SEASONAL CHANGES IN SOME ULTRASTRUCTURAL INDICES OF THE VENTRICULAR MYOCYTES OF *Rana ridibunda*

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Certain ultrastructural characteristics of the ventricular myocardium of *Rana ridibunda* (lateral contacts, intercalated disks, mitochondria) were studied in the spring and fall by electron microscopy coupled with morphometric analysis. The results point to seasonal differences in the ultrastructure of the cardiomyocytes and the conclusion was confirmed by the results of quantitative analysis.

KEY WORDS: seasonal rhythms; amphibian myocardium; ultrastructure; morphometry.

Amphibians are poikilothermic animals, that is to say animals whose vital activity depends directly on the external environment. Their motor activity and the metabolic basis for their physiological functions are directly connected with seasonal rhythms. Information on this subject is sufficiently widespread in the literature [5, 6, 8, 10, 11]. However, the question of the structural basis of the seasonal dynamics of metabolic processes in amphibians has received little attention in the literature, especially in the case of the cardiovascular system. Correlation between morphofunctional indices of the cardiovascular system and the seasons has virtually not been studied. Nevertheless, such correlation evidently exists. For instance, there are reports that the heart rate in amphibians depends on seasonal cycles [9]. Some workers [1] have also observed that the concentration of certain ions (Na^+ and K^+) in the frog's heart also depends on the time of year.

It was decided to make a comparative analysis of some morphological indices of the ventricular myocardium of *Rana ridibunda* at different times of year. Certain morphological characteristics were chosen for investigation. Ultrastructural indices of the cardiomyocytes, which play an important role in the maintenance of structural and functional unity of the myocardium, also were studied: lateral contacts, intercalated disks, and also organelles concerned with the provision of energy — mitochondria.

In the course of analysis of the data obtained by electron-microscopic study of these various parameters, morphometric methods were used to obtain an objective quantitative assessment of the state of the ultrastructures examined.

EXPERIMENTAL METHOD

Frogs (*R. ridibunda*) weighing 40-70 g were used. The hearts of 10 intact animals were studied. All the animals were subdivided into two groups depending on the season (spring and fall) and the animals were killed in May and September. Material was taken at the same time of day — from noon to 3 p.m. After sacrifice, fragments of the ventricular heart wall were fixed with 2-6.25% glutaraldehyde solution in phosphate buffer by the immersion method. After rinsing in four portions of buffer, the blocks were postfixed with osmic acid. Dehydration was carried out in alcohols of increasing concentration, after which the tissue was embedded in Epon-812. Sections were cut on the LKB-111 Ultratome, stained with 2-5% uranyl acetate and lead citrate, and then examined in the Hitachi 11-E-2 electron microscope with an accelerating voltage of 75 kV.

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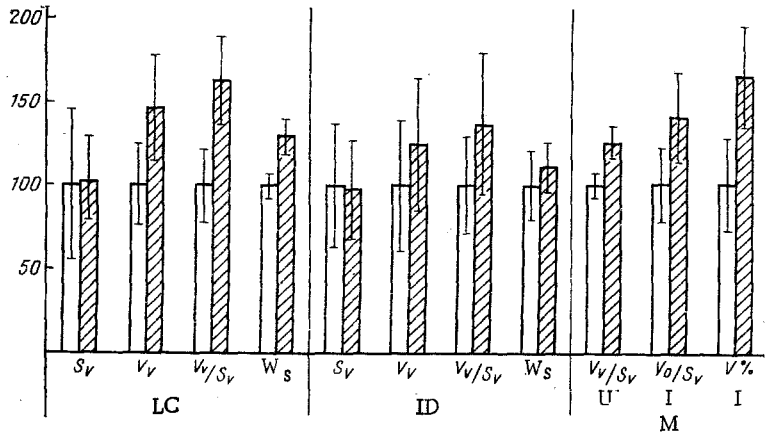

Fig. 1. Relative magnitudes of parameters studied. Ordinate, relative values in % (data for animals in spring taken as 100%); unshaded columns represent animals in the spring; shaded columns — in the fall. LC) Lateral contacts; ID) intercalated disks; M) mitochondria (U — unchanged, I — changed). Remainder of legend in text.

