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ORGANIZATION OF HISTO-HEMATIC BARRIERS OF THE LIVER IN ANGLO-NUBIAN GOAT

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Supporting Information

ABSTRACT: The aim of this research was to establish features of the liver histo-hematic barriers ultrastructural organization of the Anglo-Nubian goat. The liver of an adult Anglo-Nubian goat was used as the material. The work was carried out using the electron microscopic method. Liver parenchymal tissue fragments were selected. These samples were fixed in a 2.0% glutaraldehyde solution on a cacodylate buffer for two hours. They were then washed in three portions of the same buffer and post-fixed in a 1.0% solution of osmium tetrachloride for one hour. The samples were then dehydrated in alcohols of ascending concentration and absolute acetone. The subsequent filling of the fragments was carried out in Epon-812. Ultrathin sections were obtained on an ultramicrotome, contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate. The ultrathin sections were photographed with a Jem-1011 electron microscope at magnifications of 2500-3000. Two histo-hematic barriers are detected in the liver of the studied animals hemato-hepatic and hepatobiliary. The hemato-hepatic barrier is formed by the plasmalemma of the apical end of the hepatocyte, covered by the glycocalyx, the perisinusoidal space of the Disse, the endotheliocyte of the sinusoid capillary, as well as Kupfer cells located in the lumen of the latter. The hepatobiliary includes all of the above structures, with the exception of Kupfer cells, as well as the plasmalemma of the basal end of the hepatocyte. All of the above structures in their organization have characteristic species features for Anglo-Nubian goats.

Keywords: Anglo-Nubian goat, Digestive organs, Hepatobiliary barrier, Hemato-hepatic barrier, Liver.

SHORT COMMUNICATION

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INTRODUCTION

Mammals have numerous histo-hematic barriers (Wong et al., 2013; Mruk and Cheng, 2015). Due to their presence, the regulation of metabolic processes between blood and tissues is regulated, which ensures the constancy of the composition, as well as the physicochemical and biological properties of the tissue fluid (Alekhin et al., 2023). At the same time, histo-hematic barriers ensure the timely excretion of cellular metabolic products and also block the release of certain substances into the bloodstream (Baryshev et al., 2022; Drozdova, 2004; Drozdova and Kundryukova, 2010). The structure of histo-hematic barriers is distinguished by its specific features and is mainly determined by the structure of the organ (Akmalova et al., 2018; Ponamarev et al., 2022). It is known that the main element that is part of any histo-hematic barrier is the hemocapillary, whose endotheliocytes in different organs and tissues have characteristic morphological features. The study of structural features of histo-hematic barriers is extremely important for both theoretical and practical veterinary medicine (Prusakov et al., 2019; Kuznetsov et al., 2022). This is due to the fact that the most pathological processes basis, both at the organ and tissue levels, are structural changes in these systems (Yashin et al., 2021). Considering the foregoing, we set ourselves the task of determining the features of the histo-hematic barriers ultrastructural organization of the Anglo-Nubian goat liver.

MATERIALS AND METHODS

The material for the study was the liver of an adult Anglo-Nubian goat. Liver parenchyma tissue fragments, no larger than 2.0 mm³, were selected for electron microscopic examination. The selected samples` fixation was carried out in a solution of 2.0% glutaraldehyde in cacodylate buffer (pH 7.2-7.4) for two hours. Then they were washed out three times in the same buffer and post-fixed in 1.0% osmium tetroxide solution (prepared in cacodylate buffer, pH 7.2-7.4) for one hour. Then the samples were dehydrated in ascending alcohols and absolute acetone. The subsequent filling of the selected tissue fragments was carried out in Epon-812 according to the generally accepted method (Weekly, 1975). Ultrathin sections were obtained on an ultramicrotome (LKB-III - Sweden), contrasted with a 2.0% aqueous solution of uranyl acetate and a solution of lead citrate (Reynolds, 1963). The resulting ultrathin sections were photographed using a Jem-1011 electron microscope (JEOL, Japan) at magnifications of 2500–3000. The terminology used is in accordance with the International Histological Nomenclature (Semchenko et al., 1999).

