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*Short Communication*

# ULTRASTRUCTURAL ORGANIZATION OF LIVER HEPATOCYTES OF THE ANGLO-NUBIAN GOAT

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**Abstract**

The aim of the study was to establish the ultrastructural organization of hepatocytes of the Anglo-Nubian goat.Livers of adult goats of the Anglo-Nubian breed served as the material for the study. The study was carried out using the electron microscopic method. For this purpose, fragments of liver parenchyma tissues, no larger than 2.0 $mm^3$, were selected. The tissue samples were fixed in a 2.0% glutaraldehyde solution on a cacodylate buffer (pH 7.2-7.4) for 2 hours. Then, they were washed in three portions of the same buffer and post-fixed in a 1.0% solution of osmium tetrachloride (prepared in cacodylate buffer, pH 7.2-7.4) for 1 hour. Samples were dehydrated in increasing concentrations of alcohol and anhydrous acetone. The tissue fragments were then embedded in Epon-812. Ultrathin sections were obtained using an ultramicrotome (LKB-III, Sweden) and processed with 2.0% aqueous uranyl acetate and lead citrate solutions. The resulting ultrathin sections were photographed using a Jem-1011 electron microscope (JEOL, Japan) at 2500-3000× magnification.It was established that two morphological types of hepatocytes can be distinguished in the liver parenchyma of the Anglo-Nubian goat at the ultrastructural level – dark and light, both with characteristic morphological features. Dark hepatocytes have a high electron-optical density due to the presence of a larger number of organelles. Light hepatocytes are characterized by a smaller number of organelles and a lower electron-optical density than dark hepatocytes.

**Key Words:** electron microscopy, hepatocyte, goat, morphology

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## INTRODUCTION

Playing a leading role in the normalization of metabolic processes, such an organ as the liver, directly or through other vital organs, affects absolutely all systems of the animal body (Drozdova and Kundryukova, 2010; Drozdova, 2004). In addition, the liver, the most important element of homeostatic functionality, is involved in most of the body's abnormal (pathological) reactions that weaken the parenchyma cells (hepatocytes) (Caglayan and Gulcin, 2018; Liu et al., 2018) Such processes trigger extremely severe damage to the organ (tissue destruction, functional failures, problems with nutrition and weight – dystrophic deviations), which are explained by the appearance of various types of pathologies (Zhao et al., 2022; Lu et al., 2019). The multifaceted importance of the liver for the body indicates the complexity of its structure and the presence of significant detailed differences from the general structural organization characteristic of other glands (Prusakova and Zelenevsky, 2020; Baryshev et al., 2022). The liver's functions are able to occur due to the spatial organization of the tissue components of the liver and their relationships with the intraorgan blood vessels (Prusakov et al., 2017; Kuznetsov et al. 2022).

To reveal the life processes occurring in animals' bodies, and to be able to control them in order to increase productivity, a comprehensive study of the liver is necessary (Yashin et al., 2021; Ponamarev et al., 2022). In particular, this applies to the ultrastructural organization of liver tissues. We set the goal of establishing the ultrastructural organization of hepatocytes in the liver of the Anglo-Nubian goat breed.

## MATERIALS AND METHODS

The material for this study was the liver of adult Anglo-Nubian goats. Livers were collected from 10 animals (5 males (74 ± 3.2 kg) and 5 females (62 ± 4 kg)). Liver parenchymal tissue fragments no larger than 2.0 $mm^3$ were selected for electron microscopy. Selected samples were fixed in a solution of 2.0% glutaraldehyde in cacodylate buffer (pH 7.2-7.4) for 2 h. They were then washed three times in the same buffer and postfixed for 1 h in 1.0% osmium tetroxide solution (prepared in cacodylate buffer, pH 7.2-7.4). The samples were then dehydrated in ethanol and anhydrous acetone. Selected tissue fragments were subsequently embedded in Epon-812 according to generally accepted methods (Weekly, 1975). Ultrathin sections were obtained using an ultramicrotome (LKB-III - Sweden) and processed with 2.0% aqueous uranyl acetate and lead citrate solutions (Reynolds, 1963). The resulting ultrathin sections were photographed using a Jem-1011 electron microscope (JEOL, Japan) at 2500-3000× magnification. The terminology used corresponds to the International Histological Nomenclature (Semchenko et al., 1999). The study was approved by the Ethics Committee of FSBEI HE SPbSUVM.

