Histological definition for the gray scale ultrasonography of the rabbit liver

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Abstract: The aim of the present study is to prove that the morphological and histological features of the rabbit liver are base for the creation of proper anatomical US image. For the purpose, we use 12 clinically healthy New White Zealand rabbits. In the histological study, we use the routine staining with Hematoxylin/Eosin. The US study was carried out with ultrasonic equipment for 2D visualization. The US image of the rabbit liver was produced by the different acoustic impedance of the tissues, which composed the organ. The variability of the grey and white nuances when observing the anatomical US image of the rabbit liver is produced by its histological features. It is not relative to the orientation of the transducer to the field of study. There was a variability of the US acoustics of the liver at the same intensity of the US wave. This is also owing to the histological features of the liver and biliary ducts. US visualization of the rabbit liver is because of the dispersion character of the echo-signal, generated by parenchyma, perivascular connective tissue and extrahepatic biliary ducts. The different acoustics of *capsula fibrosa* and liver parenchyma is related to the following US indices: brightness and contrast, in accordance to the grey-white scale, a variety of the grey nuance and speed of the US wave. We present the following conclusion: The US morphological character of the studied organ is defined by its histological features. These histological features of the liver could be accepted as "Golden standard", because they define the US anatomical visualization of the organ.

Keywords: Rabbit, liver, histology, ultrasonography.

Tavşan karaciğerinin gri skala ultrasonografisi için histolojik olarak tanımlanması

Öz: Bu çalışmanın amacı, tavşan karaciğerinin morfolojik ve histolojik özelliklerinin uygun anatomik ultrason (USG) görüntüsünün elde edilmesi için referans teşkil edebileceğini kanıtlamaktır. Bu amaçla, çalışmada 12 adet klinik olarak sağlıklı Yeni Zelandalı tavşanı kullanılmıştır. Histolojik incelemede, Hematoksilen / Eozin rutin boyama yöntemi kullanılmıştır. Ultrason çalışması ise 2D görselleştirme için ultrasonik ekipmanlarla gerçekleştirilmiştir. Tavşan karaciğerinin ultrason görüntüsü, organı oluşturan dokuların farklı akustik empedansı ile üretilmiştir. Tavşan karaciğerinin anatomik USG görüntüsü gözlemlenirken oluşan gri ve beyaz nüansların değişkenliği ise histolojik

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özellikleri yardımıyla üretilmiştir. Karaciğerin USG akustiğinde USG dalgasında aynı şiddette değişkenlik gözlemlendi. Bu durumun karaciğer ve safra kanalının histolojik özelliklerine bağlı olarak şekillendiği düşünülmektedir. Tavşan karaciğerinin USG görüntülemesi, parankim, perivasküler bağ dokusu ve ekstrahepatik safra yolları tarafından üretilen yankı sinyalinin dağılma karakteri nedeniyle gerçekleşmektedir. Fibröz yapıdaki kapsülün ve karaciğer parankiminin farklı akustiği; parlaklık ve kontrast, gri-beyaz ölçeğe göre, çeşitli gri nüans ve USG dalgasının hızı gibi USG indeksleriyle ilgilidir. Dolayısıyla bu çalışmada çalışılan organın USG morfolojik karakteri, histolojik özellikleri ile tanımlanmakta olduğu sonucuna varılabilir. Karaciğerin bu histolojik özellikleri, USG'nin organın anatomik olarak görselleştirilmesini tanımladığı için "Altın Standart" olarak kabul edilebilir.

Anahtar sözcükler: Tavşan, karaciğer, histoloji, ultrasonografi

Introduction

The histological features of the tissues determine the ultrasonographic (US) features of the organs. This fact corresponds to the application of the imaging methods for the anatomical study of the organs. The histological interpretation for studying of the given soft tissue finding is perceived in the world medical practice as "Golden standard", which is necessary for the interpretation of the obtained US image (8, 14, 29).

Data from the studies on the acoustic impedance of the tissues show that the histological features of the tissues are key factors to obtain of objective US anatomical results (7, 16).

The loose connective tissue between the hepatic lobules in the man and cat is hardly developed, while the same in the swine is well constructed (3). Thus, the different echogenicity at the same frequency of the US wave is a result of the tissue specificity in the structure of the liver of these biological species.

US image of the organs is resulted by some indices: image contrast in accordance with the grey-white scale, a variety of the grey nuance, alteration of the microstructural features of the organ to its macrostructure and speed of the US wave (2, 24, 25).

