რეზიუმე

კოვიდ-19-ის გენომის თავისებურებანი და კორონავირუსის რნმ-ის ტრანსლაციური პროცესი, როგორც პოტენციური სამიზნე ადენინით და ნუკლეოტიდების სხვადასხვა ანალოგებით ეტიოტროპული თერაპიისთვის (მიმოხილვა)

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COVID-19-ის სამკურნალოდ მოწოდებული მედიკამენტების მრავალმხრივი ეფექტების მიუხედავად, ინფიცირებულთა რიცხვი და პაციენტთა სიკვდილიანობა მატულობს, რაც ცხადყოფს კორონავირუსის ინფექციებთან ბრძოლის სამკურნალოდ გამოყენებული მედიკამენტების არასაკმარის ეფექტურობას და მკურნალობის ახალი ტაქტიკის შემუშავების აუცილებობას. სტატიაში სუმირებული და გაანალიზებულია ლიტერატურული მონაცემები, რომლებიც ეხება კორონავირუსის სპეციფიკურ ნიშნებს, კერძოდ, განსაკუთრებული ყურადღება გამახვილებულია ამ ვირუსის გენეტიკურ მახასიათებლებზე, ადამიანის ორგანიზმში მისი ინვაზიის, რეპლიკაციის მექანიზმზე და ამფ-2 რეცეპტორებთან ურთიერთქმედებაზე, ისევე როგორც კორონავირუსის საწინააღმდეგოდ მოქმედი ანტივირუსული ეფექტის მქონე არსებული პრეპარატების ძირითად სამიზნეებზე.

ამჟამად COVID-19-ის სამკურნალოდ გამოიყენება შემდეგი მედიკამენტები: რემდესივირი, ქლოროქინი, ჰიდროქსიქლოროკინი (HCQ), რიბავირინი, ლოპინავირი/რიტონავირი. კორონავირუსის მკურნალობის ბოლოდროინდელი თეორიის თანახმად, პოტენციური ეტიოტროპული პრეპარატის მოქმედების მექანიზმის ამოსავალი წერტილი არის კორონავირუსის მთავარი პროტეაზას (Mpro/3CLpro) და პაპაინის მსგავსი პროტეაზას (PLpro) დათრგუნვა. ზემოთ ჩამოთვლილ მედიკამენტებს შორის ლოპინავირი მოქმედებს ამ მექანიზმის საშუალებით, მაგრამ მას ახასიათებს მწვავე გვერდითი მოვლენები.

წინამდებარე ნაშრომში განხილულია კორონავირუსის საწინააღმდეგო პოტენციური ეტიოტროპული პრეპარატის მოქმედების მექანიზმი, რაც გულისხმობს ვირუსის რეპლიკაციის პროცესში ჩართული ნუკლეოტიდების ჩანაცვლებას მათი ანალოგებით რიბოსომის "დათრგუნვის" და ვირუსული ცილების წარმოების ბლოკირების მიზნით.

LIVER EXTRACELLULAR MATRIX PECULIARITIES IN MAMMALS AND AVIANS

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The extracellular matrix - the connective tissue framework of the liver - on the one hand, determines the shape of the organ, and on the other hand, creates specialized compartments for blood and lymphatic vessels and nerves, as well as cell populations, the synergy of which determines the various functioning of the organ. The liver is the largest and heaviest parenchymal organ, and an appropriate matrix design is required to maintain its shape and fix it on the abdominal walls [1]. The liver has a dual blood supply (arterial and portal), and the connective tissue spaces containing these vessels are built with this factor in mind. Unlike other organs, in which there are three circulating fluids and, therefore, there are three compartments for the microcirculation, four fluids circulate in the liver: blood, bile, interstitial juice, and lymph [2]. At the same time, the liver produces more lymph than any other organ (up to 50% of the total amount of lymph in the body). Thus, the liver matrix forms a highly complex but strongly regulated labyrinth in which liver cells, blood vessels, bile ducts, lymphatic ducts, and tissue fluid have their own but closely interconnected compartments [3-5].

The study of the liver connective tissue skeleton dates back to the 17th century. Pursuant to Couinaud [6], in 1640 Walaeus described the connective tissue sheath, which wraps the portal vein, the hepatic artery, the bile duct, the lymphatic duct, and the nerves entering and leaving the liver connects to the capsule of the liver and hepatoduodenal ligament. Walaeus sheath originates from the vasculobiliary sheath (Glisson's capsule) and is not derived from the peritoneum or the capsule of the liver (Laennec's capsule). Besides, the separation between Laennec's capsule and the Walaeus sheath can be seen microscopically at the hepatic hilum [6], where the Walaeus sheath forms a thick plate at the inferior part of the liver referred to as the hilar (portal) plate [7].

The portal pedicle wrapped by the Walaeus sheath continues inside the organ, as the so-called Glissonian Pedicals [8].

Until the late 1980s, it was thought that the branches of the portal vein and the hepatic veins in the liver were mutually autonomous and that their connective tissue sheaths did not touch each other [9-11]. There is the established opinion in the modern textbooks of Hepatology that Glisson's portal pedicles and the main branches of the hepatic veins spatially intersect, but the parenchymal area remains between them and, therefore, they are anatomically independent of each other [12]. But Ilia Chanukvadze showed that in the human liver the connective tissues of the main portal complexes and hepatic veins might merge in some zones of the intersection. The regions of such merging were called Portacaval Fibrous Connections (PCFC). Besides, the various forms of PCFC - as are the complete merging, touching merging, septal- and fibril-like connections - were described [13,14]. It has been revealed that PCFC, as an anatomical structure, is formed on the 11th-12th weeks of pregnancy [15].

A new wave of research on the connective tissue structures of the liver has been observed over the past five years. This "revisiting" is based on the introduction of new methods and computer technology in morphological studies [16]. This is due, on the one hand, to the creation of the possibility of endoscopic anatomical liver resections and, as a consequence, to the need to clarify the intercommunications of the connective tissue structures of the liver [17], and on the other hand, to the prospects of using human and animal liver matrices as the scaffolds for the creation of bioartificial livers (in turn related to the development of stem cells technology and bioengineering) [18-21].