The manipulations performed as part of the study were allowed by the Committee of Bioethics of the FSBEI HE SPbSUVM, and corresponded to the European

Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, adopted in Strasbourg in 1987; the study was in accordance with Directive 2010/63/EU.

## RESULTS

It was established that the Anglo-Nubian goat hepatocytes, which formed the bulk of the liver parenchyma in the studied animals, are epithelial parenchymal cells with a polygonal or rounded shape. Hepatocytes accompanied auxiliary cells that are actively involved in metabolic processes, i.e., sinusoidal endotheliocytes, stellate macrophages, and perisinusoidal fat-accumulating cells.

The cytoplasmic membrane of the hepatocyte in the Anglo-Nubian goat consisted of outer and inner layers, between which there was a light osmiophobic layer 2.0 to 3.0 nm wide. Hepatocytes tightly adjoining to each other with their lateral surfaces formed chains of hepatic beams (lamellae), radially diverging from the central vein. In the middle part of each of the beams, a space was noticeable, a bile capillary (bile canaliculi) (Figure 1).

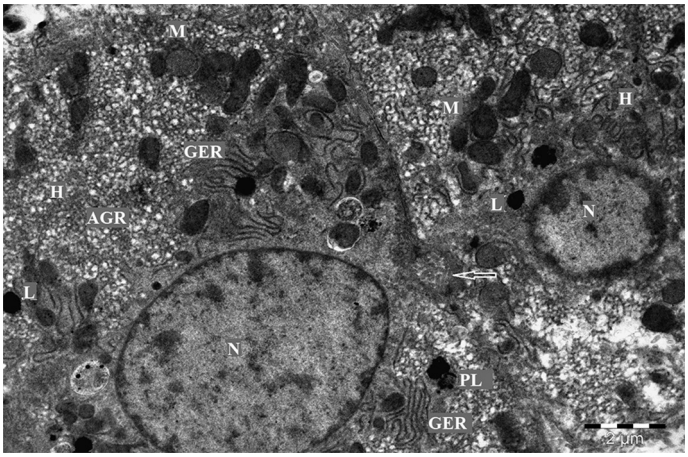


**Figure 1.** Ultrastructure of the hepatic beam area. Electron micrograph: H – hepatocyte body; N – the nucleus of the hepatocyte; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; L – lysosomes; PL – phagolysosome; ↑ – bile capillary.

The structures of two other mutually opposite surfaces of hepatocytes differed from each other. Differences in their ultrastructure are due to differences in their functions.

The apical (vascular, sinusoidal) surface of the hepatocyte (Figure 2) pointed toward the sinusoidal capillaries (sinusoid). Between the apical surface and the wall of the latter, the Disse space was defined. The apical surfaces of the hepatocytes were covered with short and long microvilli. A thin layer of glycocalyx was seen on the apical surfaces

under a high-power microscope. The short microvilli faced the perisinusoidal space. The long microvilli penetrated this space and, at the pores of the endotheliocytes forming the sinusoidal capillary, penetrated its lumen, where they came directly into contact with blood.

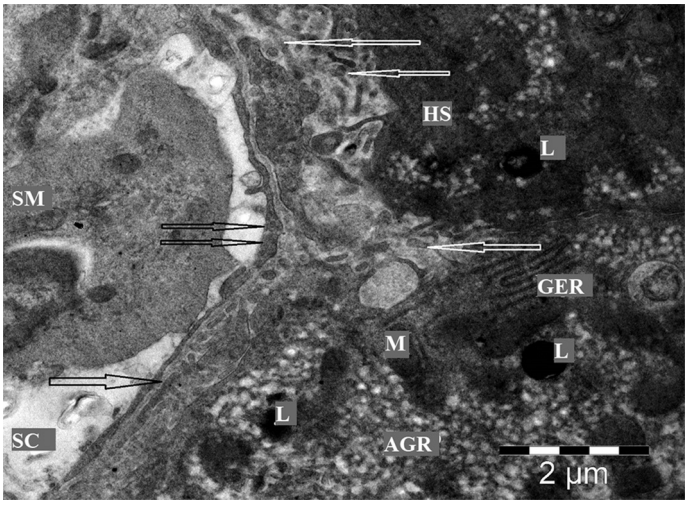


**Figure 2.** Vascular side of the hepatocyte. Electron micrograph: HS – sinusoid surface of a hepatocyte; SC – sinusoid capillary; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; L – lysosomes; SM – stellate macrophage; ↑ – Disse space; ↑↑ – endotheliocyte process.