US images give information for the micro morphological features of the tissues. The acoustic impedance of the US wave determines the degree of its penetration in the tissues. US wave which reaches the border between two biological mediums is reflected partially by the first medium and in the second the wave demonstrates fragmented transmission. The amplitudes between the reflection and transmission of the wave are proportionally dependent on the inertial and the elastic properties of the tissues (5, 13, 15, 19).

The histological specifics of the human organs correspond to their US visualization. The force of the reflected US signal depends on the direction, in which the biological object is orientated to the US wave. When the transducer is posed perpendicularly to the tissues, the echo signal is stronger (14).

The connective tissue in the capsule, parenchyma and the blood vessels in the internal organs is a morphological mark, which defines the intensity of the acoustic impedance (6, 9, 23, 27).

The connective tissue structures have higher echogenicity, compared to the other tissue components. The US image of the connective tissue components, in accordance with the grey-white scale, is intensely white (hyperechoic) (1, 10, 20).

The relative amplitude of the US wave and the characteristics of the US image, in accordance with the grey-white scale, are connected to the presence of elastic and collagenous fibers. The connective tissue in liver, heart, kidneys, spleen and urinary bladder

has similar US characteristics. So, these organs are used as soft tissue norm for echogenicity (12, 26).

The homogeneous character of the rabbit liver echogenicity makes it ideal US morphological model (17, 18).

US signal, generated by tissues with equal acoustic impedance possesses dispersion character (hypoechogenicity), while the same produced by tissues with different acoustic impedance is markedly reflective (hyperechoic) (14, 21).

The histological features of the liver are used as a base for the US substrate of the liver image. The scarce data for the histological definition of the US anatomical visualization of the rabbit liver motivate us to conduct the present comparative histosonographic study.

Materials and Methods

Materials: Twelve sexually mature, clinically healthy New Zealand white rabbits, at age 8 months and weighed from 2.8 kg to 3.2 kg were included in the present study. Six animals were euthanized with 150 mg Thiopental® (50 mg/kg, I V) (thiopental sodium 1000 mg) Biochemie, Austria i v (22) (protocol 209/24.10.2012; 213/14.11.2012; 220/12.12.2012; 231/04.02.2013) and used for anatomical and *postmortem* US studies. Six rabbits were supplied by a commercial slaughterhouse regulated for meat processing of lagomorphs in compliance with the legal requirements regarding the hygiene and inspection at slaughter (Regulation No 36 of 23/03/2006, prom. SG No. 35 of 28/04/ 2006). The study was carried out in accordance with the European convention for protection of vertebrates used for experimental and other scientific purposes (Strasbourg/16/05/1986), the European convention for protection of companion animals, and the Law on Animal Protection in Bulgaria (Section IV - Experiments on animals, art. 26, 27 and 28, adopted on 24.01.2008 and published in SG. 13 of 2008). The study was conducted in strict compliance with the university ethical guidelines.

Methods:

a. Hematoxylin (Ehrlich) Eosin staining: Liver and gall bladder were dissected. The samples were with size 5 mm and taken from *lobus hepatis sinister lateralis*; *lobus hepatis sinister medialis*; *lobus quadratus*; *lobus hepatis dexter*; *lobus caudatus* (*proc. papillaris* and *proc. caudatus*) and *vesica fellea*. After fixation in 10% formalin solution (Merck KGaA, Darmastadt, Germany), the tissue samples were rinsed with water, dehydrated in an ascending ethanol series, cleared in xylene and paraffin embedded. The slices' thickness was from 5 to 7 μm. We worked with rotary microtome YD-335A (J. Y. M. A. Ltd., China). The staining was performed after the sections were double-deparaffinized for 30-60 s in xylene (two cuvettes) and passed through series of graded alcohols (from absolute to 70% ethyl alcohol), then they were transferred in water and hence in hematoxylin (in Erlich) for 2-5 min. Once stained, the slides were rinsed in water, stayed in the distilled water for 5-10 min and stained by Eosin for a few seconds. The sections were rinsed in water, dehydrated with increasing alcohol sequence and cleared in xylene for a few seconds. Finally, the slides were mounted with Canada balsam (4, 28).

The slides were observed with light microscope – VDN-200M (LUMENLAB, China), and the results were analyzed by employing digital micro camera CMOS. Data were processed by software - ScopeImage Advanced Micro-Image Process Software.