At the same time, the analysis of these studies makes it obvious that the knowledge on the connective tissue skeleton of the liver lacks systematization, the terminology is inconsistent, and sometimes the construction of this or that component of the liver matrix is addressed controversially in the literature [22]. All of the abovementioned confirms the necessity of the further study of the liver matrix and complex analysis of the results obtained by different methods.

We set a goal to study peculiarities of the construction of connective tissue matrices of the livers of different mammals and birds for the identification and systematization of the general and specific regularities of this structure.

Material and methods. We have studied the relation of the connective tissue sheaths, covering the portal complexes and hepatic veins to each other and to the liver capsule and intralobular connective tissue network – in the livers of mammals with a gallbladder (pigs, sheep) and with no gallbladder (rats) and birds (domestic hens with gallbladder). The livers of the named animals and birds were studied by the anatomical preparation, histological, histotopographical, histochemical, immunohistochemical methods, scanning electron microscopy (SEM) of corrosion casts, and fluorescence microscopy. The number, age, and distribution of studied subjects according to the research methods are shown in Table 1.

Histology. Liver tissue sections of 3-5 μm were stained by the standard H&E method and studied microscopically with different magnification.

Histochemistry. Liver tissue sections of 3-5 μm were stained using Masson's Trichrome kit (Sigma Aldrich Catalog Number: C970D37) according to the recommendation of the manufacturer.

Immunohistochemistry. Liver sections of 3-5 um thickness obtained from the parragin-embedded blocks were stained with pan-Cytokeratin antibody [AE1+AE3] (neat) (ab961, Abcam, plc, Cambridge,UK) using appropriate protocols provided by antibody suppliers. Sections were counterstained with haematoxilin.

Histotopography. For Histotopographic examinations, histological samples of the liver of experimental animals and birds with a thickness of 3-5 micrometers were prepared.

Area of a tissue sample is up to 2cm x 4 cm. Samples were colored with Hematoxylin and Eosin (H&E) and Masson's trichrome stains. Tissue samples were digitized with MoticEasyScan Pro 6-FS scanner with x40 magnification (0.26 μm/pixel resolution). As a result, the original tissue sample was increased 1 000 times. High resolution digital images were visualized by Motic DSAssistant software, in which different types of distance measurement and morphometry tools were used for the analysis of slide images.

Corrosion Casting. The corrosion casts of the portal and hepatic veins of the pigs were prepared by injecting the solidifying liquid latex "Nairit" (Yerevan, Armenia). Latex was injected by the 20-gram graduated standard syringe under the manual pressure, through the catheter fixed a) into the extrahepatic part of the portal vein and b) into the inferior vena cava. The corrosion of liver tissue was performed in 40% sulfuric acid, during 3 days with the following washing in running water. The obtained casts were studied macro- and microscopically by using a light stereomicroscope (ProScope HR device, Bodeline Technologies, US).

The corrosion casts of the portal and hepatic veins of the rats were prepared by injecting the "Protacryl-M" (see below). The abdominal cavity of the Wistar rat, weighing 200-220 g was opened under general ether anesthesia. The catheters with appropriate diameters were inserted in the portal vein and caudal vena cava and fixed with ligatures. The liver vessels were washed out via portal vein catheter with the cocktail including 100 ml 0.9% NaCl, 1,0 ml Atropine, 1,0 ml No-Spa, 1ml Heparin, and 1 ml 2% Novocain. Outflow was achieved through the catheter inserted in the caudal vena cava. Cra-

Table 1. The number, age, and distribution of animals according the research methods

Research method / Mammal/bird	Anatomical preparation	Histologiy, histotopography	Histochemistry	Immunohistochemistry	Fluorescence microscopy	Corrosive specimen	SEM Corrosive specimen	Total
Pig (1 year old)	2	2	2	-	2	2	-	4
Sheep (1 year old)	2	2	2	-	2	-	-	3
Rat (6 months old)	2	6	6	2	2	2	2	14
Hen (6 months old)	3	3	3	-	2	-	-	3

note: Several research methods were used on the same livers

nial vena cava was ligated preliminary. After washing out the liver, the injection of the"Protacryl-M" cocktail ("Protacryl-M" powder 3 cm3 dissolved in 7.5 ml of its liquid component) was performed through both catheters under manual pressure. Cocktail injected in portal vein was colored by red pigment, while the cocktail injected in hepatic veins – by the blue pigment (Protacril powder, liquid component and the pigments are the components of standard "Protacryl-M" kits – Kharkiv, Ukraine). The liver tissue was corroded in 20% KOH solution, in-room temperature after Protacril cocktail polymerization. After 2 hr fragments were washed out in distilled water for 10 min three times, the process was repeated 6 times. Dried casts were studied with the ProScope HR device.

Fluorescent Microscopy. Immunofluorescence images were were obtained by the imunofluorescence microscope Nikon H550L (Japan). The digital images were captured by Infinity 2 camera provided with Infinity software version 6.5.6 – for measuring and analyzing.

Results and discussion. In all investigated livers, around the portal and hepatic veins, including their thinnest branches/tributaries, there are connective tissue sheaths of various thicknesses, consisting of different ratios of different types of connective tissue fibers. These sheaths communicate with each other, as well as with the liver capsule and the interlobular mesh of connective tissue, creating a single extracellular matrix - the connective tissue skeleton of the organ.

Porcine. In the porcine liver, both the portal and caval ports are located close to each other at the dorsal (posterior) surface of the liver (Fig 1a). Large-caliber portal and hepatic veins are arranged on the planes situated more or less parallel to each other. The above-mentioned blood vessels intersect with each other, but only within the mentioned planes. At the same time, their thin branches intersection might happen with different angles (Fig 1b). The hepatic veins are located above the portal vein branches (cranially).

In porcine liver, the mesh-like structure of the connective tissue fibers links the portal tracts with each other and separates the liver lobules of different sizes (from 0.5 mm to 2,2 mm in diameter) and shapes (irregular polygons). The connective tissue septs positioned among the liver lobules are quite thick (5-15 mm). In addition, these septs in some areas involve the connective tissue sheaths of the hepatic vein tributaries. Due to this feature, not rare the connective tissue framework enveloping the classic lobules includes not only the portal tracts but also the tributaries of the hepatic veins, which makes the architecture of the soft skeleton of the liver even more complex. Thus, the connective tissue sheaths of the portal tracts and the hepatic vein tributaries are connected to each other by numerous connective tissue septs separating the liver lobes. The numbers of liver lobules situated between the hepatic veins and the portal tracts might vary from one to several (Fig 1c,d,g,k).

