Baku State University Journal of Life Sciences & Biology ***2024****, v.1 (2), p. 31-38*

journal homepage: http://bsuj.bsu.edu.az/en

HISTOLOGICAL STUDY OF LUNGS AND LIVER OF *OPHISOPS ELEGANS* (MENETRIES, 1832), *LACERTA STRIGATA* (EICHWALD, 1831), *TENUIDACTYLUS CASPIUS* (EICHWALD, 1831) (REPTILIA, SAURIA)

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Received 25 december 2023; accepted 17 april 2024

Abstract

The objective of this study was histological structure of lungs and liver of lizards. The septal air sacs inside the lizard's lungs are much larger than the alveoli of mammals and the air ducts of birds. Despite the large volume created by the septa in the lungs of lizards, the surface area in contact with air is much less than that of birds and mammals. Muscles on the front side of the lungs locate in the transverse direction of the lung, and due to their constriction, the entrance to the air sacs located in front is narrowed. But the direction of the smooth muscles in the middle part of the lungs is along the length of the partitions, and due to their constriction, the volume of the air sacs decreases. İn the experiments carried out by us, the main part of gas exchange occurs at the front part of the lungs, and air collects at the back part of the lungs. Gas exchange in the posterior sections of the lungs is very weak. Inside the lungs of geckos, there are tubes similar to the air ducts of birds. In lizards exposed to stress, most of the blood is retained in various organs (lung, liver) and the amount of circulating blood decreases sharply. When we observe the livers of lizards by stationaries, the diameter of sinusoids is determined to decrease as the height above sea level increases, but the number of sinusoids per unit area increases.

Keywords: histology, cytology, lungs, liver, lizard.

1. Introduction

The lizard's body contains epithelial, connective, nervous and muscular tissues [1]. All organs are formed from the combination of these four types of basic tissue [2] in different proportions. Tissue consists of cells and the extracellular matrix produced by these cells. Apart from the local cells in each organ of the lizard, there are also cells coming to this tissue through the blood. Cells brought to the tissues of the body through the blood are mainly mobile and leave the tissue after completing their function in this part. Extracellular matrix is synthesized by cells [3] or transported among cells through the blood. These substances constitute the external environment of cells. Substances brought between cells by the blood are mainly used by the cells [4] or returned back to the blood through the lymphatic system [5]. The extracellular matrix synthesized by cells consists of amorphous material and fibers. The fibers present here are of collagenous [6], elastic and reticular type.

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2. Materials and Methods

The object of the study was lungs and liver of *Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831), *Tenuidactylus caspius* (Eichwald, 1831). These species are included in the list of LC (Least concern). Specifically, the International Union classifies it as an unprotected species for Conservation of Nature (IUCN) because of its large number in nature [7]. This research was carried out on the basis of a scientific work registered at BSU on April 04, 2021 under the number 3/262. Researches were conducted at the Department of Zoology and physiology of BSU and the Medical biology and genetics of AMU in 2021-2024. Making a histological preparation from a lizard means bringing the removed tissue to a condition that can be viewed with a microscope [8]. This process begins with tissue fixation [9]. The fixed tissue should first be prepared into 3-5 μm thick sections. We provide it by infiltrating the paraffin [10]. Since the tissue contains water, it is not infiltrated with paraffin. Paraffin is a substance insoluble in water. To this end, first we need to remove the water from the fabric. In this case, we first use 50%, 70% and 95% ethyl alcohol, remaining each for 1 hour [11]. In the process of tissue dehydration, we keep it in 100% ethyl alcohol for 15-16 hours in the end. It is needed to be careful when removing tissue water. If the water is not removed gradually, the tissue may shrink and harden [12]. Along with ethyl alcohol, we use methanol and acetone for dehydration. Acetone is a very good dehydrating agent [13]. It is more effective to use this substance in adipose tissues. Following the dehydration, the tissue should be made transparent. The substances used for this include xylene, toluene, benzene, chloroform, limonene. The water is kept in a bath of undiluted toluene for 3.5 hours and then for 0.5 hours. After being transparent, the preparation should be infiltrated with paraffin so that it can be cut using a microtone device. We remain the tissue in a paraffin-toluene mixture for 45 minutes to inject the paraffin into the tissue slowly and not directly. After that, we put the tissue in melted paraffin three times at a temperature of 55-60C and put it in a thermostat. Afterwards, blocking is performed. After blocking, the finished blocks are kept in a cold place for a while. It is placed in a microtone device for cutting. A section of 3-5 μm thickness is made in the microtome. We place the cut pieces in distilled water with a temperature of 30-35C prepared in a container with a dark bottom. When preparing this water, we add 5 ml of 95% alcohol to each 100 ml of it [14]. After the submerged cuts are finished, they are captured through the object glass. The object glass and the preparation on it are dried at a temperature of 50-60C [15]. At this time, part of the paraffin melts and separates from the tissue, and the tissue sticks to the object glass. The preparation on the dried object glass is placed in two xylene baths for 5 minutes each. This is very important to remove the paraffin and make the tissue transparent. The tissue is then placed in 100%, 95% and 70% alcohol dishes and kept for 5 minutes each [16]. The preparation is placed in distilled water for 10 minutes. The tissue is now ready for dyeing. We use hemotoxylen-eosin dyes to stain most tissues. The tissue is kept in a bath containing hematoxylin and eosin dyes for 4 minutes and then kept under water for 1-2 minutes. In this staining, the nucleus is blue, fibrous tissue is light blue, cartilage is blue depending on its density, cytoplasm is pink, and muscles are red. The dyed preparation washed in water is covered with cover glass [17].

