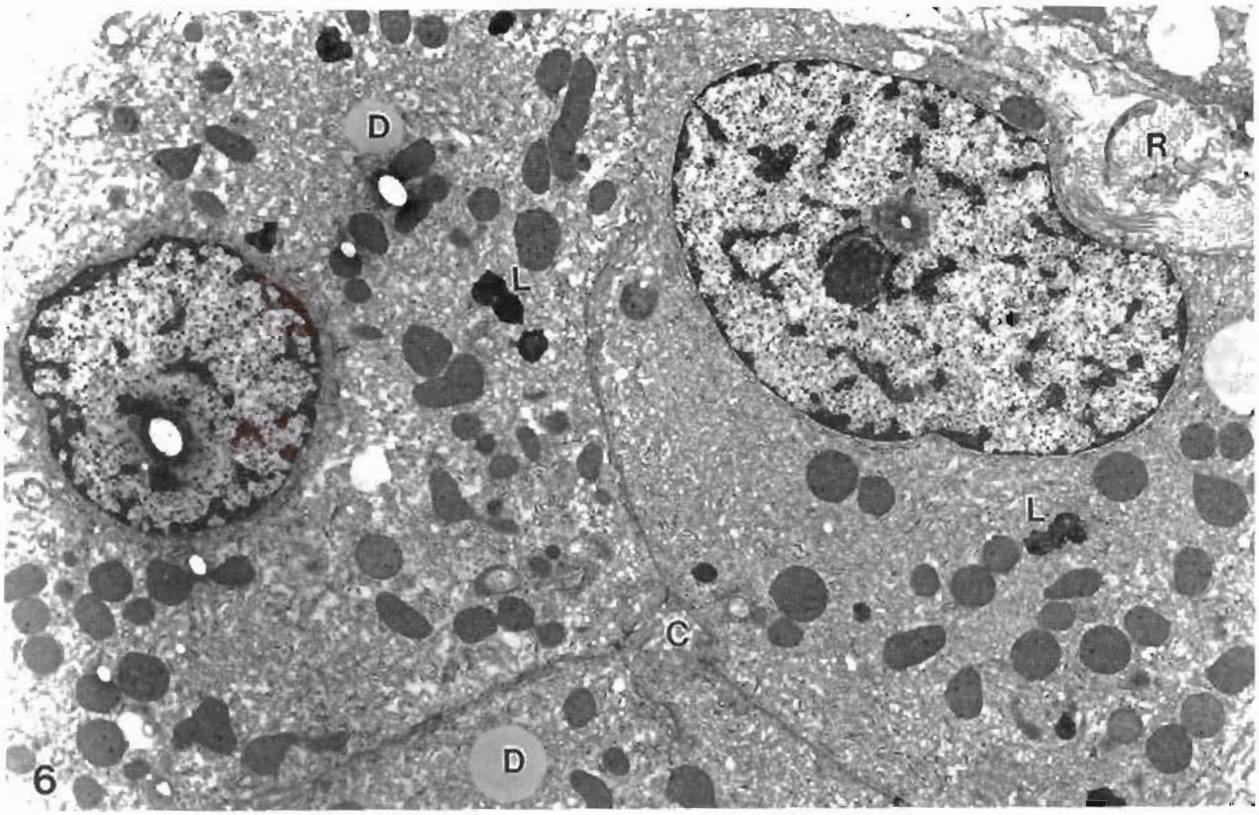

Fig.6. Electron micrograph of hepatocytes limiting a canaliculus (C). The grey structures are mitochondria. Observe few lysosomes (L) and lipid droplets (D). In the upper left corner a reticular collagen fiber (R). Smooth endoplasmic reticulum (S) occupies most of the area. X 7.200

