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# Ultra-structural organization of the gallbladder mucous membrane of Anglo-Nubian goat

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## Abstract

Currently, studies devoted to establish the anatomical and histological patterns of the internal organs organization in animals depending on their species and breed, as well as conditions of detention are the most relevant. The liver morphology in representatives of the ruminant family has not been sufficiently studied. Questions regarding the micro- and ultra-structural organizations of the gallbladder wall remain open. The aim of the study was to establish the ultra-structural organization features of the gallbladder mucous membrane of an Anglo-Nubian goat. The material for the study was the gallbladder wall's fragments of an adult Anglo-Nubian goat. Further processing of the obtained samples was carried out to acquire histological preparations. Ultra-thin sections were photographed in a Jem-1011 electron microscope at magnifications of 2,500 - 3,000. It was found that the gallbladder mucous membrane of an Anglo-Nubian goat is formed by the epithelial layer and its own lamina. The epithelial layer is represented by a single-layer prismatic epithelium. The nuclei are light, with clear contours. The apical surface of epithelial cells forms microvilli and the cytoplasm of the apical pole of cells contains many electron-dense secretory granules. The lateral surfaces of the cells in their apical part are interconnected by tight contacts. The lamina propria is formed by loose connective tissue containing many blood vessels and nerve fibers. Referring to the scientific literature describing possible pathologies of the gallbladder, we can conclude that the picture presented in the results generally corresponds to the position of the gallbladder without pathologies.



## Introduction

Practicing veterinarians in their daily work quite often encounter diseases of the biliary system.[1-3] As a rule, they are caused by gallbladder pathologies.[4,5] At the same time, cholelithiasis is most often recorded; the mechanism of which is not fully understood, as well as the role of the gallbladder in the digestion process.[6,7] In veterinary medicine, the pathophysiology of bladder disorders in biliary pathologies has been practically not studied.[8,9] It has also been noted in the scientific literature that some animals including zebrafish, lamprey and Rhesus monkey with biliary pathologies may have normal gallbladder contractility.[10,11] In this regard, to understand the pathogenesis of cholecystitis and to establish the functions of the gallbladder in the digestion process, comprehensive knowledge of its structural organization is required.[12,13] Taking into account the above, we set a goal to establish the ultra-structural organization features of the gallbladder mucous membrane of the Anglo-Nubian goat. The choice of this animal species was due to the fact that the liver and biliary tract pathologies are extremely common, posing an acute problem in goat breeding, since the liver is susceptible to a large number of parasitic diseases being dangerous to humans and animals. These include echinococcosis, fasciolosis, opisthorchiasis, alveococcosis and dicraceliosis. The liver lies on the migration route of the larval stages of nematodes such as roundworms and some Strongylides.[1]

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## Materials and Methods

The material for the study was the gallbladder of an adult goat (male, 1 year old) of the Anglo-Nubian breed. The bladder wall was selected during the planned slaughter of goats according to the technological cycle of the farm. Fragments of its wall, not more than 2.00 $mm^3$ in size, were selected for electron microscopic examination. The selected samples were fixed in a solution of 2.00% glutaraldehyde (LenReactiv, Saint Petersburg, Russia) in cacodylate buffer (pH: 7.20 - 7.40; LenReactiv) for 2 hr. Then, they were washed in three portions of the same buffer and post-fixed in 1.00% osmium tetroxide solution (being prepared in cacodylate buffer with pH of 7.20 - 7.40) for 1 hr.[10] After that, the samples were dehydrated in ascending concentrations of alcohols and absolute acetone. Subsequent embedding of selected tissue fragments was carried out in Epon-812 according to the generally accepted procedure.[14] Ultra-thin sections were obtained by an ultra-microtome (LKB-III; LKB-Produkter AB, Stockholm, Sweden), being contrasted with 2.00% aqueous solution of uranyl acetate and lead citrate solution.[15] The resulting ultra-thin sections were photographed using an electron microscope (Jem-1011; JEOL Ltd., Tokyo, Japan) at 2,500 - 3,000 magnifications. Terminology was used in accordance with the International Histological Nomenclature.[16] Calculations were made in the open source program for image analysis and processing by ImageJ Software (National Institutes of Health, Bethesda, USA). Using ImageJ, we calculated the areas and statistics parameters of the pixel values of the image regions. This makes it possible to determine the histological structures, as well as compare them with each other. The work was carried out on the basis of the Saint Petersburg State University of Veterinary Medicine, Saint Petersburg, Russia. This study was carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, the rules of Good Laboratory and Clinical Practice, as well as the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union dated 22 September 2010 for the Protection of Animals Used for Scientific Purposes. The study design was approved by the Bioethics Committee of the Saint Petersburg State University of Veterinary Medicine, Saint Petersburg, Russia (Permission Number: 1, dated February 12, 2023).