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Ethical regulation of study

The studies were 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 (GLP and GCP), 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 St. Petersburg State University of Veterinary Medicine.

RESULTS AND DISCUSSION

In the liver of the studied animal, two histo-hematic barriers are revealed – hemato-hepatic and hepatobiliary. It has been established that the hemato-hepatic barrier is formed by the plasmalemma of the hepatocyte apical end, covered with glycocalyx, as well as the endothelial cell of the sinusoidal capillary, and Kupffer cells (stellate macrophages) located in the lumen of the perisinusoidal space of Disse. The composition of the hepatobiliary barrier of the Anglo-Nubian goat includes all of the above structures, with the exception of Kupffer cells, and the plasmalemma of the hepatocyte basal end.

The cytoplasmic membrane of hepatocytes in the studied animals consists of two clearly defined layers - outer and inner. Between the layers, a light osmiophobic layer is clearly visible, the thickness of which varies within 2.0-3.0 nm.

The apical (vascular, sinusoidal) surface of the hepatocyte (Figure 1) faces the sinusoidal capillary (sinusoid). Between them, the perisinusoidal Disse space is determined. The cytoplasmic membrane of the cell apical part forms a multitude of short and long microvilli covered with a thin layer of glycocalyx. Short microvilli face the perisinusoidal space. Long microvilli penetrate this space and through the pores of endotheliocytes, forming the sinusoid capillary, follow into its lumen, where they are in direct contact with the blood.

The endothelial cells of the liver sinusoid capillaries have flattened nuclei (Figure 2). Their cytoplasm contains well-developed organelles (short channels of the granular endoplasmic reticulum, many ribosomes and polyribosomes, slightly rounded mitochondria, lysosomes, microvesicles), and microfilaments.

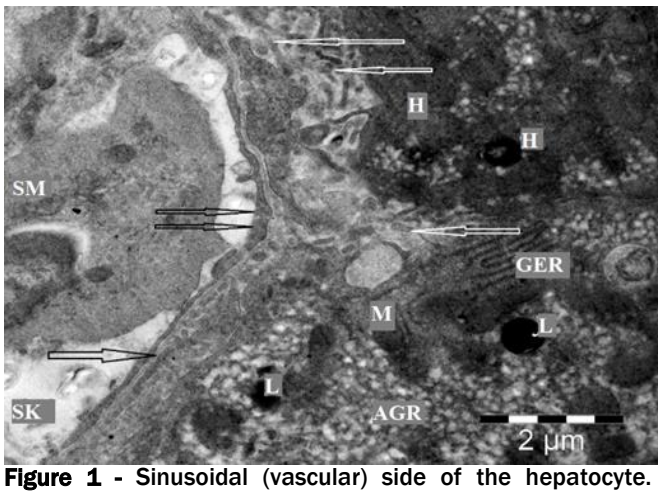

Figure 1 - Sinusoidal (vascular) side of the hepatocyte. Electron microphoto: H - sinusoidal surface of the hepatocyte; SC - sinusoidal capillary; M - mitochondria; GER, granular endoplasmic reticulum; AGR - agranular endoplasmic reticulum; L - lysosomes; SM - stellate macrophage; ↑ – Disse space; ↑↑ – Endotheliocyte process.

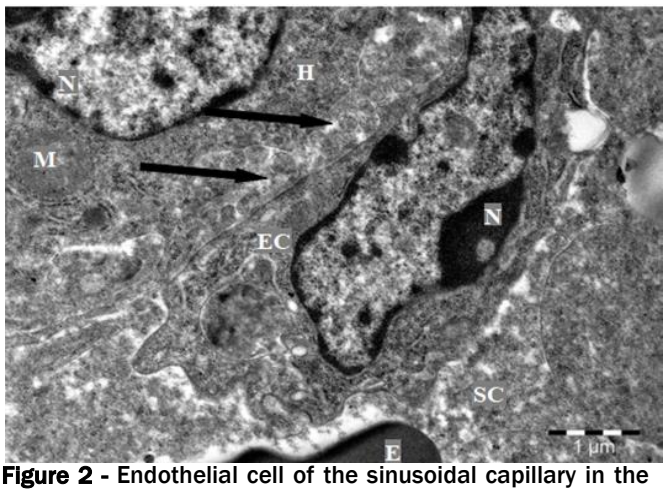

Figure 2 - Endothelial cell of the sinusoidal capillary in the liver. Electron microphoto: H - hepatocyte; N - nucleus; M - mitochondria; EC - endotheliocyte cytoplasm; ↑ – Disse space; SC - lumen of the sinusoidal capillary; E - erythrocyte.