3. Results and discussion

The main functions of the organs of the respiratory system of lizards include entering air into the body [18], cleaning the air entering the body from microorganisms and dust, enriching the blood with oxygen in the lungs, removing carbon dioxide from the body, removing excess nitrogen from the blood, cooling the body in very hot times, and immunological protection. Apart from these, air and blood can be stored in the lungs. In the lizards we studied, gas exchange takes place in the front part of the lungs, but air is collected in the back part of the lungs. It can be considered as an analogue of air sacs of birds. But in the experiments carried out by us, it was found that the enrichment of blood with oxygen can also occur due to the air in the back part of the lungs. In a lizard respiratory movements of which seem to be at rest, the heart continues to beat and carry oxygen from the residual air in the lungs mainly to the head. Despite the fact that respiratory movements in lizards are mainly performed by the intercostal, chest and abdominal muscles, the limbs are also involved here. The more the lizard moves, the more intense the respiratory movements of the body bending to the left and right, especially the opening and closing of the front limbs. Heart rate also plays a major role in regulating respiratory movements. Unlike humans, the body in these animals is

not divided into the thoracic and abdominal cavity [19]. It is more appropriate to call the cavity in their body the coelom cavity. The amount of respiratory movements of a lizard depends on the concentration of carbon dioxide in the lungs, temperature, age, and stress. In lizards, although the internal volume of the lungs is larger than in mammals, the amount of surface area for internal gas exchange in the lungs is very small. Gas exchange is difficult in the caudal part of the lungs. A lizard's lung is very flexible (Fig. 1). It is known that some lizards, when they see an enemy, inflate their lungs and increase their body size to make themselves look bigger. In the Cyrtopodion caspium, both lungs have the same structure. Apart from having the same structure, they are also the same in size.