## Results

Electron microscopic studies of the Anglo-Nubian goat's gallbladder wall showed layers of mucous, muscular and serous membranes. The mucous layer consists of epithelium and lamina propria. The serous membrane covering the gallbladder from the outside is formed from loose connective tissue covered with mesothelium.

The epithelial layer of the mucosa is represented by a single-layer prismatic epithelium. The highly prismatic epithelial cells that form it reach a height of 30.00 - 50.00 μm, and their basal part lies on a dense basement membrane bordering the lamina propria (Fig. 1).

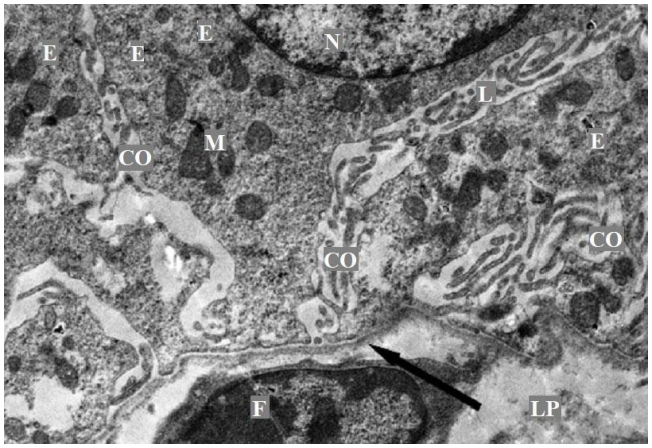


**Fig. 1.** Ultra-structure of the epithelial cells basal pole of the gallbladder wall. E: Prismatic epithelium; LP: Lamina propria; L: Lysosomes; N: Epitheliocyte nucleus; M: Mitochondria; CO: Cytoplasmic outgrowths; F: Fibroblast. Arrow indicates basement membrane (magnification = 3,000×).

The basal part of the epitheliocytes contains one large oval or elongated nucleus with clear contours. Sometimes one dense small nucleolus is visible in the composition of the nucleus. A small amount of condensed hetero-chromatin is distributed along the inner karyolemma, and finely dispersed euchromatin is determined throughout the nucleus. The cytoplasm of the basal part of the cell contains a large number of small oval mitochondria. Among them, there are mitochondria with a dark dense mitochondrial matrix and lighter mitochondria with distinct thin cristae. Mitochondria mostly lie around the nuclei, although they are also partially detected in the apical part of the cell. Also, in the cytoplasm of the epithelial cells of the gallbladder wall, a small Golgi complex, short cisterns of the granular endoplasmic reticulum, ribosomes and polyribosomes, rare lysosomes and numerous small vesicles are determined.

The free apical surface of the prismatic epitheliocytes forms many relatively short microvilli directed into the gallbladder cavity (Fig. 2).

The lateral surfaces of the epitheliocytes apical parts are connected to each other by dense contacts with the presence of dark dense bands, desmosomes, approximately over a distance of 2.50 - 4.00 μm. Further deep into the epithelial layer, the lateral surfaces of the epitheliocytes form numerous finger-like processes and cytoplasmic outgrowths. With the help of these structures, inter-cellular spaces can expand.

In the cytoplasm of the epithelial cells apical pole, a large number of secretory granules and vesicles of various sizes containing electron-dense finely dispersed material is determined. Their presence makes the cytoplasm optically dark. The secretion accumulated in them is excreted into the gallbladder lumen, forming a thin layer of glycocalyx on the surface of epithelial cells. The latter protects the gallbladder wall epithelium from the effects of bile salts. Besides secretory granules at the apical pole, numerous pinocytic vesicles, vacuoles, single small dark mitochondria, single lysosomes and ribosomes are found in the cytoplasm of epithelial cells.