The described fibers of the connective tissue structure continue into the interlobular connective tissue, located in the space of Disse, which proves the formation of a single connective tissue labyrinth, likewise, it was described in cat liver [3] (Fig 1e,f). The liver capsule (the Laennec capsule), consisting of thin elastic and I-type collagen fibers, is connected with the above-mentioned connective tissue interlobular septs, which fibers extend into the intralobular extracellular matrix (Fig 1h). At the same time, in some areas, between the thin connective tissue plates (0.2-0.5 μm) covering liver lobules, from the one hand, and the Glisson's capsules of the large portal pedicles, from the other hand, were found the fissures, which were crossed over by the single connective tissue fibers establishing the communication between the Glisson's plates and the derivates of Laennec's capsule (Fig 1i,j). These plates are similar to those described in the human liver 23,24]. However, we couldn't find the "proper liver ligament" identified by Ikeda et al., between the Laennec's and Glisson's plates using the computer program processing of liver histotopogramms [16]. We think that such identification of "new structure" is somewhat artificial which is resulted from the "ability" of the used computer program.

The described fissure, as well as the connective tissue fissures inside of the Gleason capsule, according to our previous studies, represents one of the compartments of the pre-lymphatic circulation basin [5].

Masson's trichrome differently dyes different connective tissue fibers and muscles (the elastic fibers in pomegranate color, I-type collagen – in blue (flesh-color), and the III type collagen – in brown colors). Besides these structures are characterized by different self-fluorescence lightening in fluorescent microscopy. Considering the above mentioned we can conclude that the connective tissue structures of the liver capsule, interlobular septa, and hepatic vein tributaries sheaths are mainly represented by the interweaving of the I-type collagen and elastic fibers. hepatic vein tributaries sheaths additionally contain the longitudinal smooth muscle fibers, the individual bundles of which are separated from each other by thin connective tissue inserts (Fig 1k,l).

The single smooth muscle fibers are found in portal vein adventitia as well. In addition, the portal vein has its own connective tissue coat enveloping its adventitia. A narrow fissure (gap) can be detected between the adventitia and the mentioned coat. The arteries, bile ducts, and nerve trunks (vagus branches) in the portal tracts are characterized by similar covers (Fig 1m,n). These findings fully agree with the data obtained in the 60s and 70s of the last century by several researchers and summarized by Kovanov and Anikina [25] identifying the fissures around the tubular structures of portal tracts. These fissures represent the areas for the interstitial fluid and pre-lymph circulation and confirm that the extracellular matrix of the liver represents a single basin for the extravascular microcirculation [5].

The walls of the portal vein and bile duct contain elastic fibers. In the walls of bile ducts, these fibers are accompanied by single muscular bundles. Elastic fibers are abundantly represented also in the perineurium, as well as in the subendothelial and external layers of the branches of the hepatic artery.

A significant portion of the connective tissue skeleton of the portal tract is represented by thick, well-structured fibers of the I-type of collagen (Fig 1i,m).

In the porcine liver, we couldn't identify some connective tissue structures described in the human portal tract. In particular, the human portal tract contains 2 connective tissue layers located around the portal vein, the 1st of which, located close to the portal vein, contains the branches of the hepatic arteries, and the other, located peripherally - contains the bile ducts and extramural peribiliary glands. There are no similar layers in the portal tracts of the porcine liver.

The intramural biliary (mucosal) glands are quite common in the bile ducts of the porcine, while the extramural peribilliary glands are rare. Besides, it is notable, that these glands are opened into the biliary lumen from all sides, more or less evenly (Fig 1o), while in humans these glands are usually located along the two opposite sides of the ducts [13,26,27].

Porta-caval connective tissue connections by the merging of the elements of the connective tissue sheaths of large portal tracts and the hepatic veins, we could not find in pig livers. In addition, with some assumptions, the fibril-like or septal connections of the portal and venous sheaths, consisting of connective tissue fibers, can be considered as analogs of Porta-caval connections described in humans (Fig 1c,d,g).

