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The histological and cytological analysis of muscles of lizards (*Reptilia, Squamata*)

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All lizard cells possess microfilaments. However, there are many microfilaments in muscle cells, and these cells provide contraction. In our research, we have used three species (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831), *Tenuidactylus caspius* (Eichwald, 1831)) of lizards. In the embryonic period of lizard, myoblast cells are formed from the myotomes. Migration of myoblasts are essential to generate the skeletal muscles. When myoblasts reach their target area, they first form the muscle plate. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form a large syncytium for producing myosymplast. During the further development of the embryo, related parts of membranes of myoblasts disintegrate and become the sarcoplasmic reticulum. As a result, a long myotube is formed. When we investigated skeletal muscles of hind limbs, according to their colour, three types of muscle fibers were found in the skeletal muscles of the lizard. Red fibers were observed in *Lacerta strigata* more commonly than that in other two lizards. In the same muscles, light fibers were observed in *Tenuidactylus caspius* more frequently. That allows them to move on vertical surfaces very quickly. *Ophisops elegans* had more intermediate colored fibers than the other two lizards. Diameter of myotube depended on species of lizards, muscle location, age of animal and its size. Thickness of epimysium, perimysium and endomysium, observed in the same lizard, also varied depending on type of muscles, the animal's age, weight and environmental conditions where it lived. Myofibrils of cardiomyocytes occupy up to half of total volume of the cytoplasm. In lizards, force of the smooth muscle is more than that of the skeletal muscle. Structural characteristics of skeletal muscle fibers are influenced by many factors such as species, genotypes, nutritional and environmental factors.

Keywords: *Muscle cell, myofibril, histological structure, Ophisops elegans, Lacerta strigata, Tenuidactylus caspius*

INTRODUCTION

The micromorphological structural features of the separate tissues and organs of a lot of species of herpetofauna have not been studied in detailed yet. One of the such kind of unstudied tissue is muscle tissue of lizard. This tissue plays a vital role in ensuring the dynamics of reptiles in nature, additionally maintains the balance of the biocenosis and trophic communication of animals. Considering that reptiles carry a number of parasitic and infectious diseases, the relevance of the study becomes even more obvious. The object of research - Caspian bent-toed gecko, is naturally distributed in the rocks of the semi-deserts, in all biotops where it can hide. They have become synanthropic animal by living in residential areas, gardens and fences. Spending this type of nocturnal lifestyle protects them against predators. Snake-eyed lizard lives in semi-desert areas with rocky, hard soils and

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drought-resistant drought-tolerant plants. The caspian green lizard inhabits in pastures and bushes in the areas where are rich in plants. As it can be seen, the histological study of types of muscles of lizards, which live in different environmental conditions and with different physiological characteristics, worth to study scientifically and practically.

MATERIALS AND METHODS

Researches were conducted at the Department of Medical Biology and Genetics of AMU and the Central Scientific Research Laboratory of AMU in 2009-2020. Snake-eyed lizard - *Ophisops elegans* (Menetries, 1832), Caspian green lizard - *Lacerta strigata* (Eichwald, 1831) and Caspian bent-toed gecko - *Tenuidactylus caspius* (Eichwald, 1831) spread in the territory of the Azerbaijan Republic widely (Najafov et al., 2014) were used for histological researches. For this purpose, expeditions were organized to various areas. Appropriate methodological methods were used to conduct histological analysis of slides from different organs of lizards. Incisions from the muscles tissue of lizards were sampled and fixed in 5-10% formalin. In preparation of muscle tissue slides histological incisions were made both longitudinally and transversely. Paraffin-impregnated blocks were prepared from these samples, and 5-10 µm thick incisions were made using a digital microtome and transferred to the glass. Subsequent histological and cytological studies were performed in the laboratory. Initially deparaffinized samples of muscles were stained with Giemsa stain, hematoxylin and eosin stain. After the preparation the slide observed under the microscope.

RESULTS AND DISCUSSION

Cells of muscle tissue of lizard are rich in microfilaments, which provide muscle contraction. Muscle tissues of lizards, according to their morphofunctional structure, divided into skeletal, cardiac and smooth muscles. Skeletal muscles located on the bones or attached to the skeleton of lizard. An example of smooth muscles are muscles of walls of various internal organs, blood and lymphatic vessels. Cardiomyocytes are observed in the myocardium.