In places, among the prismatic epithelial cells on the basement membrane, there are single lighter and smaller basal cells. They contain a large rounded nucleus, well-developed organelles including small rounded mitochondria, short cisterns of the granular endoplasmic reticulum and a small number of dense vesicles around the nucleus.

Loose connective tissue forms its own plate of the mucous membrane of the gallbladder wall. It consists of scattered thin collagen and elastic fibers, as well as elongated fibroblasts, being immersed in an amorphous base substance (Fig. 3).

These cells contain elongated nuclei, in the karyoplasm of which there is a large amount of heterochromatin. Elongated channels of the granular endoplasmic reticulum are found around the nuclei in the cytoplasm (Fig. 3).

In addition to fibroblasts, phagocytic macrophages with phagolysosomes and solitary mast cells with dark granules in the cytoplasm are sometimes detected in the lamina propria (Fig. 4).

Along with abundant blood supply, one can also note the presence of good innervation of the lamina propria due to non-myelinated nerve fibers. Their profiles are often found on sections close to blood vessels (Fig. 5).

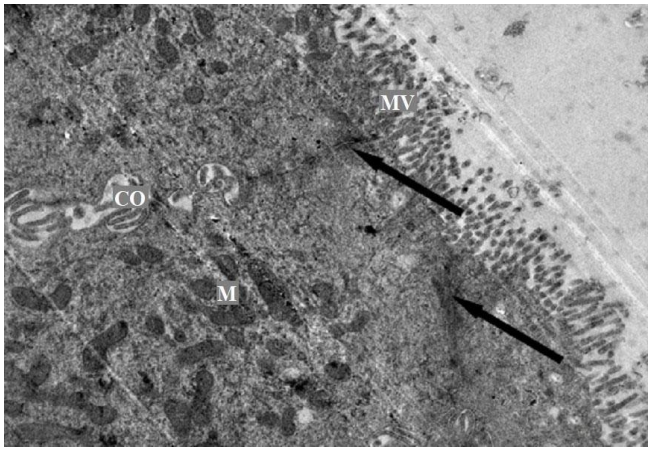


**Fig. 2.** Ultra-structure of the apical pole of epithelial cells in the gallbladder wall. MV: Microvilli; M: Mitochondria; CO: Cytoplasmic outgrowths (pseudopodia). Arrows indicate desmosomes (magnification = 2,500×).

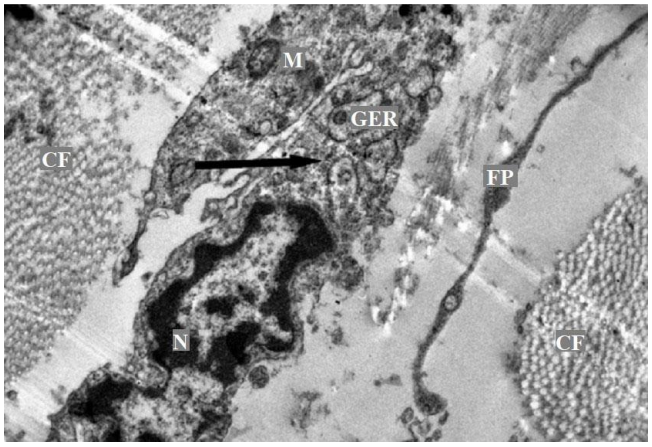


**Fig. 3.** Ultra-structure of the lamina propria of the gallbladder wall mucosa. N: Fibroblast nucleus; CF: Collagen fibers; GER: Granular endoplasmic reticulum; M: Mitochondria; FP: Fibroblast process. Arrow indicates ribosomes and polyribosomes (magnification = 2,500×).