The study was conducted in the Unit of Cytology, Histology and Embryology, Department of Veterinary Anatomy, Histology and Embryology,

Faculty of Veterinary Medicine, Trakia University, Stara Zagora.

b.2D US method for histosonographic analysis: The study of the rabbit liver was *post-mortem*. It was carried out with ultrasonic system Diagnostic Ultrasound System (model DC-6V SHENZHEN MINDRAY BIO-MEDICAL, Electronics CO. LTD, CHINA) and 7 MHz microconvex and multifrequent probe (6C2 and radius 20 mm). The used US approach was transabdominal hypochondral. The results were interpreted in accordance with NAV (30).

The US data (graininess, echogenicity, contours, US attenuation of parenchyma, the variability of the grey nuance, brightness, contrast and acoustic impedance) were interpreted, considering the histological features of the liver (8, 11).

Results

In the histological study of the rabbit liver parenchyma have been obtained data for its structure. The epithelial parenchyma was composed of liver epithelial cells (hepatocytes) in the form of plates. The Central vein was observed centrally. The liver veins were positioned irregularly radially against the central vein. Between the hepatocytes' plates were situated sinusoidal capillaries. These capillaries flew into the central vein of each lobule and separated the hepatocytes' plates (Figure 1).

The epithelial origin of the radially located hepatocytes, the sinusoidal capillaries, and the scarce connective tissue in the capillaries defined the direction and reflection of the US beam. That resulted in the biological visualization of the liver parenchyma, the biliary ducts and fibrous capsule. There was not a sharp distinction between the parenchyma components. The US structure of the liver was grainy finely and nuanced in dark grey color. The parenchyma's acousticity was related to its structure and tissue contrast. The dark gray nuance defined the low acoustic (hypoeogenicity) of parenchyma and the fine-grained character of its structure (Figure 2).

The histological features of the portal tributaries presented three tissue layers: *tunica intima, tunica media* and *tunica adventitia*. The precapillary position of the portal tributaries determined the distinction and position of the three vascular layers and the clearer differentiation of tunica adventitia. The hepatic veins were intra organ vessels. They

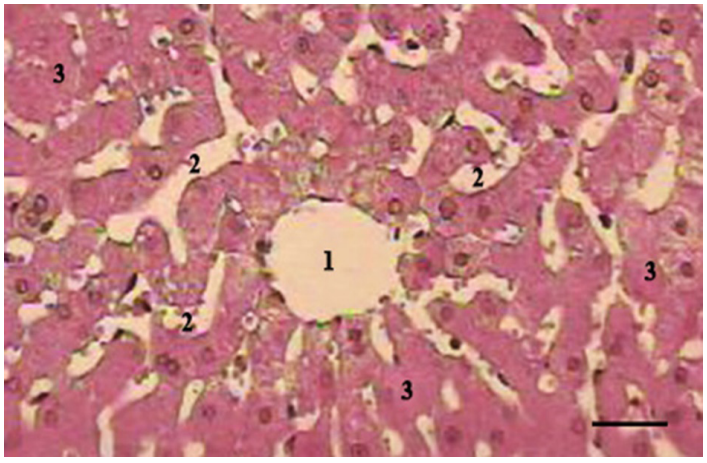

Figure 1: A photomicrograph of the cut surface of the rabbit liver. (1) V. Centralis, (2) sinusoidal capillary, (3) hepatocytes. Hematoxylin/Eosin. Bar = 50 µm.

***Şekil 1*:** *Tavşan karaciğer kesit fotomikrografisi. (1) V. Centralis, (2) sinuzoidal kılcal, (3) hepatosit. Hematoksilen/Eosin. Bar = 50 µm*

had less developed layers of the wall, because of the postcapillary localization. Tunica intima was the most inner layer. The adventitia was less developed, compared to the same layer in the wall of the portal tributaries (Figure 3).

The histological structure of portal branches and hepatic veins was used as a "gold standard" for ultrasound imaging of vascular morphology. The results obtained presented the morphological features of parenchyma, hepatic vessels and biliary structures. The clearer distinctness and positioning of the three vascular layers and the clearer differentiation of the tunica adventitia were a prerequisite for higher acoustics and the higher echogenicity of the portal vein's wall, compared to the walls of the intra-organ hepatic veins. This US morphological feature was owing to the more developed connective tissue and smooth muscle in the wall of the portal vein. The longitudinal ultrasound profile of the portal branches was sharply differentiated from the transversal contours of the hepatic veins. The sharpness of the resulting vascular images were further defined by the fine-grained heterogeneous character of the hepatic parenchyma. The US presentation of the portal vein and hepatic veins was related to the microscopic structure of the wall, as the differences in their acousticity were defined by the structure of parenchyma. The weak differentiation of the interlobular connective tissue increased the tissue contrast of the liver vessels, the portal wall was hyperechoic to the wall of the hepatic veins and. According to the gray-white scale, it was visualized in intensely white color (Figure 4).