The biliary surface of the hepatocytes, opposite to the apical surface, faced the bile capillary (Figure 3). In the region of the mouth of the bile capillary, the cells of the

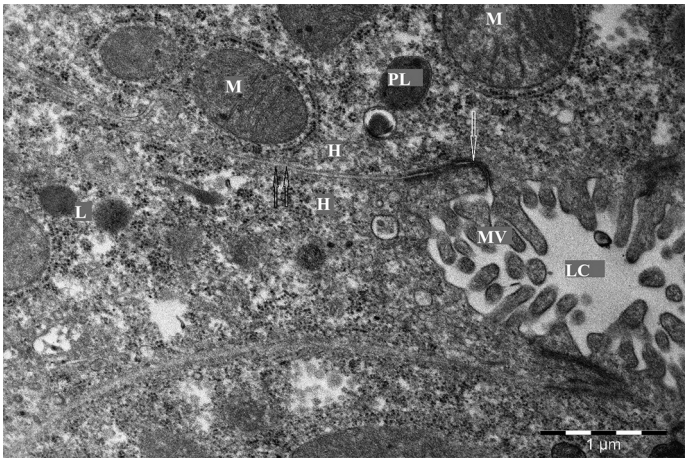


**Figure 3.** Biliary capillary. Electron micrograph: H – hepatocyte; M – mitochondria; L – lysosomes; PL – phagolysosome; LC – bile capillary lumen; MV – microvilli; ↑ – desmosome; ↑↑ – hepatocyte membrane.

hepatocytes were firmly linked by dense junctions, desmosomes, which ensure stable adhesion of the cells. The sections of the cytolemma which formed the wall of the biliary surface were distinguished by pronounced invaginations and microvilli facing the lumen.

Bile acids and other substances (bilirubin, cholesterol, phospholipids, etc.) from the blood enter the bile capillary, as well as bile acids, proteins, and carbohydrates produced in the hepatic cells. From the bile duct, these substances enter further into the excretory ducts of the liver.

The size of hepatocyte nuclei depends on their ploidy. As for the composition of hepatocytes, they were located mainly eccentrically, had a rounded shape, and contained 1-2 nucleoli. At high magnifications, the bypass karyolemma was clearly visible. The main volume of the nuclear matrix was filled with light karyoplasm containing finely dispersed euchromatin. Small lumps of heterochromatin were mostly concentrated along the internal karyolemma (Figure 4).

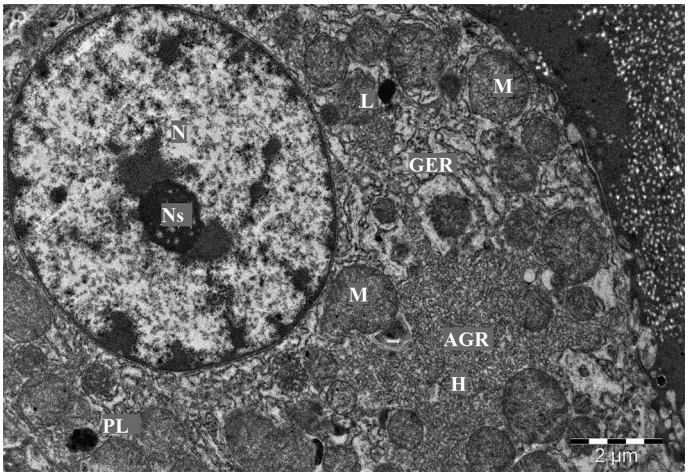


**Figure 4.** Ultrastructure of the liver hepatocyte. Electron micrograph: H – hepatocyte; N – nucleus; Ns– nucleolus; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; L – lysosomes; PL – phagolysosome.