In the embryonic period of lizard, myoblast cells are formed from the myotomes of the paraxial mesoderm in the somites (Najafov, 2007). These cells are proliferatively active. Migration of myoblasts are essential to generate the skeletal muscles. It has been discovered by us that, when myoblasts reach their target area, they first form the muscle plate. The existence of this structure in lizard takes a short time. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form a large syncytia for producing myosymplast. During the further development of the embryo, related parts of membranes of myoblasts disintegrate and become the sarcoplasmic reticulum. As a result, a long myotube is formed. Each myotube contains many nucleises, which are initially located in the center of the tube. New myofibrils formed in the periphery of the myotube. After the growing myofibrils in the myotube push the nucleies into the cytoplasmic membrane to perform their functions well. If the nucleus retains in the center, it impedes contraction of skeletal muscle fibers and slows down the differentiation of muscle fibers. Thin (actin) and thick (myosin) filaments can be seen under a microscope in the myotube of the lizard (Fig. 1).

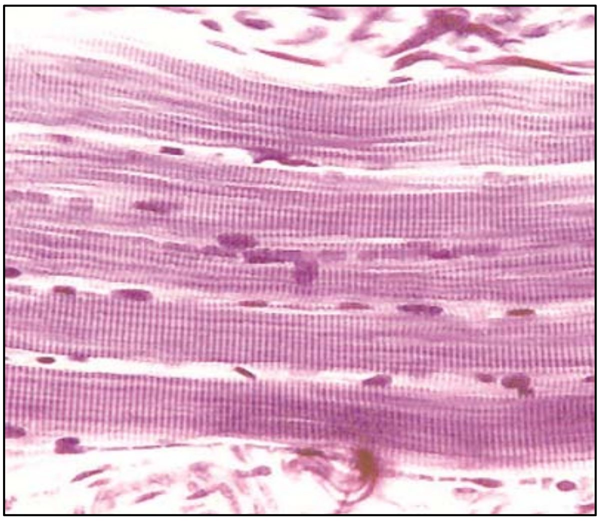

Fig. 1. Microscopic view of thin and thick filaments in the myotube of skeletal muscle.

Some myoblasts do not involve in the formation of myotubes. These cells are located outside of the sarcolemma and beneath the surrounding basal lamina to form myosatellite cells. These satellite cells are observed in the muscle fibers in the tail of the lizard more. When lizard's tail is cut off for any reasons, these myosattelites are involved in muscle regeneration actively. On the other hand, along with myosatellite, some red bone marrow cells also divide and participate in the restoration of damaged skeletal muscle tissues (Najafov et al., 2014). It is not easy to see myosatellite by light microscope staining techniques. These cells have branched elongated cytoplasmic processes.

Completely formed myotube and satellite cells are surrounded by basal lamina and form the basis of the muscle fiber. Inside of the myosimplast, the nuclei divide and multiply by mitosis. Axon and myotube membrane are closer together significantly on the sarcolemma (Garland et al., 1994). This area is myoneural junction consisting of the axon terminal, the synaptic interval and the plasmalemma of the myotube.

We have identified three different types of muscle fibers in the skeletal muscles of lizard by their color. Red fibers of lizards contain a large amount of myoglobin and lower amounts of myoglobin. Through these fibers have rich capillary network. They are slow but can to do repetitive contraction over long periods of time. White colored fibres fatigue more easily but can sustain more intense activity. These fibres have more myofibrils and a large diameter. Intermediate fibers, as it may be seen by their names, are intermediate in their colour and function. All three of these fibers are found in every skeletal muscle of lizard. When we examine skeletal muscles (*Triceps humeralis*) of same type of forelimb (left), red fibers are more frequent in the Caspian green lizard than in the other two lizards. In Caspian bent-toed gecko light-colored fibers are more frequent in the same muscle. This allows the gecko to move very quickly over vertical substrates in short time. Intermediate fibers in the left forelimb of snake-eyed lizard dominate over than other two lizards. The reasons for these differences in skeletal muscle fiber types are: capillary density, oxidative capacity, myoglobin content, glycolytic capacity and ATPase levels (Nelson et al., 2001). The diameter of the muscle fiber varies depending on species of lizard, age, size of the animal and the functional state of the muscle. The diameter of the muscle fiber varies in different parts of each lizard. Sarcomers of skeletal muscle fibers are seen in lizards quite well. Muscle fibers structural characteristics can be modulated by environmental factors independently. Skeletal muscle of lizard consists of 88% muscle tissue and 12% of connective, vascular, nervous and fat tissues. The connective tissue in skeletal muscle is divided into endomysium, perimysium and epimysium. Near the tendon of lizard the connective tissue are all thicker than in other regions of the muscle.