The histological study of extrahepatic biliary pathways provided data on the construction of their wall. *Lamina epithelialis* was constructed by a monolayer prismatic epithelium. *Lamina propria* was the second sublayer that formed the wall of the large biliaryducts. *Lamina propria* and tunica adventitia were well-developed connective tissue components, with formed aggregations of smooth muscle cells observed between them. *Tunica adventitia* was bordered by hepatocytes plates and was observed in the perivistular space (Figure 5).

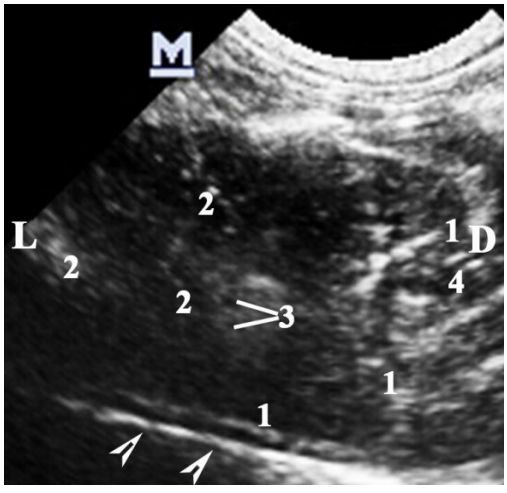

Figure 2: 2D US image of the rabbit liver (7 MHz multifrequent microconvex transducer; B-mode). (L-left; D-right). (1) *capsula fibrosa*, (2) parenchyma, (3) *ductuli biliferi*, (4) *pars cranialis duodeni*, (arrowheads) *diaphragma*.

Şekil 2: *Tavşan karaciğerinin 2D USG görüntüsü (7 MHz çok-fazlı mikro konveks prob; B-mod). (L-sol; D-sağ). (1) fibröz kapsül, (2) paranşim, (3) safra kanalı, (4) duodenumun ön kısmı, diafram (ok başları).*

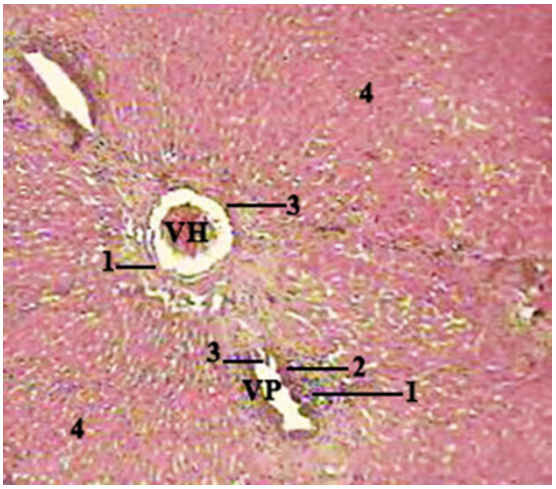

Figure 3: A photomicrograph of the cut surface of liver. (VP-*v. portae*; VH-*v. hepatica*). (1) *tunica adventitia*, (2) *tunica media*, (3) *tunica intima*, (4) hepatocyte plates. Hematoxylin/Eosin. Bar = 50 µm.

Şekil 3: *Karaciğer kesitinin fotomikrografisi. (VP-v. portae; VH-v. hepatica). (1) tunika adventisya, (2) tnika media, (3) tunika intima, (4) hepatositler. Hematoksilen/Eosin. Bar = 50 µm.*

The US visualization of the cystic duct and left hepatic duct demonstrated US reversion. The variability of the gray nuance was defined by *lamina propria* and *tunica adventitia*. They defined the brightness and sharpness of the US image of the extrabiliary ducts. The wall of the cystic duct and the left hepatic duct was hyperechoic compared to the relatively hypoechoic parenchyma, with increased acoustics being related to the echo signal strength produced by *lamina propria* and *tunica adventitia*. The increased acoustics was related to the strength of the echo signal, which was produced by *lamina propria* and *tunica adventitia*. The echo-signal was generated by tissues with different acoustic impedance (*lamina epithelialis*, *lamina propria* and *tunica adventitia*) and possessed a dispersion character. The echo-signal generated by the liver's parenchyma was homogenous (the hepatocytes' plates were with identic acoustic impedance) (Figure 6).