In the cytoplasm of hepatocytes, cisterns of the granular endoplasmic reticulum and agranular (smooth) endoplasmic reticulum, ribosomes, polyribosomes, the Golgi apparatus, polymorphic mitochondria, lysosomes of various types, glycogen granules, and sometimes lipid inclusions were detected. Depending on the amount of content and degree of concentration of intracellular organelles in the cytosol, at the electron-microscopic level, hepatocytes could be divided into two types: dark and light (Figure 5). Dark hepatocytes were mostly located around the portal tracts and were characterized by pronounced synthetic activity. Light hepatocytes were larger than dark ones and were situated around the central veins.

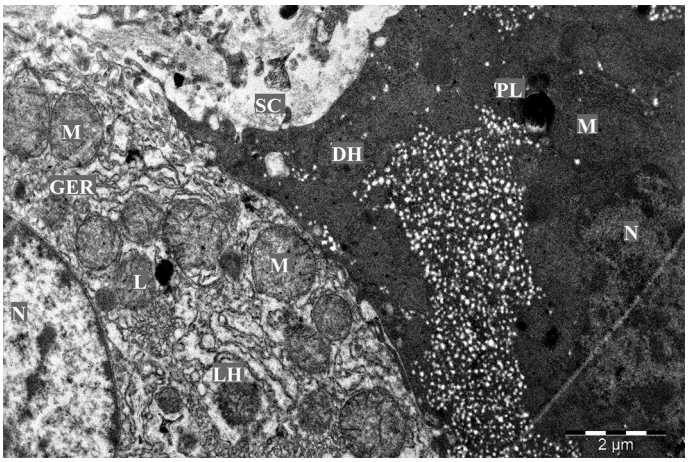


**Figure 5.** Hepatocytes of dark and light types. Electron micrograph: LH – light hepatocyte; DH – dark hepatocyte; N – nucleus; M – mitochondria; GER – granular endoplasmic reticulum; L – lysosomes; PL – phagolysosome; SC – sinusoidal capillary.

The dark hepatocytes had a high electron-optical density. Their cytoplasm contained a large number of elongated cisterns of the granular endoplasmic reticulum with beads of ribosomes on the outer side of the membranes (Figure 6).

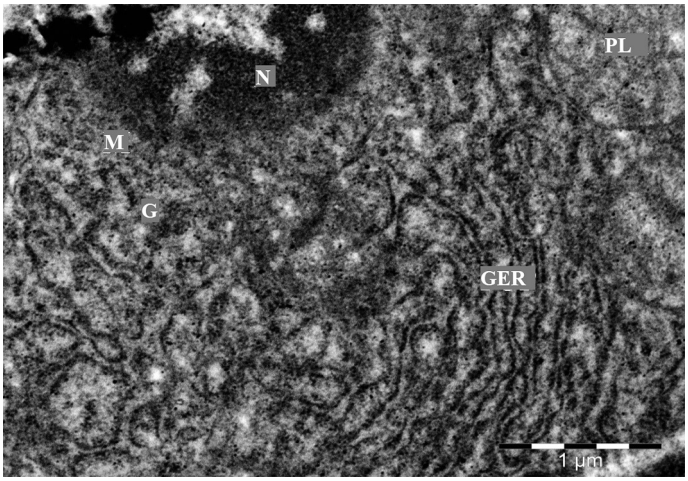


**Figure 6.** Dark hepatocyte. Electron micrograph: N – nucleus; M – mitochondria; GER – granular endoplasmic reticulum; G – glycogen; PL, phagolysosome.

Between them, a large number of ribosomes, polyribosomes, and glycogen granules were visible in the cytosol. The cisternae of the agranular reticulum were also numerous, and they looked like thin tubules, ornately folded in separate sections of the cytoplasm (Figure 7). This figure also shows small dark and heterogeneous secondary lysosomes, visible in the biliary pole region of the hepatocyte.