Each skeletal muscle fiber is surrounded with an endomysium. It separate fibers from each other (Higham et al., 2010). Within the endomysium are present capillaries and neuronal branches. When we examined the cross section of muscles, we saw that endomysium were connected to each other. İt also connected to the perimysium by intermittent perimysial junctional plates. Perimysium is connective tissue partition that is thicker than endomysium. İt surrounds a group of fibers to form a fascicle. Blood vessels and nerves also located in the perimysium (Vitt et al., 2003). Endomysial and perimysial network acts to transmit muscle force. Epimysium surrounds collection of fascicles that constitutes the muscle organ. İt consists of dense connective tissue and connects to perimisium. Near the articular capsule and disc, endomysium, perimysium, and epimysium were all thicker than in other regions of the muscle. We noticed that the thickness of endomysium, perimysium and epimysium varies depending on the type of muscle, the age of animal and the environmental conditions in which it lives.

Lizard cardiogenic area are located in the mesodermal component of the splanchnopleural layer of the plate. Cardiomyocytes are differentiated from cardiac mesodermal primordia. Some cardiomyoblast cells multiply and mature into cardiomyocytes, all other cardiomyoblasts do apoptosis. All cardiomyocyte cells of adult lizard arrested at G_0 cell cycle. It means, they never divide. However, we have observed a large number of polyploid chromatin fibers in their nucleus. Some cardiomyocytes may have 2 or 3 nucleuses. The nucleus is located in the center of the cytoplasm of the cardiomyocyte cell and is oval in shape. In the cytoplasm of cardiomyocytes with the proteins provide contraction accumulate myoglobin, fat drops and glycogen. Myofibrils are the protein complex that provides contraction. In lizards, the percentage of cell volume that myofibrils occupy in cardiomyocytes is being approximately half of the total cytoplasm. These cells are highly resistant to fatigue. Cardiac muscle cells are branched and combine with neighboring cells to form a muscle network (Fig. 2).

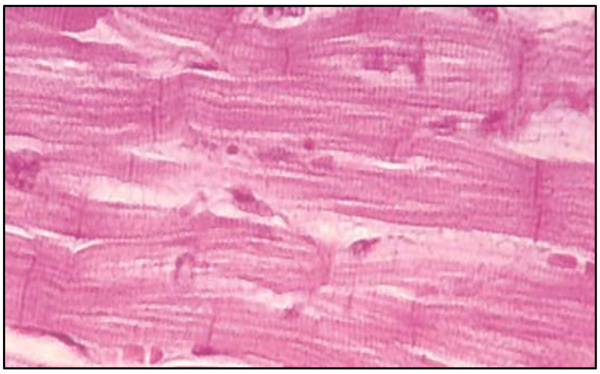

Fig. 2. Cardiac muscle cells form long, branching muscle fibers.

Dark layers appear in the areas where these cells touch each other. Sarcolemma does not disappear in areas where there is a contact between cardiomyocytes cells. The intercalated discs are formed on the contacting areas. The surface of cardiomyocytes is covered by basal lamina. There are no satellite cells under the basal membrane. The endomysium is present on cardiac muscle cells. Elements of connective tissue, rich in capillaries are identified.

We have seen during our observations that cardiomyocyte sarcomeres are differs from skeletal muscle sarcomeres. The sarcomere lies between two Z-lines, A and I bands are not clearly defined (Curtin et al., 2005). There are dyads instead of triads. Cardiomyocyte sarcoplasmic reticulum is better developed than the fibers of skeletal muscles.