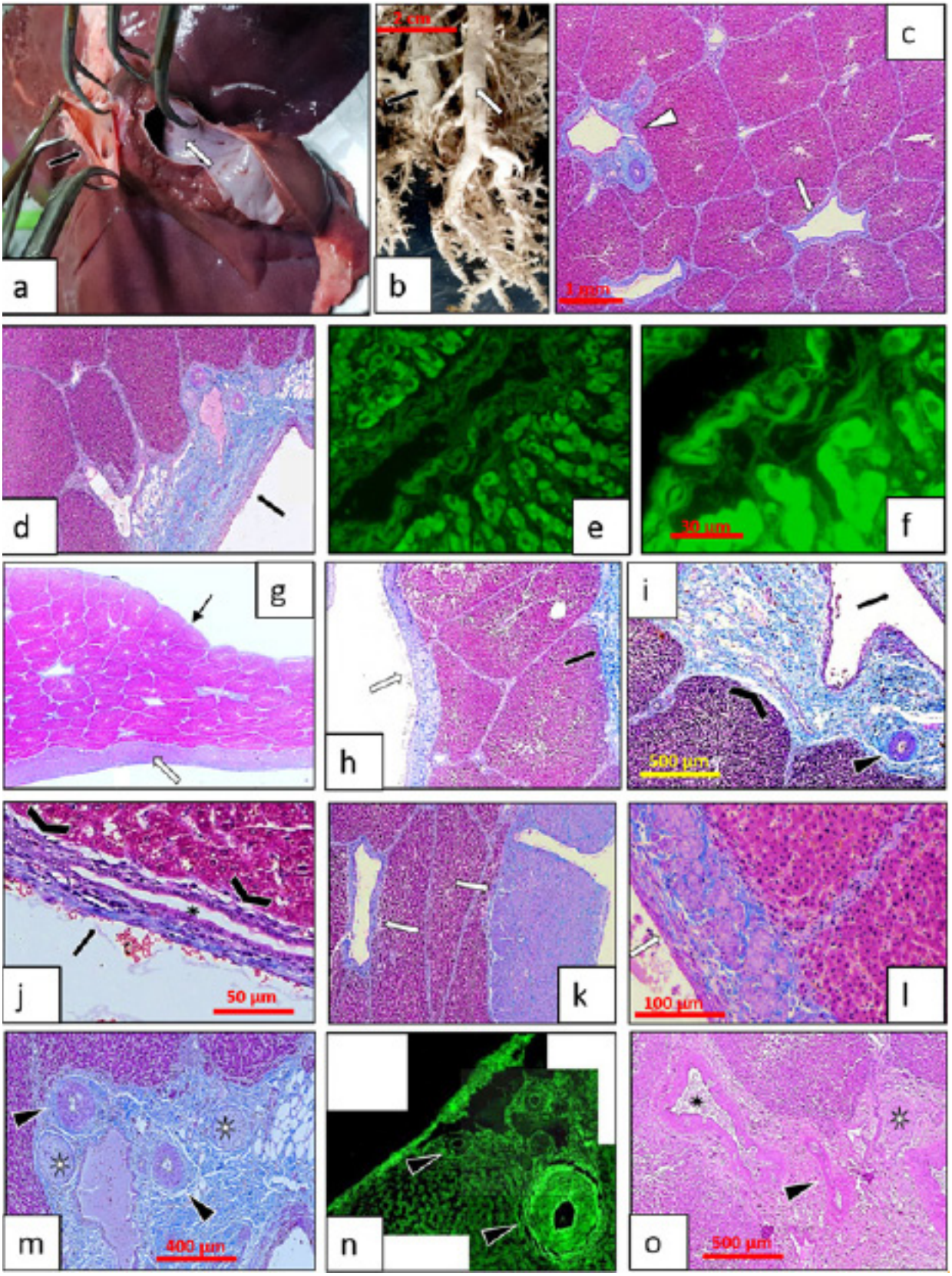

Fig. 1. Porcine Liver. a - the macro-anatomical specimen; b - corrosion casts of portal and hepatic vein; c,d,g,h,i,j,k,l,m – histotopograms of liver tissue (Masson's trichrome staining); e,f,n – fluorescent microscopy of liver tissue (self-fluorescence); o – histology of liver tissue (H&E). White arrow – hepatic veins; black arrow – portal vein; white arrowhead – portal tract; free arrow – hepatic capsule (Laennec's capsule); black arrowhead – hepatic artery; pterygoid arrowhead – fissure; white asterisk – nerve (the branch of vagus); black asterisk – bile duct (bile ductule)

Hen. The portal and caval ports of the hen liver are separated from each other to some extent. The hepatic veins have an extrahepatic section of considerable length (not found in mammals) and the hen liver is connected to the dorsally located inferior (caudal) vena cava by 3-5 hepatic veins covered with peritoneum (Fig 2a). Posteriorly, liver is connected to the small intestine by the ligament, which is created from the duplication of the peritoneum as well, and in the depth of which the bile duct, hepatic portal vein, hepatic artery and the gallbladder are located [28].

Intrahepatic sections of portal complex and hepatic veins are usually located on planes at different angles to each other and therefore spatially intersect with each other.

The structures made of different types of connective tissue fibers are more sharply differentiated within the hen portal tracts in comparison with the porcine liver. In particular, the own capsule (sheath) of the portal vein is formed by the type-I collagen and circular elastic fibers, which are tightly twisted with the portal vein adventitia. The thickness of this capsule does not exceed 20-30 μm. The portal vein with its connective tissue sheath is situated in the connective tissue of the portal tract, which is mainly composed of type-I collagen fibers of different sizes and directions (Fig 2b,c,d,e,f). Sometimes, in the immediate vicinity of the portal vein, they form small areas (spaces) in which longitudinal smooth muscle fibers are located (Fig 2c,g).

In those regions where thin daughter branches separate off the portal vein, the amount of type -I collagen gradually decreases, rarely "disappears momentarily" and the thin branch of the portal vein remains surrounded by a thin sheath (3-5 μm) of type-III collagen and elastic fibers (Fig 2b,c). The fissures between the sheath and adventitia of the portal vein, similar to those found in pig liver might be also found in the hen liver but less frequently (Fig 2g).

The arteries located in the portal tract are characterized by a well-defined muscular layer. At the same time, their adventitia is so thin that it is often impossible to identify. The connective tissue covering around the artery, as it was described in the human and porcine livers, is practically absent in hens. The study of the histotopograms gives the impression that the arteries with a well-developed muscular layer are directly "inserted" into the connective tissue structure of the portal tract, mainly formed by type-I collagen fibers. At the same time, the contact between the