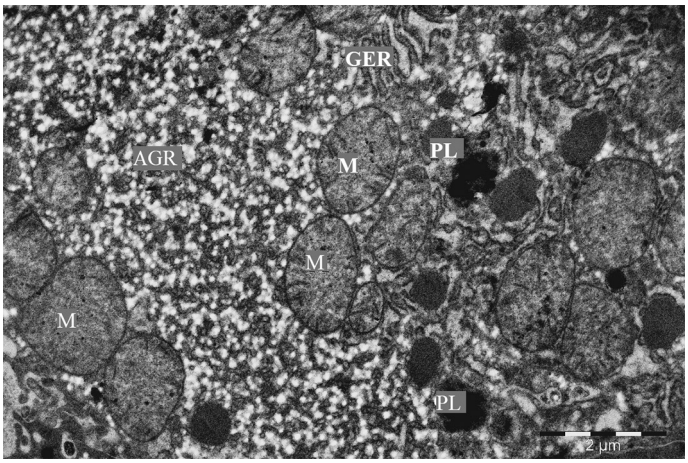


**Figure 7.** Dark hepatocyte. Electron micrograph: M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; PL – phagolysosome.

In dark hepatocytes located near the bile ducts, the Golgi complex and inclusions of bile pigments were often found (Figure 8), as well as numerous mitochondria with a double-membrane and rare, thin cristae. The internal matrix of the latter was characterized by the presence of fine granularity.

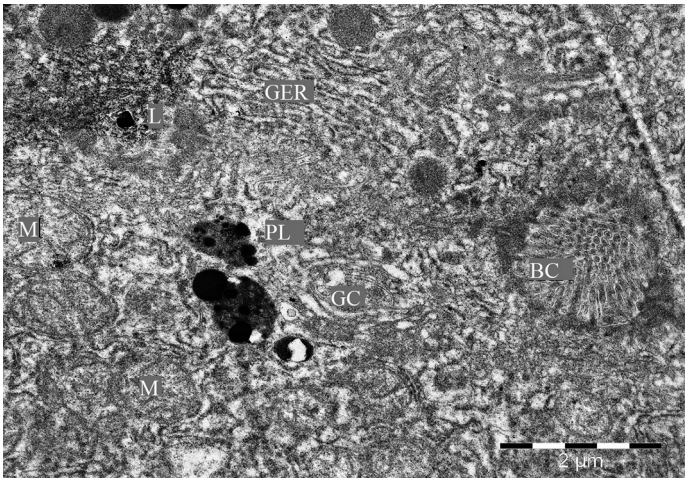


**Figure 8.** Dark hepatocyte. Electron micrograph: M – mitochondria; GER – granular endoplasmic reticulum; GC – Golgi complex; L – lysosomes; PL – phagolysosome; BC – bile capillary with microvilli.

In the vast majority of dark hepatocytes, the mitochondria were small, elongated, and with a very dense dark mitochondrial matrix (Figure 9). They differed from the above-described mitochondria in their smaller size and more elongated shape, and the cristae in them were not clearly visible.

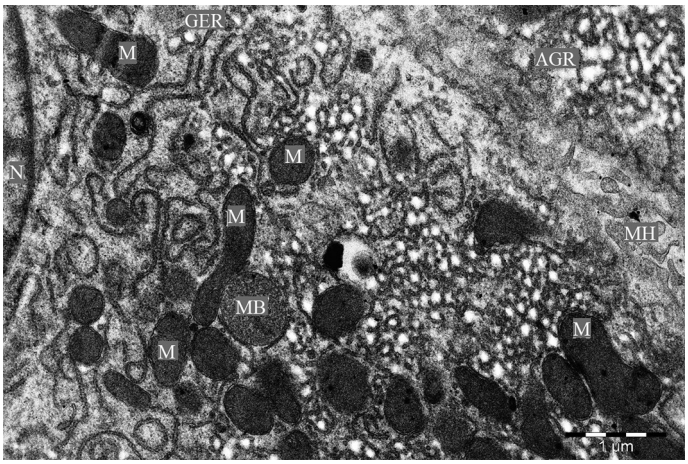


**Figure 9.** Dark hepatocyte. Electron micrograph: N – nucleus; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; MB – multivesicular body; MH – microvilli of hepatocyte.

There were fewer organelles in light perilobular hepatocytes than in dark hepatocytes (Figure 10). The mitochondria in the light perilobular hepatocytes were large and rounded. Their mitochondrial matrix was of medium density and was permeated with thin, short cristae. In the cytoplasm, a moderate amount of thin elongated flattened sacs of the granular endoplasmic reticulum, and ornate accumulations of flattened sacs of the agranular endoplasmic reticulum were clearly visible. Sometimes, quite large inclusions of bile pigments and lipid drops were notable (Figures 11, 12).