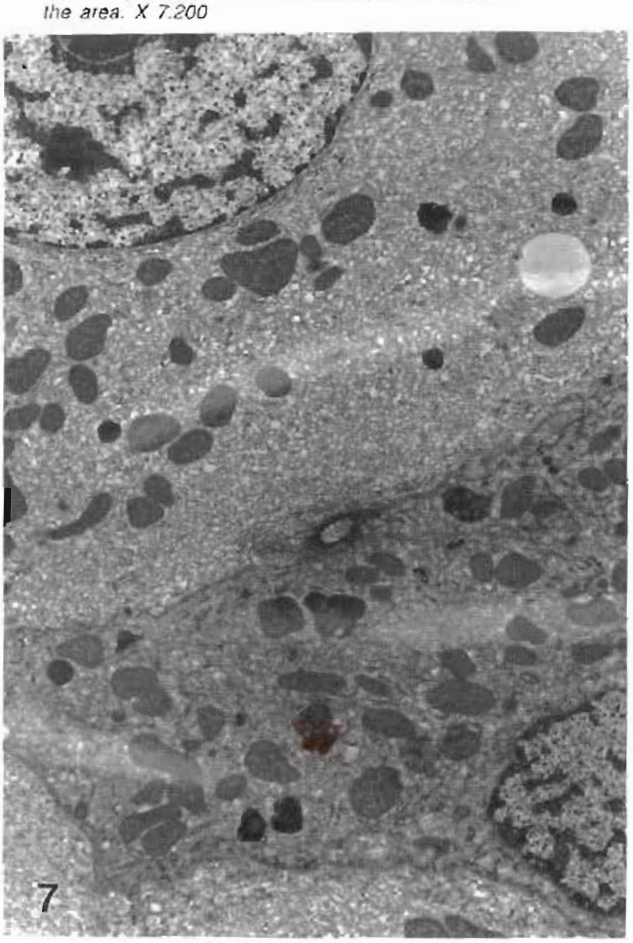

Fig.7. Hepatocytes with scant amount of glycogen (above, light) and much glycogen (below dark), illustrating the diversity of glycogen content in these cells. X 6.900

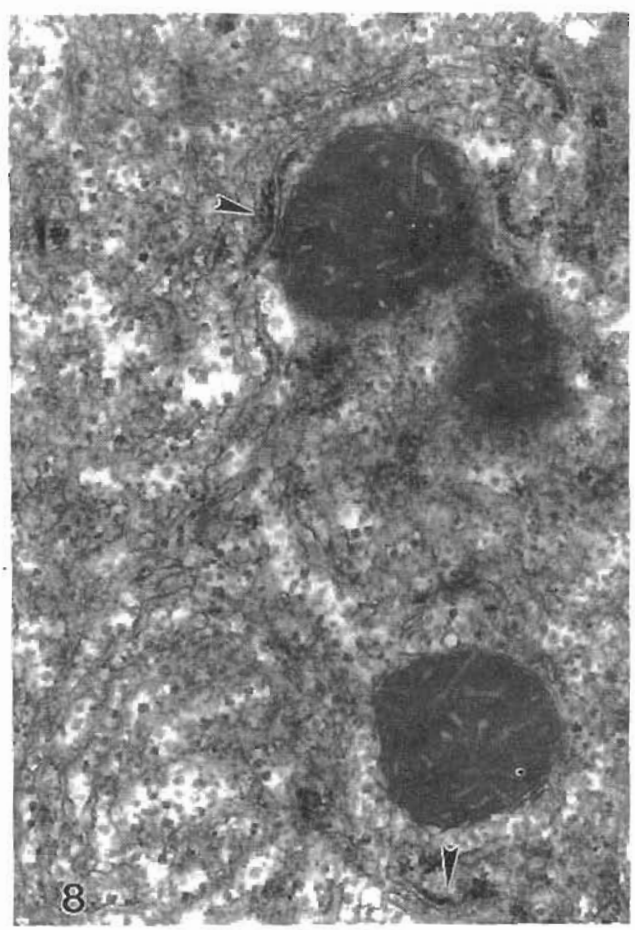

Fig 8. Showing an area of smooth endoplasmic reticulum and the small amount of rough endoplasmic reticulum (arrowheads). Scarcity of cristae in the hepatocytes mitochondriae. The dark granules are beta particles of glycogen. X 41.700