Discussion and Conclusion

By the comparative analysis of our results with those (14) for the liver in humans, we argue that the US image of the rabbit liver is due to differences in the genesis of the acoustic impedance of the tissue. It is not relative to the orientation of the transducer to the field of study.

The variability of the grey and white nuances of the rabbit liver is produced by its histological features. The findings of our study contradict the arguments for man (1, 10, 12), according to which the connective tissue is the only morphologic substrate that determines the acoustic properties of the US image.

According to us, the histosonographic study shows that there is a variability of the US acoustics of the liver at the same intensity of the US wave. This is also due to the histological features of the liver and biliary ducts. These facts contradict the arguments (6, 9, 23, 27), according to which only connective tissue defines the acoustic impedance.

Tissue differentiation of the acoustic impedance which is found by our study correspond to the results (7, 16), showing that the histological structure of the liver defines its US visualization.

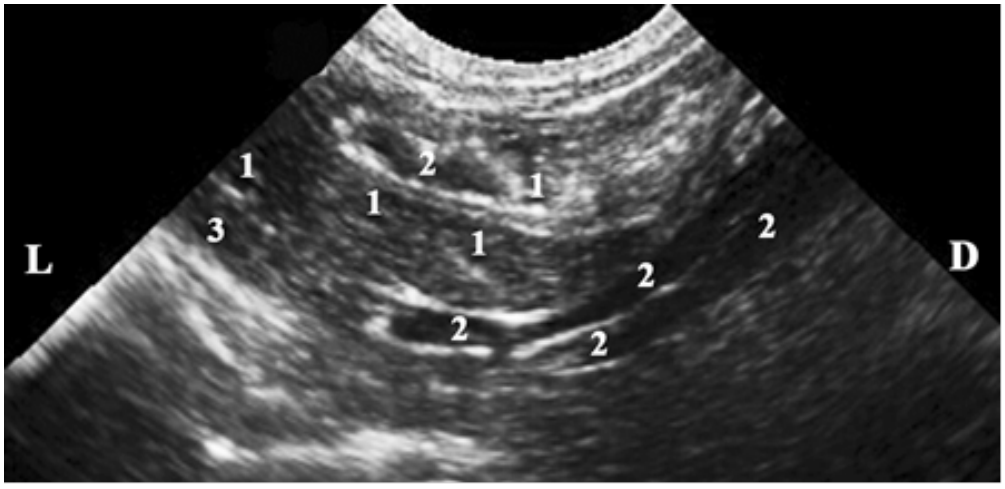

Figure 4: 2D US image of liver (7 MHz multifrequent microconvex transducer; B-mode). (L-left; D-right). (1) *Vv. Hepaticae*, (2) *V. portae*, (3) biliary vessels.

Şekil 4: *2 Boyutlu karaciğer ultrason görüntüsü (7 MHz çok-fazlı mikro konveks prob, B-mod). (L-sol; D-sağ). (1) Vv. hepaticae; (2) V. portae, (3) safra kanalları.*

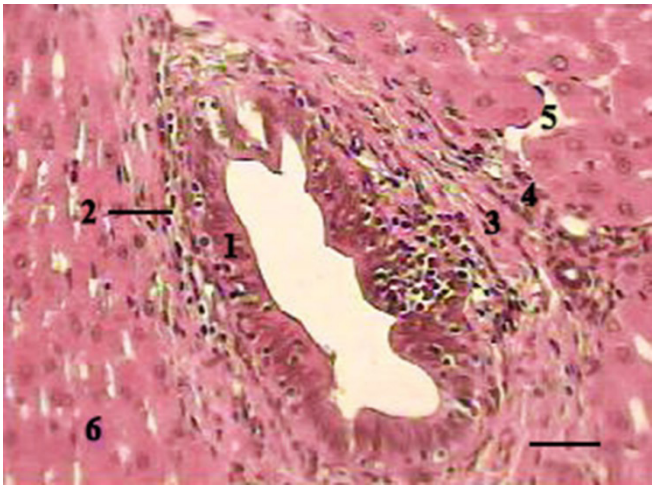

Figure 5: Histological cut through a major biliary vessel. (1) *lamina epithelialis mucosae*, (2) *lamina propria mucosae*, (3) congregations of myocytes, (4) *tunica adventitia*, (5) sinusoidal capillary, (6) hepatocytes. Bar = 35 μm.