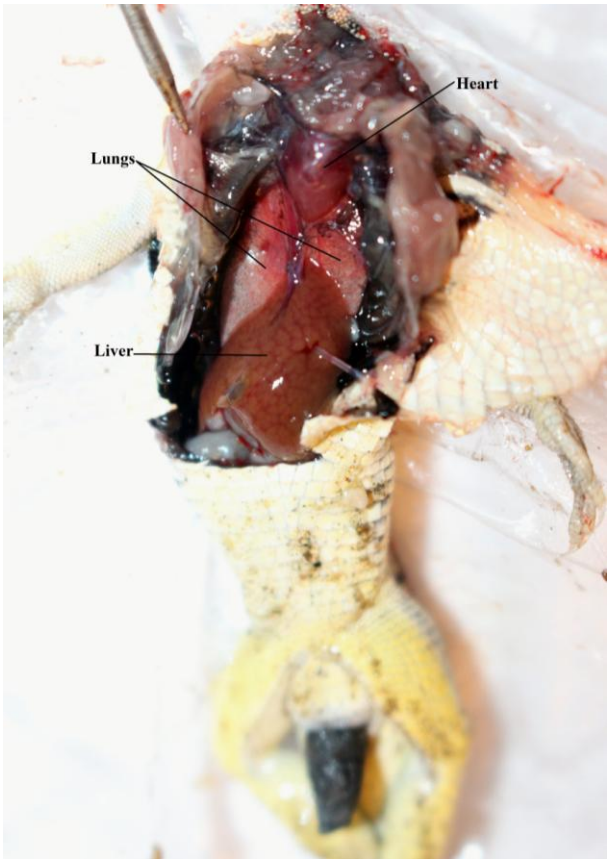

Fig 1. *Heart, lungs and liver of Ophisops elegans (Menetries, 1832)*

The lining epithelium of the lung is thin, and therefore its internal parts are partially visible when viewed from the outside. Inside the lung are many thin partitions attached to the wall of the lung. When we cut a lizard's lung longitudinally and veiw it with a light microscope, there are three types of partitions depending on the length. The longest partitions form protrusions towards the edges. Medium- and small-sized partitions divide the protrusions of long partitions into smaller parts which extend to the edges. These partitions, in cross-section of the lung, resemble the cells of a bee's comb. The septated air sacs inside the lizard's lung are much larger than the alveoli of mammals and the air ducts of birds. Despite the large volume created by the partitions in the lungs of lizards, the surface area in contact with air is much smaller than that of birds and mammals. Inside the lungs of geckos, there are tubes similar to the respiratory tubes found in birds. When we view the wall of these partitions, we can observe a collagenous layer, elastic tissue and thigh muscles. Smooth muscles locate in small groups in the free parts of the air sacs arising from the partitions. These muscles on the front side of the lungs locate in the transverse direction of the lung, and due to their constriction, the entrance to the air sacs located in front is narrowed. But the direction of the smooth muscles in the middle part of the lungs is along the length of the partitions, and due to their constriction, the volume of the air sacs decreases (Fig. 2). Apart from these, there is also a dense capillary network on the inner surface of the partitions. The inner surface of the lung is covered with epithelial

tissue. Two types of pneumonocytes are observed here. Flat-shaped type-1 pneumonocyte cells have a wide central part, but the outer parts are thinned. These cells, which are directly involved in gas exchange, can be in contact with several capillary vessels. The nucleus of these cells is partially depressed from above and elongated to the sides, like a chicken egg. The partially thin peripheral parts of the cells are also combined with type-1 or type-2 pneumonocyte cells. Type-2 pneumonocyte cells are roughly cube-shaped and have microvilli on their apical surfaces. One of their main functions is secretion. These secretory substances facilitate gas exchange and protect the inner surface of the lung. The nucleus of type-2 pneumonocytes is in the center. A thin-walled capillary network locates under the epithelium composed of pneumonocytes. These capillaries form small convexity towards the lumen of the lung. This network of convexitys in the air sacs increases the surface area where gases are exchanged. The lumen of capillary vessels is surrounded by endothelial cells. In some parts of blood vessels, endothelium cells are surrounded by pericytes. Endothelium and pericytes are covered with basal lamina. Fibroblasts and macrophages are rarely observed in the lizard's lung. The partitions of the lung are also rich with nerve fibers. However, these fibers rarely locate close to capillary endothelium and pericytes. We have never observed the passage of nerve fibers into the basal lamina of capillaries.

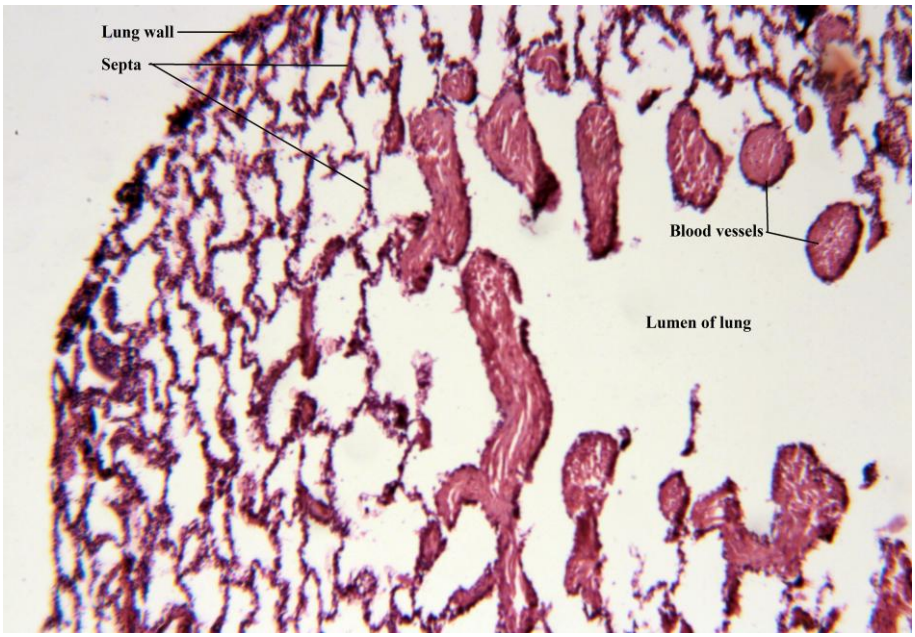

Fig 2. *Histology of lung of Lacerta strigata (Eichwald, 1831). Hematoxylin and eosin stain, original magnification x 10.*