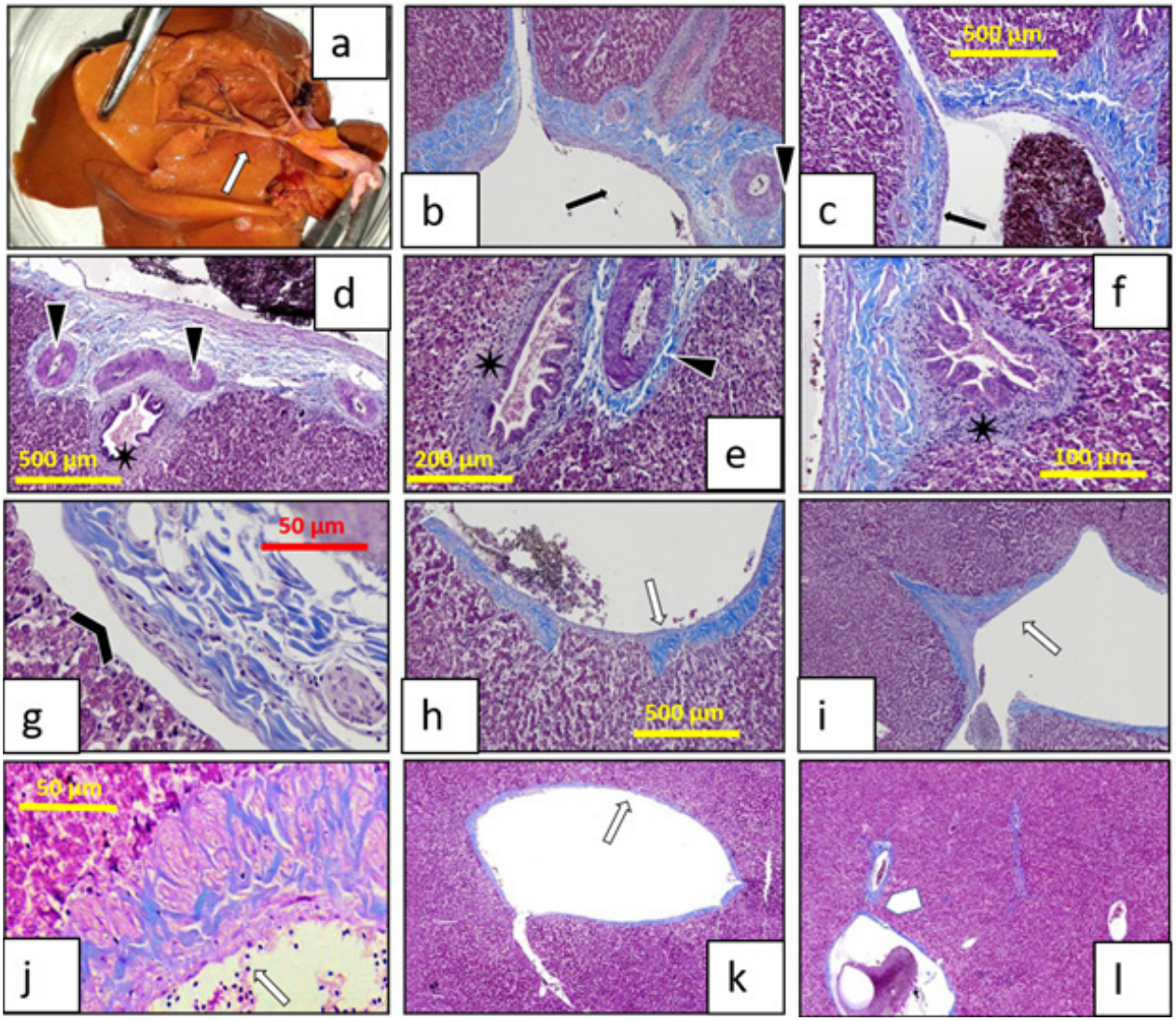

Fig. 2. Hen Liver. a - the macro-anatomical specimen; b - l – histotopograms of liver tissue (Masson's trichrome staining). White arrow – hepatic veins; black arrow – portal vein; white arrowhead – portal tract; free arrow – hepatic capsule (Laennec's capsule); black arrowhead – hepatic artery; pterygoid arrowhead – fissure; white asterisk – nerve (the branch of vagus); black asterisk – bile duct (bile ductule); pentagonal arrow – fibril-like porta-caval fibrous connection

artery wall and surrounding collagen fibers is loose, sometimes to such an extent that it becomes possible to identify thin (1-5 microns) gaps (Fig 2b,d,e).

The wall of the bile ducts is thick. They are covered with a dense peribiliary capsule of type-I collagen fibers, which contains a large number of fibroblasts. The epithelial layer is wrinkled to such an extent, that the lumen of the bile ducts is often star-shaped on the slices. Furthermore, small-diameter ductular profiles lined with epitheliocytes were found in the peribiliary connective tissue around the lumens of bile ducts (Fig 2d,e,f,). This indicates that the hen bile ducts are also supplied by the peribiliary mucous glands. Considering our previous studies it was expected [29] as the hens have gallbladder. The high degree of wrinkling of the epithelial lining of the bile ducts co-exists with the multi-rowed positioning of epithelial cells, which imitates the proliferation.

The bile ducts are always located at the periphery of the portal tract. Its connective tissue sheath is connected to the connective tissue skeleton of the portal tract constituted by type-I collagen fiber from one side, while from the other side directly adjoin to the liver parenchyma. In such regions, the connective tissue fibers of the duct membrane continue directly into the adjacent lobules, thus creating a unified structure of the extracellular matrix of the liver (Fig 2e,f). But in regions where instead of the bile ducts the connective tissue of the portal tract is adjoined to the liver parenchyma, it is possible to identify fissures between these structures. Such fissures are bordered on the one hand by the fibers of the portal tract and on the other, by the capsule directly covering the parenchyma, the separate fibers of which extend into the intralobular matrix (Fig 2g).

The connective tissue fibers, surrounding the large hepatic veins (both type-I and type-III collagen and elastic fibers), are tightly intertwined and form the sheaths of these veins. Besides, the greater the venous lumen the greater the thickness of the connective tissue sheaths (Fig 2h,i). The thickness of the connective tissue sheath around the main veins of the liver reaches 100 μm. The type-I collagen fibers pass through the surrounding layer of the III type collagen and elastic fibers, thus giving the impression of making septums in the mentioned layer. Longitudinal muscle fibers (running alongside the veins) are located in some spaces bordered by these septums (Fig 2 h,j). The thickness of the connective tissue sheath containing such elastic and muscular fibers increases locally at the junctions of the tributaries with the hepatic veins (Fig 2 I,k). It should has a certain sphincter-like and blood-flow-supporting function. The connective tissue sheaths formed around the smaller veins are much thinner and mainly contain type-III collagen fibers, part of which extends directly into the connective tissue skeleton of adjacent lobules.

Generally, PCFC is not observed in chicken liver. The interconnection of portal tracts and separate connective tissue fibers, covering the hepatic veins, is observed only occasionally. Considering their shape, these connections can be called fibrillar-like, or at most, plate-shaped PCFC (Fig 2l).

Rat. The portal and caval ports are located close to each other at the dorsal (posterior) surface of the rat liver (similar to those described in the porcines). The hepatic veins are located above (cranially) the portal veins. The large portal and hepatic veins are located in planes more or less parallel to each other. They intersect with each other, but within the specified plane. In addition, thin branches of the portal vein and hepatic veins can intersect at different angles (Fig 3a).