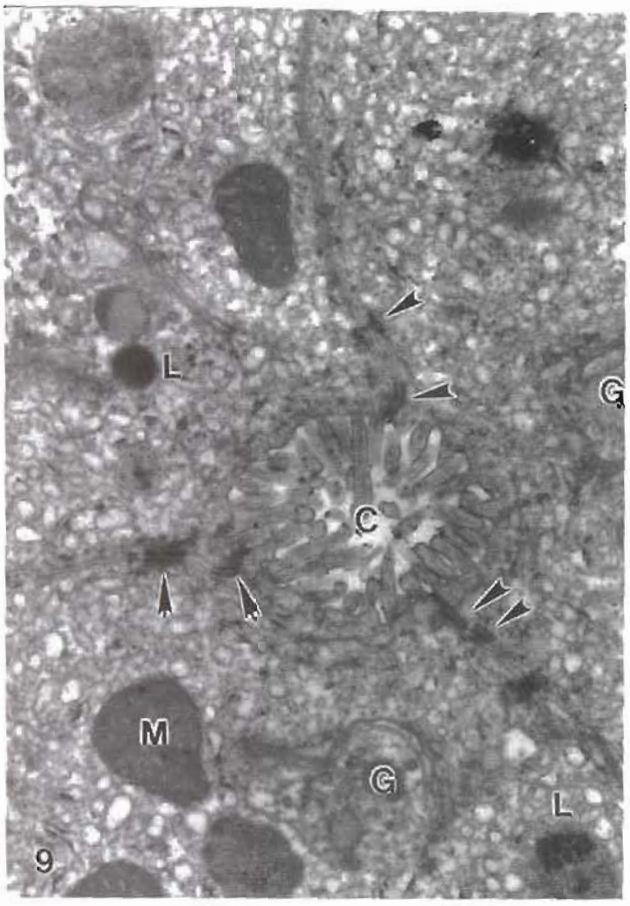

Fig.9. Micrograph showing a biliary canaliculus (C) with the cell junctions involved in this structure (arrowheads). Discreet Golgi regions (G), mitochondriae (M) and lysosomes (L) are present. X 37.300

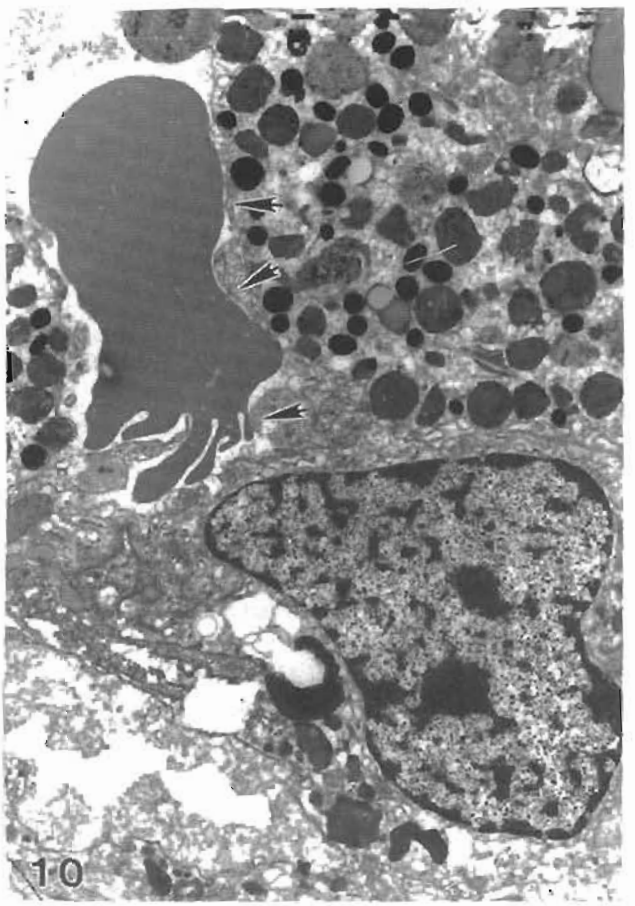

Fig.10. Showing two macrophages (M) making close contact (arrowheads) with a deformed erythrocyte (E). X 6.570