The liver is one of the organs most affected by external influences. This is due to its high functionality. The lizard's liver is the largest of the digestive glands and is a dark brown organ. The liver of lizard is covered with Glisson's capsule, which is a thin layer of connective tissue. It is composed of fibrous connective tissue. The components of this connective tissue also enter the liver. The arteries, veins, and lymphatics that enter the liver are located within a tree root-like network formed by these connective tissue components. But the veins coming out of the liver lack that connective tissue. Blood and lymphatic vessels, bile ducts and nerves pass through the hilum, located at the bottom of the liver. The capsule covering the liver consists of two layers that are firmly connected to each other. The outer layer of the capsule is the serous, and the inner one is fibrous. Although the outer serous layer covers only surface of the liver, the collagens of the inner fibrous layer extend to the sinusoids. In the lizards under the study, it is not possible to observe the formation of lobules inside the parenchyma tissue of the internal collagens of the capsule with a light microscope. Masson's trichrome is the dye we use to study collagen fibers. In this case, collagens are dyed blue, muscles red, and nuclei black.

The hepatic artery and the veins called the hepatic portal vein enter the liver and branch internally. Since the bile duct is always observed next to the veins, these structures together are called the triad. Apart from these vessels, there are also lymphatic vessels in the liver. Arteries in the triad have a smaller diameter than that of veins. In the wall of the veins of the triad, the smooth muscles very poorly developed, but in the parts of these veins before the branching, normally developed smooth muscles can be observed. Blood capillaries branch off from the veins of the triad and form a network called sinusoids within the veins. The blood in the sinusoids moves from the periphery of the veins to the central vein. The diameter of sinusoids can be in the range of 0.05-25 µm. Typically, up to half of the blood capillaries in the lizard's other organs are too narrow in diameter to enable only plasma to flow. However, such a situation is not observed in the sinusoids of the liver. Since the diameter of the sinusoids is large, the speed of the blood flow here is also low. Blood can be stored in the liver of stressed lizards. In stressed lizards, most of the blood is stored in various organs and the amount of circulating blood drops sharply. When we cut the head of a stressed lizard to take blood, we notice that very little blood was injected into the head. The chemical composition of the blood flowing in the capillary vessels of the liver also differs from other organs and has a mixed character. When we observe the livers of lizards by stationaries, the diameter of sinusoids is determined to decrease as the height above sea level increases, but the number of sinusoids per unit area increases. We also analyze the liver of lizards using the EPR method [20].

Hepatocytes and sinusoidal endothelial cells, which are gathered around the veins [21], together are considered the main structural unit of lobules (Fig 3). Unlike the human liver, these lobules also contain melano-macrophages. In lizards, the network of thin fibers extending from the central canal to hepatocytes and endothelial cells develops very weakly. The adjacent side walls of the endothelial cells of the sinusoids in the liver of the lizard also contain micropores. Kupffer cells locate between the endothelial cells [22]. The blood coming through the sinusoids accumulates in the central vein, which in turn accumulates in the sublobular veins. There are fewer sinusoids towards the periphery of the liver. But in the center of the liver, a dense capillary network is visible. Lizards do not have bile ducts near the sublobular veins. The sublobular veins and central veins in lizards are always open because they must constantly receive blood. It is due to the fact that these vessels are tightly connected to the surrounding tissue and almost no muscles of the thigh are found in their wall. The wall of the veins of the central nervous system of the lizard has the same structure, and the muscles of the jaw are not found inside. Endothelial cells covering the inner wall of these veins are large sized and form depressions and protrusions. The endothelium is covered by a basement membrane, which is connected to fibrous connective tissue [23].