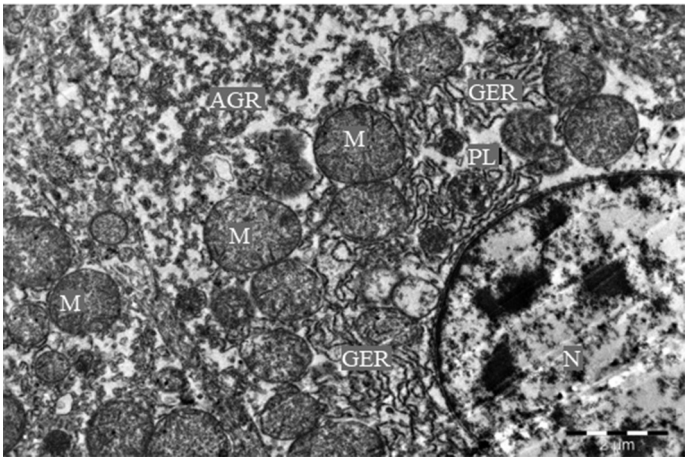


**Figure 10.** Light hepatocyte. Electron micrograph: N – nucleus; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; PL – phagolysosome.

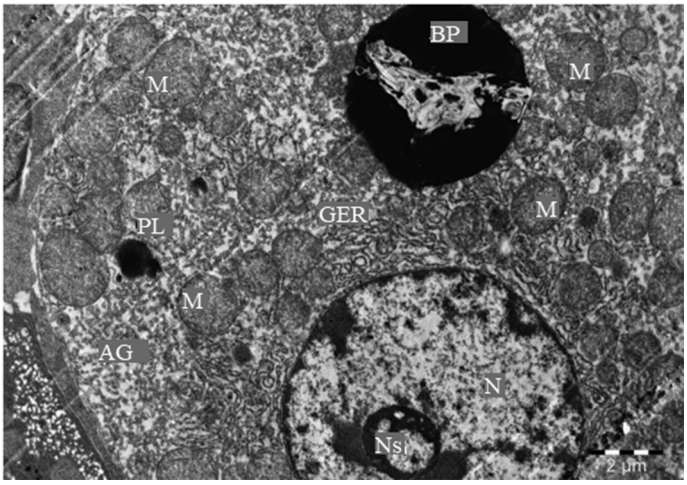


**Figure 11.** Light hepatocyte. Electron micrograph: N – nucleus; Ns – nucleolus; M – mitochondria; GER – granular endoplasmic reticulum; AGR – agranular endoplasmic reticulum; PL – phagolysosome; BP – inclusion of bile pigments.

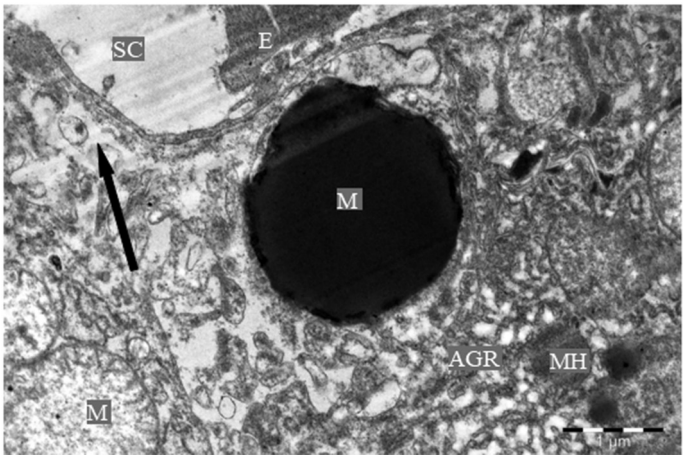


**Figure 12.** Fragment of a light hepatocyte. Electron micrograph: M – mitochondria; AGR – agranular endoplasmic reticulum; ↑ – Disse space; SC – sinusoidal capillary lumen; E – erythrocyte.

## DISCUSSION

Information about the ultrastructural organization of the liver is of great scientific and practical importance, as it opens up new opportunities in the diagnosis of hepatopathy of various geneses through the more detailed study of the pathogenesis and pathological changes in cells and tissues, and also allows us to further propose new methods for assessing the effectiveness of hepatotropic pharmacocorrection.