During the embryonic development of lizards, mesenchyme gives rise to connective tissue, bone, cartilage, circulatory and lymphatic systems and smooth muscles. Smooth muscle cells are elongated and spindle-shaped. They are tapered at both ends and round at the center. The nuclei are located closer to one lateral membrane of the cell along the midline of myositis. Smooth muscle cells of the lizard retain ability to reproduce throughout their life and have capacity to regenerate. By morphofunctional properties smooth muscles of lizard is divided into two subgroups: single-unit and multi-unit smooth muscles. Each multi-unit smooth muscle cell innervated by a neuron and receive synaptic input. Their contraction does not spread from one cell to the another. These types of smooth muscle is found in the large blood vessels and in the respiratory airways of lizard. İn the walls of all visceral organs single-unit smooth muscle fibers are connected via gap junctions. Few of single-unit smooth muscle cells are innervated by neuron. The smooth muscle cells of lizard are much smaller than that skeletal muscle cells. They have in the cytoplasm actin and myosin filaments but do not have sarcomeres (Fig. 3). During lizard's myositis contraction, it can remain 20% of its volume comparing in the quiet situation. During contraction, the nucleus also alters its shape considerably. İf we compare with the skeletal muscles, smooth muscles contraction are weak.

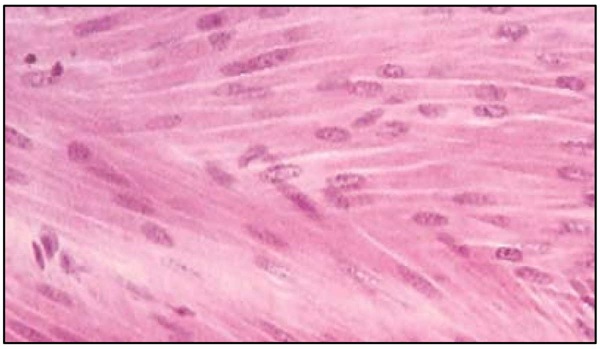

Fig. 3. Location of myocytes in the smooth muscle tissue.

Smooth muscles can remain contraction position for a long time. But contraction strength of smooth muscle observed in lizards is greater than that of skeletal muscle. The reason of this is the transverse bridges from the myosin that can form a strong bond with the actin (Garland et al., 2005). These compounds separate from each other hard. Smooth muscle cells are innervated autonomic nervous system. Another feature of the lizard's smooth muscle is that it does not lose its former strength in spite of being in a stretched or shortened position for a long time.

CONCLUSION

In the embryonic period of lizard, myoblast cells are formed from the myotomes of the paraxial mesoderm in the somites. Migration of these cells are essential to generate the skeletal muscles. When myoblasts reach their target area, they first form the muscle plate. The existence of this structure in lizard takes a short time. The number of nuclei in the muscle plate is small and their length is short. Muscle plates are fused with myoblasts to form large syncytia for producing myosymplast. During the further development of the embryo, myotubes are formed. Three types of muscle fibers in the skeletal muscles of the lizard were determined in the basis of their colours. When we examine the skeletal muscles of the same type locating in the forelimb, red fibers are more observed in the Caspian green lizard more commonly than in the other two lizards. The intermediate fibers are more commonly in the snake-eyed lizard in the same muscles. The light fibers of skeletal muscle of limbs of Caspian bent-toed gecko are dominant over the other two lizards. Species, age and size of lizard affects to diameter of the muscle fiber. The thickness of endomysium, perimysium and epimysium observed in the same lizard alters depending on kind of muscle, age of the animal, its weight, and the environmental conditions in which it lives. Cardiomyocytes contain up to half of the total amount of contractile proteins. On the cardiomyocyte endomysium is present. The sarcomere areas of cardiomyocytes under the microscope are almost indistinguishable from the sarcomeres of the skeletal muscles. If we compare to the skeletal muscles, the smooth muscles contraction more slowly. These muscles can stay in a contraction position for a long time. But the erection strength of smooth muscles of lizards are greater than that of skeletal muscles.

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Kərtənkələlərdə (*Reptilia, Squamata*) əzələlərin histoloji və sitoloji analizi