Şekil 5: *Büyük bilayer damarın histolojik kesiti. (1) lamina epiteliyalis, (2) lamina propria, (3) lamina muskularis, (4) tunika adventisya, (5) sinuzoid, (6) hepatositler. Bar = 35 μm.*

We accept that the different acoustics of *capsula fibrosa* and liver parenchyma is related to the following US indices: brightness and contrast, in accordance to the grey-white scale, a variety of the grey nuance and speed of the US wave. Our hypothesis confirms the attitude (29) when interpret the US soft tissue findings.

The histosonographic study of the rabbit extrahepatic biliary ducts adds the thesis (5, 14, 15) that the echogenicity's character depends on the histological features of the tissues.

Our histosonographic data for micromorphological features of the rabbit liver parenchyma, biliary ducts, portal vessels and hepatic

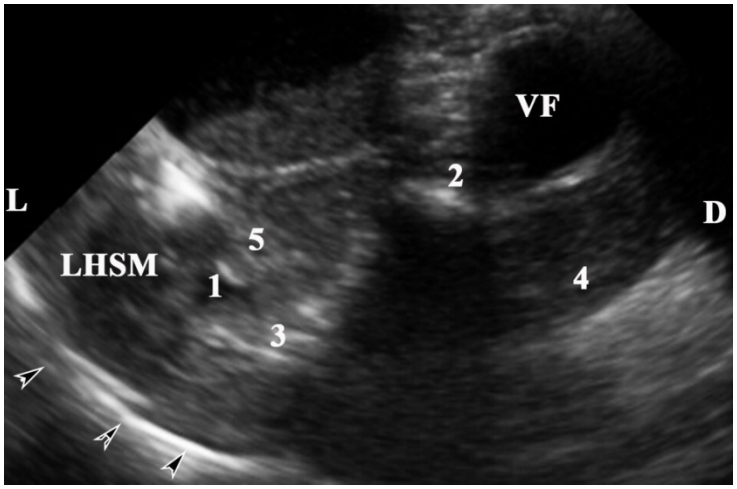

Figure 6: 2D US image of the liver. (7 MHz multifrequent microconvex transducer; B-mode). (L-left; D-right; VF-*vesica fellea*; LHSM-*lobus hepatis sinister medialis*). (1) *ductus hepaticus sinister*; (2) *ductus cysticus*; (3) *V. portae sinistra*; (4) liver parenchyma; (5) biliary vessels; (arrowheads) *capsula fibrosa*.

Şekil 6: *2 Boyutlu karaciğer 2D USG görüntüsü. (7 MHz çok kademeli mikro konveks problu B-mod ultrasonografi). (L-sol; D-sağ, VF- safra kesesi, LHSM-lobus hepatis sinister medialis). (1) ductus hepaticus sinister; (2) ductus cysticus; (3) V. portae sinistra; (4) karaciğer paranşimi, (5) safra kanalı, fibröz kapsül (ok başları).*

veins are related to the brightness and contrast of the US image. This theory confirms the hypothesis for the man (15, 19, 24, 25).

We use the connective tissue as US morphological substrate to interpret the US images, in accordance with the grey-white scale. Our algorithm for interpretation of the US images of the rabbit liver corresponds to the theory (26) for the role of the connective tissue as a morphological marker for the human liver's US visualization.

We accept as many researchers (17, 18), that the rabbit is a suitable morphological model for the study of the histosonographic features of the liver.

Our histological interpretation of US visualization of the rabbit liver is due to the dispersion character of the echo-signal, generated by parenchyma, perivascular connective tissue and extrahepatic biliary ducts. These facts add the published data for the man (8, 14, 29) that the micromorphological features of the soft tissue structures are "Gold standard" and define their US anatomical visualization.

The US anatomical image of the rabbit liver is defined by tissues with different morphological characteristics. Hepatic parenchyma has a different tissue resolution than that of perilobular connective tissue. The US morphological characteristic of the investigated organ is determined by its histological features which are related to the sharpness and contrast of the resulting image, in accordance to the gray-white scale, gray nuance's variability and ultrasound wave velocity.

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