In the liver of rats, in at least one or two lobes, we found an area where the fibers of the portal connective tissue fuse with the connective tissue surrounding the hepatic vein. Sometimes this fusion might be so intim that above-mentioned two types of veins can have one single (united) wall. Such areas, which are considered to be PCFCs, similar to human PCFCs, are the areas where not only connective tissue fibers can intertwine, but some structures of the portal complexes can be displaced into the connective tissue sheath covering the hepatic veins (Fig 3b,c,d,e,f,g).

The adjoining of the branches of the hepatic artery to the walls of the hepatic veins in the areas of close contact of the portal tracts with the branches of the hepatic veins was described many years ago [30]. More recently, we also found the bile ducts dislocated from the portal tract towards the hepatic veins. Notably, in 2014, we confirmed that translocated ducts can accompany the hepatic veins up to their small tributaries, including the sublobular and central veins; Therefore, we assumed that in some parts of the rat liver, the outflow of bile from the bile capillaries can occur not only in the interlobular bile ducts located in the portal tract but also in the bile ducts passing along the central and/or sub-lobular venules [31].

Various forms of portocaval connections have been identified in rat liver, starting from the complete merging and ending with fibrill-like connections. In the regions of complete fusion, the branch of the hepatic vein is connected with the bilio-vascular triad of the portal complex as the fourth element. This biliary-vascular quaternion are enclosed in a single common capsule - the sheath, similarely to the one described in the human livers [32].

In rat liver, the peculiarities of the location of connective tissue fibers, going along the portal tracts of different sizes and the tributaries of the hepatic veins, substantially repeat those described in the hen liver. In addition, it should be noted that in the liver of rats elastic fibers are found in smaller amounts, as well as the muscular layer of arteries is less vividly pronounced. It is also noteworthy that in the rat liver hilum and in the area of large vessels, the boundary between the Glisson's capsule and the Laennec's capsule is distinguished. The latter, as in the porcine liver, covers not only the liver surface but also separates its parenchyma from the adjoint portal tracts and tributaries of the hepatic veins (Fig 3h,i). Besides, we have confirmed the opinion of other researchers, that the fissures, between the sheats of small portal tracts and hepatic veins, on the one hand, and the connective tissue sheaths of the liver parenchyma, on the other hand, is no longer identified. The fibers of all the named connective tissue structures intertwine, forming a single extracellular carcass of the liver (Fig 3j,k). At the same time, gaps are maintained between the adventitia and connective tissue capsule of individual blood vessels, on the one hand, and the above-mentioned capsule and Glisson's sheath covering the entire portal tract, on the another hand. The results of scanning electron microscopy of the corrosion casts of the hepatic blood vessels presented in Fig 3l,m and described and discussed in detail in our previous papers [33], confirm the above-mentioned. In particular, in some of the corrosion casts, a "leak" of the hardening mass introduced into the portal vein was found. Some of these leaks travel around the vessels - in the spaces bounded by the perivascular capsule. These hardened leaks might form the sheaths that enveloping the casts of the blood vessels. Some sheaths contain casts of two blood vessels, but some are empty. In several samples, we have found the patterns where one empty (free from the vessel) sheath was surrounded by another. In addition, a direct continuation of these sheaths into the casts of intralobular connective tissue spaces (perisinusoidal spaces of Disse) was found. This once again confirms the existence of the extracellular matrix of the liver as a single structure.