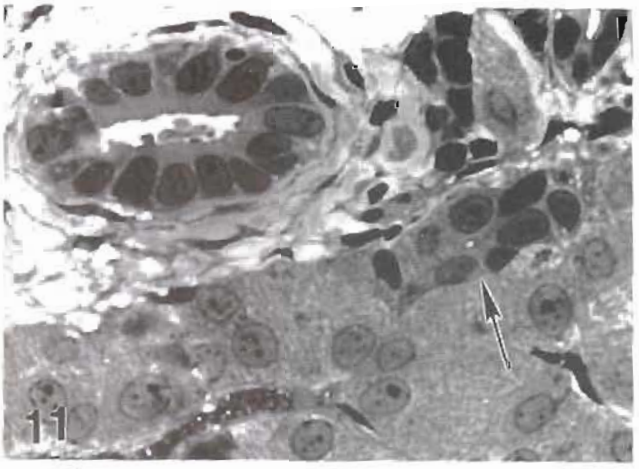

Fig.11. Photomicrograph with a small biliary duct (upper left) surrounded by connective tissue. On the right (arrow) a ductule formed by cubic epithelial cells. X 420

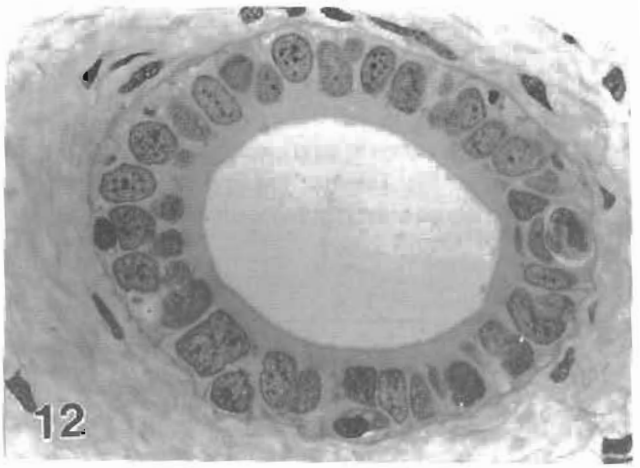

Fig.12. A larger biliary duct with prismatic epithelium infiltrated by a lymphocyte (left), a plasmocyte (below) and a neutrophil (right). X 500

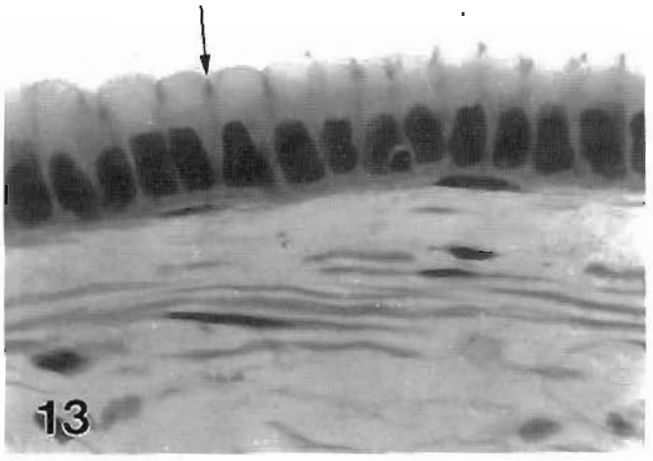

Fig.13 Section of gall bladder. Simple prismatic epithelium with cell junctions visible in their apex (arrow) covering a thin layer of smooth muscle cells and loose connective tissue. X 700

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