The ultrastructural organization of the liver is a very little-studied issue that has rarely been considered in any detail in the scientific literature. Usually such an organization is described in the context of studying changes in the liver due to parasitic pathologies (Bilqees, 2010), or in the context of toxicological studies, when a detailed study is required of the hepatotoxic effect of a toxicant or the effect of general toxic effects on the liver (Ghosh et al, 2014), or is described in adjacent sciences, such as histochemistry (**Sethi et al., 2021**) or diagnostic ultrasonography (Braun et al., 2013; Braun et al., 2011).

It should also be noted that histological examination is still the most reliable method for diagnosing hepatopathologies, often called the "gold standard" in the scientific literature for the diagnosis and differential diagnosis of liver diseases.

## CONCLUSION

In liver parenchyma of the Anglo-Nubian goat, at the ultrastructural level, two morphological types of hepatocytes can be distinguished – dark and light. The dark hepatocytes have a high electron-optical density. Their cytoplasm contains a large number of elongated cisterns of the granular endoplasmic reticulum with beads of ribosomes on the outer side of the membranes, many ribosomes, polyribosomes of glycogen granules, and agranular reticulum cisterns. In the cytoplasm of dark hepatocytes located near the bile ducts, in addition to the above organelles, the Golgi complex and inclusions of bile pigments are found, as well as numerous mitochondria with a double-circuit membrane and rare thin cristae. Light hepatocytes are characterized by a smaller number of organelles and a lower electron-optical density than dark hepatocytes. The mitochondria in light hepatocytes are large, rounded, with a medium-density matrix pierced by thin short cristae. In the cytoplasm, a moderate amount of thin elongated flattened sacs of the granular endoplasmic reticulum and ornate accumulations of flattened sacs of the agranular endoplasmic reticulum are clearly visible. Quite large inclusions of bile pigments and lipid drops appear in several cells.

### Authors' contributions

ZVN, YVA – modeled and supervised all work, overviewed the literature; PVA, PVA – conducted the research, took and prepared samples, wrote the paper; PSV – analyzed results, translated the paper, edited the paper.

### Competing interests

The authors declare that they have no competing interests.

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# ULTRASTRUKTURALNA ORGANIZACIJA HEPATOCITA JETRE ANGLO-NUBIJSKE KOZE

Anna V. PRUSAKOVA, Nikolay V. ZELENEVSKIY, Aleksey V. PRUSAKOV, Anatoly V. YASHIN, Vladimir S. PONAMAREV

**Kratak sadržaj**

Cilj istraživanja je da se utvrdi ultrastrukturalna organizacija hepatocita anglo-nubijske koze. Uzeti su uzorci jetre odrasle koze ove rase, ispitani metodom elektronske mikroskopije. U tu svrhu odabrani su fragmenti tkiva parenhima jetre, ne veći od 2,0 $mm^3$, fiksirani u 2,0% rastvoru glutaraldehida u kakodilatnom puferu (pH 7,2-7,4) tokom 2 sata. Zatim su isprani tri puta u istom puferu i naknadno fiksirani u 1,0% rastvoru osmijum tetrahlorida (pripremljen u kakodilatnom puferu, pH 7,2-7,4) - 1 sat. Uzorci su dehidrirani u rastućim koncentracijama alkohola i anhidrovanog acetona. Fragmenti tkiva su zatim stavljeni u Epon-812. Ultratanki preseci dobijeni sečenjem na ultramikrotomu (LKB-III – Švedska) postfiksirani su u 2,0% vodenom rastvoru uranil acetata i olovo citrata. Dobijeni ultratanki preseci su fotografisani korišćenjem Jem-1011 elektronskog mikroskopa (JEOL, Japan) pri uvećanju od 2500–3000 puta. Utvrđeno je da se u parenhimu jetre anglo-nubijske koze na ultrastrukturnom nivou mogu razlikovati dva morfološka tipa hepatocita – tamni i svetli sa specifičnim morfološkim karakteristikama. Tamni hepatociti imaju veću elektron-optičku gustinu zbog prisustva većeg broja organela. Hepatocite svetlog tipa karakteriše manji broj organela i niža elektron-optička gustina.

**Ključne reči:** elektronska mikroskopija, hepatocit, koza, morfologija.