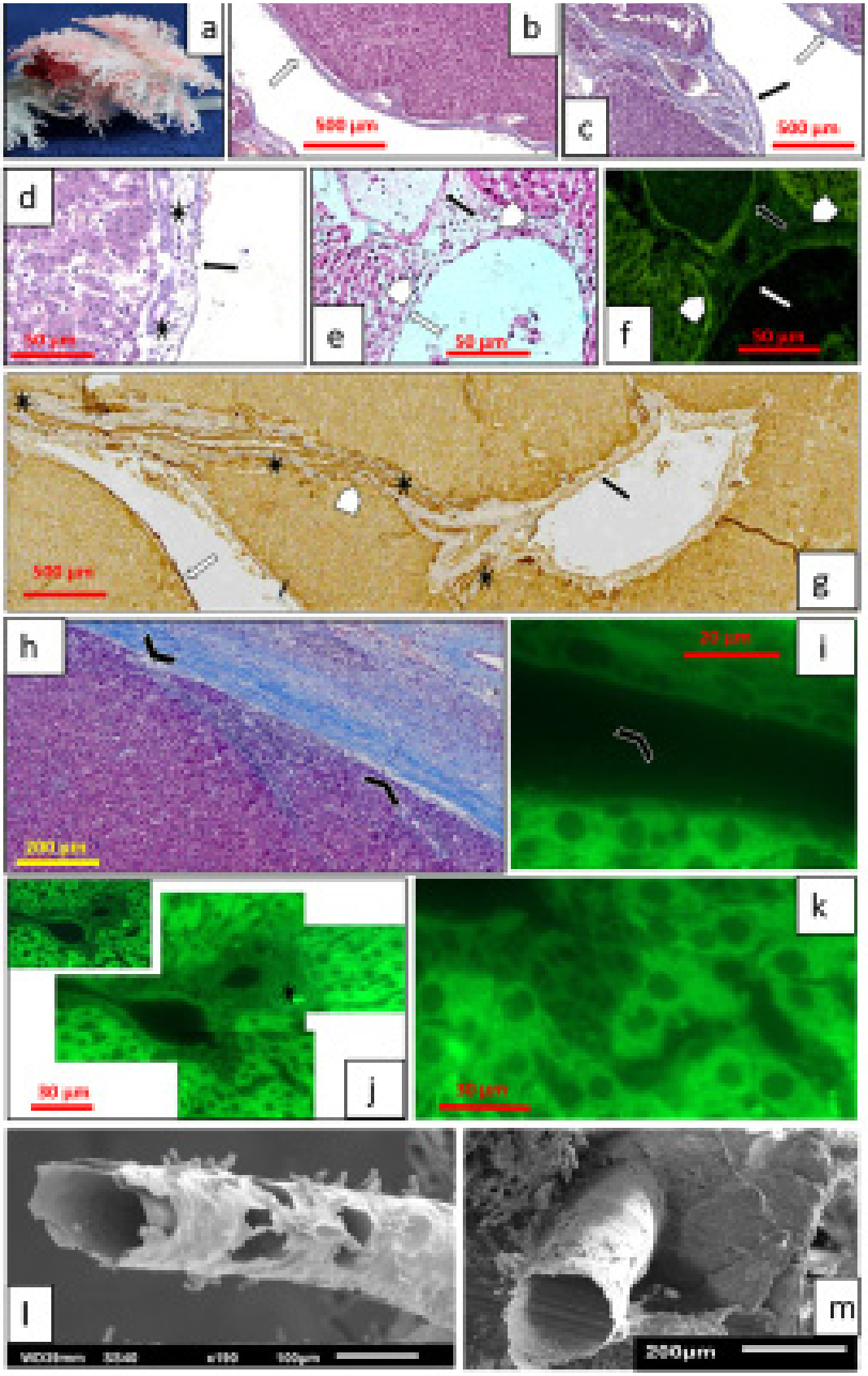

Fig. 3. Rat Liver. a - the corrosion casts of portal (red color) and hepatic (blue) veins; b,c,d,e,h – histotopograms of liver tissue (Masson's trichrome staining); f,i,j,k - fluorescent microscopy of liver tissue (self-fluorescence); g- immunohistochemistry (AE1-AE3) of liver tissue; l,m - scanning electron microscopy of corrosion casts. White arrow – hepatic veins; black arrow – portal vein; pterygoid arrowhead – fissure; black asterisk – bile duct (bile ductule)

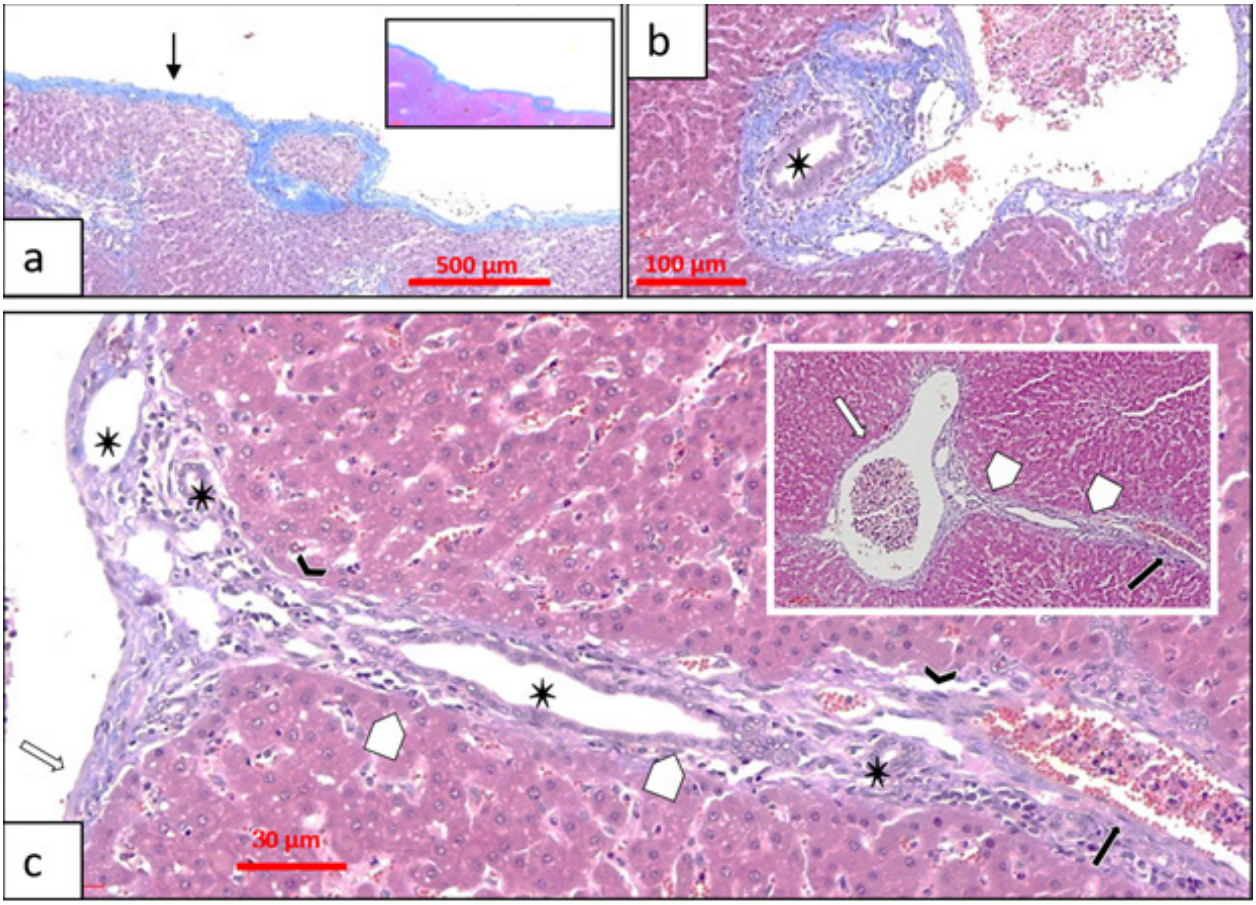

Fig. 4. Sheep Liver. a-c – histotopograms of liver tissue (Masson's trichrome staining); White arrow – hepatic veins; black arrow – portal vein; free arrow – hepatic capsule (Laennec's capsule); pterygoid arrowhead – fissure; black asterisk – bile duct (bile ductule); pentagonal arrow – fibril-like porta-caval fibrous connection

Sheep. In the liver of the sheep, the portal and the caval ports are somewhat separated from each other (the hilum of the portal vein is located more ventrally). In this respect, sheep liver, like cow liver [34], shows more similarity with the human liver. The portal and hepatic veins, respectively, are located at the planes, which are at different angles to each other and intersect spatially.

The structure and location of the connective tissue structures of sheep liver are more similar to the structure and location of connective tissue structures of rat and hen livers than that of porcine liver. Besides, there is a difference: the liver capsule (Laenek's capsule) contains a large amount of type-I collagen and a small amount of type-III collagen and elastic fibers (Fig 4a).