Long processes of endothelial cells stretched along the hepatic plates are characterized by the presence of numerous pores - fenestra, through which there is a free exchange between blood and intercellular fluid (Figure 3). It should be noted that there is no basement membrane in the sinusoidal capillaries of the liver. In the lumen of sinusoidal capillaries in many parts of the liver, there are stellate macrophages (Kupffer cells), which are large process cells with high phagocytic activity (Figure 4). Their nuclei are large, sometimes elongated oval, sometimes with strongly indented edges. In the nuclei, a large amount of heterochromatin is determined, which lies on the inner karyolemma or is located throughout the nucleus in large, coarse lumps. At one end, stellate macrophages are attached in bifurcations between hepatocytes, and the cell body with processes most often lies freely in the sinusoid lumen (Figure 5).

Macrophages "purify" the blood brought from the portal vein from toxins, antigens, microorganisms, etc., therefore their cytoplasm contains many small dark lysosomes and heterogeneous residual bodies. The cytoplasm of hepatic macrophages appears optically dark due to the presence of a large number of ribosomes, polyribosomes, small granules, and various microvesicles. In addition, many rounded small mitochondria, a well-developed Golgi complex, and short cisterns of the granular endoplasmic reticulum are found in their cytoplasm. Sometimes there are fragments of obsolete erythrocytes and inclusions of hemosiderin.

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The opposite apical, biliary surface of the hepatocyte faces the bile capillary (Figure 6). In the area of the capillary mouth, the cell membranes of hepatocytes are firmly bound by dense osmiophilic compounds - desmosomes, which provide stable adhesion between cells. The sections of the cytolemma forming the bile capillary wall create pronounced intussusceptions and microvilli facing the lumen.

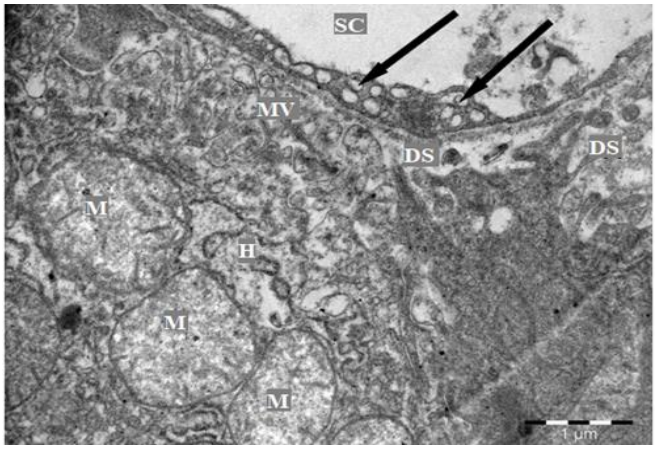

Figure 3 - Fragment of a hepatocyte and endothelial cell process of a sinusoidal capillary. Electron microphoto: H - hepatocyte; M - mitochondria; DS - Disse space; ↑ - pores of the endotheliocyte; SC - lumen of the sinusoidal capillary; MV - microvilli of the hepatocyte sinusoidal edge.

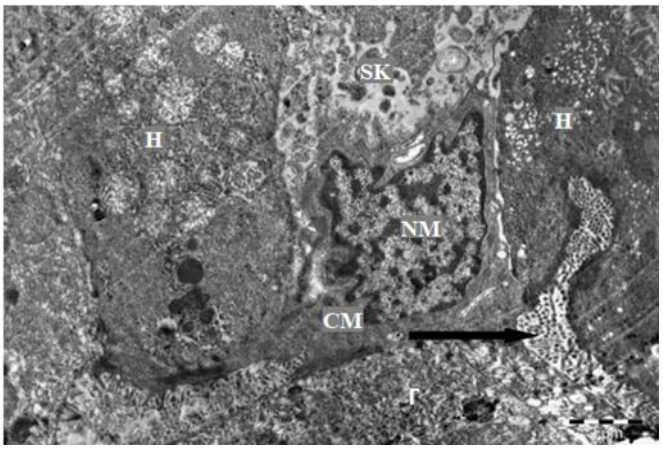

Figure 4 - Stellar liver macrophage. Electron microphoto: H – hepatocyte cytoplasm; NM - macrophage nucleus; CM - macrophage cytoplasm; ↑ - bile duct; SC - lumen of the sinusoidal capillary.