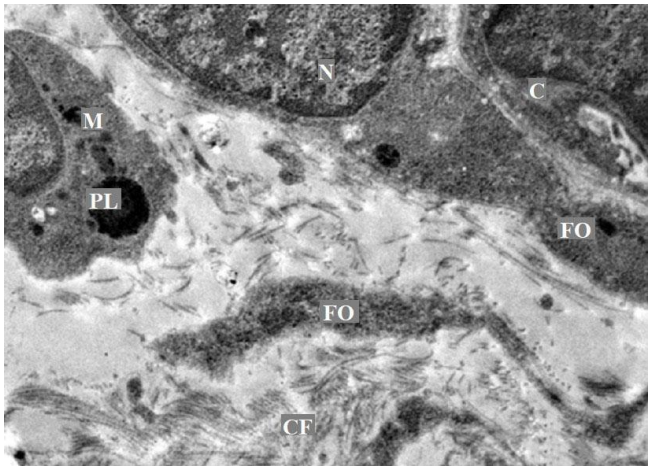


**Fig. 4.** Ultra-structure of the lamina propria of the gallbladder wall. N: Fibroblast nucleus; M: Macrophage; CF: Collagen fibers; PL: Phagolysosomes; C: Capillary; FO: Fibroblast outgrowth (magnification = 3,000×).

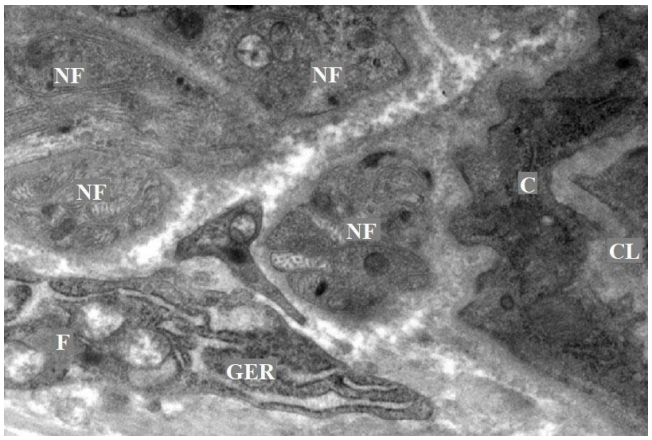


**Fig. 5.** Ultra-structure of the lamina propria of the gallbladder wall. NF: Unmyelinated nerve fiber; F: Fibroblast; C: Blood capillary; CL: Capillary lumen; GER: Granular endoplasmic reticulum (magnification = 3,000×).

## Discussion

In the scientific literature, there is very little information describing the ultra-structural organization of the gallbladder using electron microscopy, especially in productive (agricultural) animals.

Thus, electron microscopic studies showed that the gallbladder mucosa of the Anglo-Nubian goat is formed by the epithelial layer and its own lamina. The epithelial layer is represented by a single-layer prismatic epithelium, the cells of which lie on a dense basal membrane. Their nuclei are light, with clear contours, and sometimes contain one electron-dense nucleolus. In the cytoplasm, many small mitochondria, vesicles, a small Golgi complex, short cisterns of the granular endoplasmic reticulum, ribosomes, polyribosomes and rare lysosomes are detected. The apical surface of epitheliocytes forms microvilli, and the cytoplasm of the apical pole of the cells contains many electron-dense secretory granules. The secretion of the latter is excreted into the lumen of the gallbladder, forming a thin layer of glycocalyx on the surface of the epithelium, protecting it from the effects of bile salts. The lateral surfaces of the cells in their apical part are interconnected by tight contacts with the presence of desmosomes, and in the middle part, they form numerous finger-like processes and cytoplasmic outgrowths. Smaller and lighter single basal cells are found in the composition of the epithelial layer. The lamina propria is formed by loose connective tissue containing many blood vessels and unmyelinated nerve fibers.

In general, the data described above are consistent with the morpho-functional structure of the gallbladder in other animal species having a diet similar to goats.[17] Also, using various non-invasive imaging methods, we can conclude that there are correlations between the morpho-functional structure, ultra-structural organization and the displayed results of the above-described visualization.[18] Referring to the scientific literature describing possible pathologies of the gallbladder, we can conclude that the picture presented in the results generally corresponds to the position of the gallbladder without pathologies.[19, 20]

The data obtained regarding the morphology of the gallbladder wall of an Anglo-Nubian goat can be recommended for use in therapeutic practice in the diagnosis of hepatobiliary pathologies, future studying of species, comparative and breed morpho-physiology and patho-morphology of the digestive system, assessing the morpho-functional state of the gallbladder in order to determine the boundaries of normality and pathology and conducting scientific research in laboratories studying the morpho-physiology of the gastrointestinal tract.

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## Conflict of interest

There is no conflict of interest.

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