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Kərtənkələlərin bütün hüceyrələrinin tərkibində mikrofilamentlər mövcuddur. Amma əzələ toxumasının hüceyrələrində mikrofilamentlər çoxdur və bu hüceyrələr təqəllüsü təmin edir. Biz tədqiqatlarımızda üç növ kərtənkələdən istifadə etmişik (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831), *Tenuidactylus caspius* (Eichwald, 1831)). Kərtənkələnin embrional inkişafında somatik əzələ toxuması miotomlardan yaranır. Mioblastların miqrasiyası əzələlərin formalaşması üçün əhəmiyyətlidir. Mioblastlar hədəflədikləri nahiyəyə gəldikləri zaman ilk əvvəl əzələ plastinkası əmələ gətirirlər. Bu quruluşun mövcudluğu kərtənkələdə çox qısa vaxt çəkir. Əzələ plastinkasında nüvələrin sayı az olur və ölçüsü qısadır. Daha sonradan mioblastların plastinkaya birləşməsi yolu ilə miosimplast formalaşmış olur. Embrionun sonrakı inkişafının nəticəsində miotubul yaranır. Kərtənkələnin skelet əzələlərində rənginə görə üç tipdə olan əzələ lifləri müəyyən edilmişdir. Ətraflarda yerləşən eyni növ skelet əzələlərini araştırdığımızda, zolaqlı yaşılkərtənkələdə qırmızı liflər digər iki kərtənkələyə nisbətən çox müşahidə olunur. Eyni əzələlərdə xəzər nazikbarmaq gekkonunda açıq rəngli liflər daha çox müşahidə olunur. Bu da gekkonun şaquli vəziyyətdə olan substratlar üzərində qısa zaman ərzində çox cəld hərəkət etməsinə səbəb olur. Biçimli ilanbaşda ətraflarda aralıq liflər digər iki kərtənkələyə nisbətən üstünlük təşkil edir. Miosimplastın diyametri kərtənkələnin növündən, yaşından, heyvanın ölçüsündən və əzələnin növündən asılıdır. Eyni bir kərtənkələdə müşahidə olunan endomiz, perimiz və epimizin qalınlığı da əzələnin növündən, heyvanın yaşından, onun kütləsindən və yaşadığı ekoloji şəraitdən asılı olaraq dəyişir. Kərtənkələlərdə miofibrillər kardiomiosit hüceyrələrinin sitoplazmasının ümumi həcminin yarısına qədər olan hissəsini tutur. Kərtənkələdə müşahidə etdiyimiz saya əzələlərin yığılma gücü eninəzolaqlı əzələlərin gücünə nisbətən daha çoxdur.

Açar sözlər: *Kərtənkələ, biçimli ilanbaş, zolaqlı yaşılkərtənkələ, Xəzər nazikbarmaq gekkonu, histoloji quruluş, əzələ, mioblast, miotub, miosit*

Гистологический и цитологический анализ мышц у ящериц (Reptilia, Squamata)

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Во всех клетках ящериц содержатся микрофиламенты. Однако в клетках мышечной ткани, обеспечивающей сокращение, микрофиламентов много. В нашей работе мы исследовали три вида ящериц (*Ophisops elegans* (Menetries, 1832), *Lacerta strigata* (Eichwald, 1831) и *Tenuidactylus caspius* (Eichwald, 1831)). В эмбриональном развитии ящериц соматическая мышечная ткань формируется из миотомов. Миграция миобластов необходима для формирования скелетных мышц. Когда миобласты достигают необходимой области, они вначале формируют мышечную пластину. Мышечная пластинка у ящериц существует очень короткий промежуток времени, количество ядер в ней немногочисленно и она достаточно короткая. Мышечная пластинка сливается с миобластами, образуя миосимпласты. В процессе дальнейшего развития эмбриона образуется мышечная трубка. В зависимости от цвета в скелетных мышцах ящериц различают три типа мышечных волокон. Когда мы исследовали скелетные мышцы, расположенные в задних конечностях, красные миофибриллы чаще наблюдались у *Lacerta strigata* (Eichwald, 1831), чем у двух других видов ящериц. Светлые миофибриллы чаще встречались у каспийского тонкопалого геккона. Эта особенность позволяет геккону очень быстро перемещаться по вертикальным поверхностям. У *Ophisops elegans* (Menetries, 1832) больше промежуточных миофибрилл, чем у двух других ящериц. Диаметр миосимпласта зависит от типа ящерицы, ее возраста, размера животного и типа мышц. Толщина эндомизия, перимизия и эпимизия у одной и той же ящерицы также варьирует в зависимости от типа мышц, возраста животного, его веса и экологического состояния окружающей среды. В клетках кардиомиоцитов миофибриллы занимают до половины общего объема цитоплазмы. У ящериц сократительная сила гладких мышц выше, чем скелетных.

Ключевые слова: *Мышцы, миофибриллы, гистологическое строение, форма клеток, Ophisops elegans*, *Lacerta strigata*, *Tenuidactylus caspius*