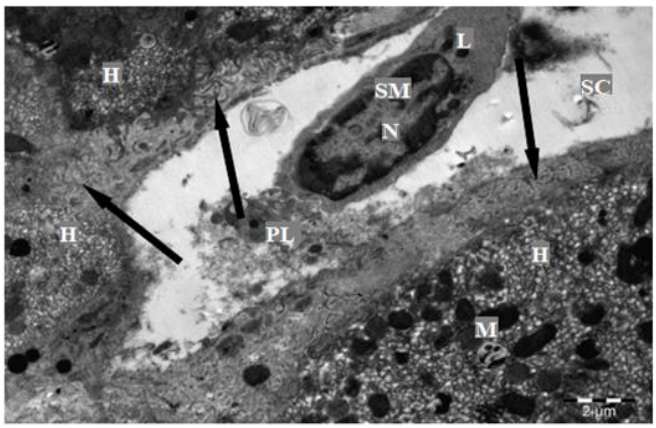

Figure 5 - Stellar liver macrophage. Electron micro-photo: H - hepatocyte; M - mitochondria; ↑ - hepatocyte microvilli in the Disse space; SC - lumen of the sinusoidal capillary; SM - stellate macrophage; N – a nucleus of a macrophage; PL - phagolysosomes in macrophage cytoplasm; L - lysosomes.

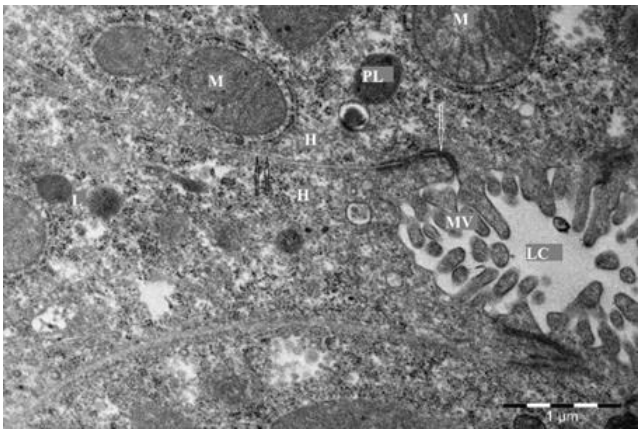

Figure 6 - Bile capillary. Electron microphoto: H - hepatocyte; M - mitochondria; L, lysosomes; PL - phagolysosome; LC - lumen of the bile capillary; MV - microvilli; ↑ - desmosome; ↑↑ – hepatocyte membrane.

CONCLUSION

In the liver of the studied animal, two histo-hematic barriers are revealed – hemato-hepatic and hepatobiliary. The hemato-hepatic barrier is formed by the plasmalemma of the apical end of hepatocytes covered with glycocalyx, as well as the endothelial cell of the sinusoidal capillary, and Kupffer cells (stellate macrophages) located in the lumen of the perisinusoidal space of Disse. The composition of the hepatobiliary barrier of the Anglo-Nubian goat includes all of the above structures, with the exception of Kupffer cells, and the plasmalemma of the basal end of hepatocytes. All of the above structures in their organization have characteristic species features characteristic for the Anglo-Nubian goat.

DECLARATIONS

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Authors' contributions
Zelenevskiy V. Nikolay, Yashin V. Anatoly: modelling and supervising all work, literature overview.
Prusakova V. Anna, Prusakov V. Aleksey: performing research, taking and preparing samples, article writing.
Ponamarev S. Vladimir: analyzing results, translating, editing.

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Competing interests
The authors declare that they have no competing interests.

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