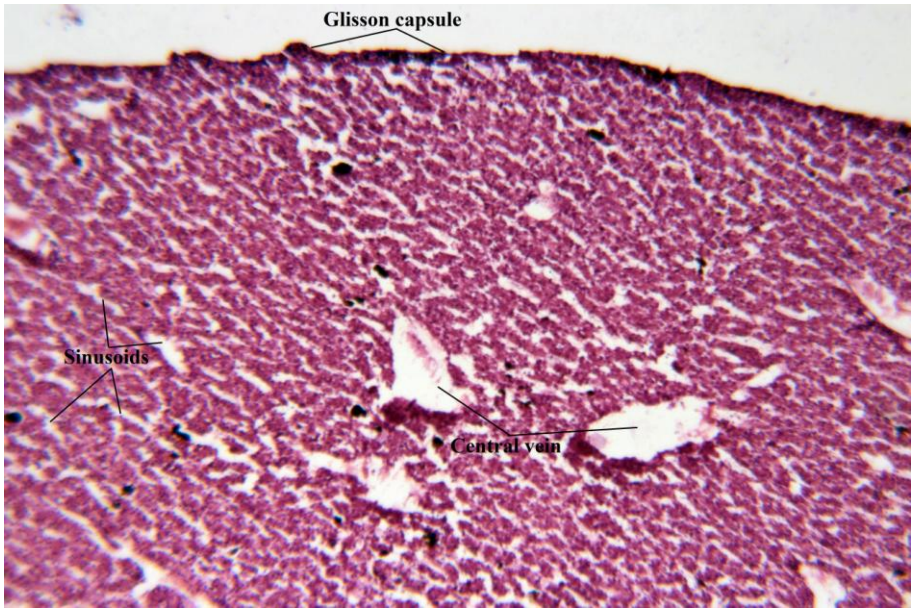

Fig 3. *Liver histology of Tenuidactylus caspius (Eichwald, 1831). Hematoxylin and eosin stain, original magnification x 10.*

Inside the liver, hepatocytes, Kupffer cells, stellate (ITO cells) fat-storing cells and endothelial cells of the liver predominate. The main structural and functional unit of the liver is hepatocyte cells. They make up to 70% of the cells observed in the liver. These cells generate ribbon-like structures located next to each other. Sinusoids are the reason for the ribbon-like structures. Most of the hepatocytes are polyhedral epithelial cells of relatively large size, round-like shape, with a large nucleus. Although most of the hepatocytes of the lizard have one nucleus, there are also those with two or more nuclei. In most of these cells, the nuclei are displaced to the periphery, and their shape is round or partially elliptical. In the lizards we study, hepatocytes produce small interconnected groups. These cells store glycogen, vitamin B12, folic acid, pigments and iron. They are involved in the transformation and transportation of substances and prepare bile. In lizards leaving their shelter mainly during the day, glycogen synthesis in the liver increases in the evening, however bile production increases during the day.

Liver sinusoids are low-pressure blood capillaries. They take blood from the terminal arteries and portal veins and slowly transport it towards the central veins. The cavity of sinusoids is called sinusoidal lumen and this cavity is mainly covered by endothelial cells. Endothelial cells may include Kupffer cells and T-lymphocytes. Perisinusoidal area (Disse area) locates between endothelial cells of sinusoids and hepatocytes [24]. Kupffer cells, which perform a protective function, are of monocytic origin and are local macrophages of the liver. When needed, these cells can enter the bloodstream and become free macrophages. Fat-storing stellate cells, pit cells (natural killers of the liver) and mesenchymal stem cells (MSC) are also observed in the Disse area. Drops of lipid origin are also observed in the cytoplasm of stellate cells. The mitochondria in these cells are very few. They also store fat-soluble vitamins [25]. Microvilli of hepatocytes are connected to the interior of sinusoids and ensure absorption of plasma substances by hepatocytes. Pit cells are the natural killers of the liver and have specific granules. These cells originate in the red bone marrow and reach the liver through the blood. Pit cells are involved in the destruction of cells damaged by cancer and viruses. Ito cells (stellar cells) are of mesenchymal origin located in the perisinusoidal space (Disse area) of liver lobules. These cells turn into myofibroblast-like cells when a pathological condition occurs, synthesize a large amount of collagens outside the cell, and cause collagenization of the area. Ito cells are also actively involved in liver regeneration [26].

4. Conclusions

Based on the results described above, it can be concluded that lungs and liver are organs that most affected by changing environmental conditions. The septated air sacs inside the lizard's lung are much larger than the alveoli of mammals and the air ducts of birds, but the surface area in contact with air is much smaller than that of birds and mammals. Gas exchange takes place in the front part of the lungs, but air is collected in the back part of the lungs. İn the experiments carried out by us, it was found that the enrichment of blood with oxygen can also occur due to the air in the back part of the lungs. Inside the liver, hepatocytes, Kupffer cells, stellate (ITO cells) fat-storing cells and endothelial cells predominate. The chemical composition of the blood flowing in the capillary vessels of the liver also differs from other organs and has a mixed character.

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