The bile ducts, which have the star-shaped lumens (similarly to hens bile ducts) and are covered with a sheath containing type-III collagen and single elastic fibers, do not directly touch the liver parenchyma but is separated from it with the type-I collagen fibers. The latter borders the liver parenchyma which in turn is covered by the derivate of Laenek's capsule (Fig 4b).

PCFC was detected only in isolated cases. For the most part, these connections are plate-shaped and contain bile ducts, making the sheep liver like the liver of a rat (Fig 4c).

Conclusion. In mammalian and bird livers, the connective tissue sheaths of various thicknesses and compositions around the portal tracts and hepatic veins are interconnected in various ways with each other, the liver capsule and intralobular connective tissue network. This system of connective tissue fibers forms the so-called liver extracellular matrix - the connective tissue skeleton of the liver.

The connective tissue sheaths around the portal tracts and the hepatic veins might be connected to each other in the form of fusion, touching, septum (plate-shaped), or thread-shaped connections when intersecting each other. Such connections form a sturdy extracellular matrix and strengthen the architecture of the liver tissue that helps the organ maintain its integrity under various pathological (including traumas) conditions. Due to the minimal number (virtually absent) of portocaval connective fibrous connections, compared to that of the livers of pigs, sheep, and rats, the hen livers seem to be more vulnerable to mechanical damage.

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SUMMARY

LIVER EXTRACELLULAR MATRIX PECULIARITIES IN MAMMALS AND AVIANS

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Analysis of liver matrix studies makes it obvious that knowledge about the connective tissue skeleton of the liver is not systematized, the terminology is contradictory, and the question of the construction of some components sometimes causes controversy. We set a goal to study the features of the construction of the connective tissue matrix of the liver of various mammals and birds in order to identify and systematize general and specific patterns of this structure.

The liver of mammals with a gallbladder (pigs, sheep) and without a gallbladder (rats) and birds (domestic chickens with a gallbladder) was studied by the methods of anatomical preparation, histology, histochemistry, histotopography, immunohistochemistry, scanning electron microscopy of corrosion replicas and fluorescence microscopy.

In the liver of mammals and birds, connective tissue membranes of various thicknesses and compositions around the portal tracts and hepatic veins are revealed. These membranes are connected in various ways with each other, the liver capsule and the intralobular network of connective tissue and form an extracellular matrix, which strengthens the structure of the liver tissue and helps the organ maintain its integrity in various pathological conditions.

Keywords: portal sheath, hepatic vein sheath, liver matrix, porta-caval fibrous connections, liver capsule.

РЕЗЮМЕ

ОСОБЕННОСТИ ВНЕКЛЕТОЧНОГО МАТРИКСА ПЕЧЕНИ У МЛЕКОПИТАЮЩИХ И ПТИЦ

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Анализ исследований матрикса печени выявил, что знания о соединительнотканном каркасе печени не систематизированы, терминология противоречива, а вопрос о строении того или иного компонента печеночного матрикса вызывает споры.

Целью исследования явилось определение особенностей строения соединительнотканного матрикса печени различных млекопитающих и птиц для выявления и систематизации общих и частных закономерностей этой структуры.

Печень млекопитающих с желчным пузырем (свиньи, овцы) и без желчного пузыря (крысы) и птицы (домашние куры с желчным пузырем) изучали методами анатомического препарирования, гистологии, гистохимии, гистотопографии, иммуногистохимии, сканирующей электронной микроскопии коррозионных реплик и флуоресцентной микроскопии.

В печени млекопитающих и птиц выявляются соединительнотканные оболочки различной толщины и состава вокруг воротных трактов и печеночных вен. Эти оболочки различными способами связаны друг с другом, капсулой печени и внутрилобулярной сетью соединительной ткани, образуя внеклеточный матрикс, который укрепляет структуру ткани печени и помогает органу сохранять целостность при различных патологических состояниях.

რეზიუმე

ღვიძლის ექსტრაცელულური მატრიქსის თავისებურებები ძუძუმწოვრებსა და ფრინველებში

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კვლევების ანალიზი უჩვენებს, რომ მონაცემები ღვიძლის შემაერთებელი ქსოვილის ჩონჩხის შესახებ არ არის სისტემატიზებული, ტერმინოლოგია წინააღმდეგობრივია და ზოგიერთი კომპონენტის სტრუქტურის საკითხი ზოგჯერ ურთიერთსაწინააღმდეგოდ არის წარმოდგენილი.

კვლევის მიზანს წარმოადგენს სხვადასხვა ძუძუმწოვრებისა და ფრინველების ღვიძლის შემაერთებელქსოვილოვანი კარკასის აგებულების თავისებურებების, მისი სტრუქტურის ზოგადი და სპეციფიკური ნიშნების იდენტიფიცირება და სისტემატიზაცია.

ანატომიური პრეპარაციის, ჰისტოლოგიური, ჰისტოქიმიური, ჰისტოტოპოგრაფიული, იმუნოჰისტოქიმიური, კოროზიული ტვიფრების მასკანირებელი ელექტრონული მიკროსკოპიის და ფლუორესცენტული მიკროსკოპიის მეთოდებით შესწავლილია ნაღვლის ბუშტის მქონე (ღორი, ცხვარი) და ნაღვლის ბუშტის არმქონე (ვირთაგვა) ძუძუმწოვრების და ნაღვლის ბუშტის მქონე ფრინველების (მამალი) ღვიძლები.

ძუძუმწოვრებისა და ფრინველების ღვიძლში პორტული ტრაქტებისა და ღვიძლის ვენების გარშემო გამოვლინდა სხვადასხვა სისქის და კომპოზიციის შემაერთებელქსოვილოვანი გარსები, რომლებიც სხვადასხა ფორმით არის დაკავშირებული ერთმანეთთან, ღვიძლის კაფსულასთან და შემაერთებელი ქსოვილის წილაკშიდა ქსელთან და ქმნის ღვიძლის ერთიან მატრიქსს, რომელიც სიმტკიცეს აძლევს ღვიძლის ქსოვილის სტრუქტურას და უზრუნველყოფს ღვიძლის სტრუქტურული მთლიანობის შენარჩუნებას სხვადასხვა პათოლოგიური ზემოქმედების პირობებში.