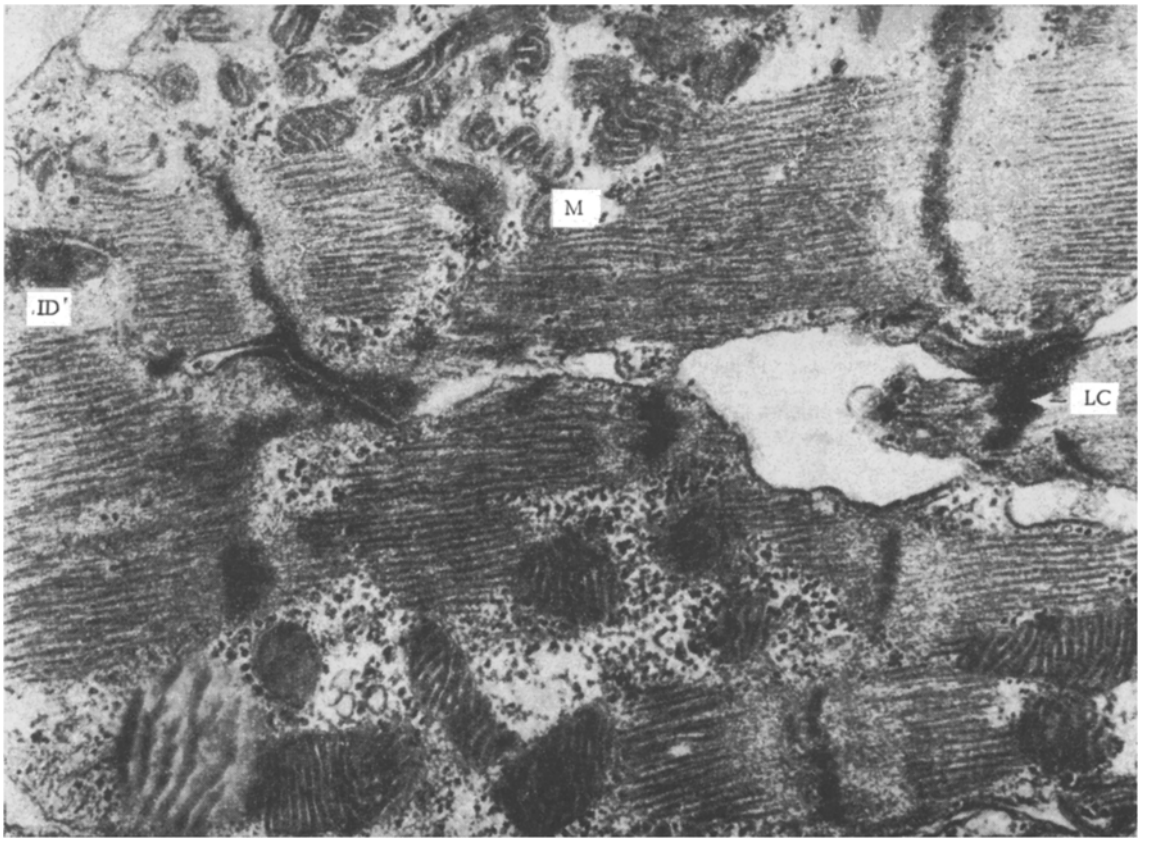

Fig. 2. Muscle cells of ventricular myocardium of spring frog. Here and in Fig. 3, legend as in Fig. 1. 30,800 ×.

The volumes and surface areas of the test structures (lateral contacts, intercalated disks, and mitochondria) were determined by a morphometric method. For this purpose, a grid with 5-mm step was applied momentarily to the photograph. The volumes of the structures in μ^3/μ^3 tissue were calculated on the basis of Glagolev's theorem [3],

$$V_{vi} = \frac{P_i}{P},$$

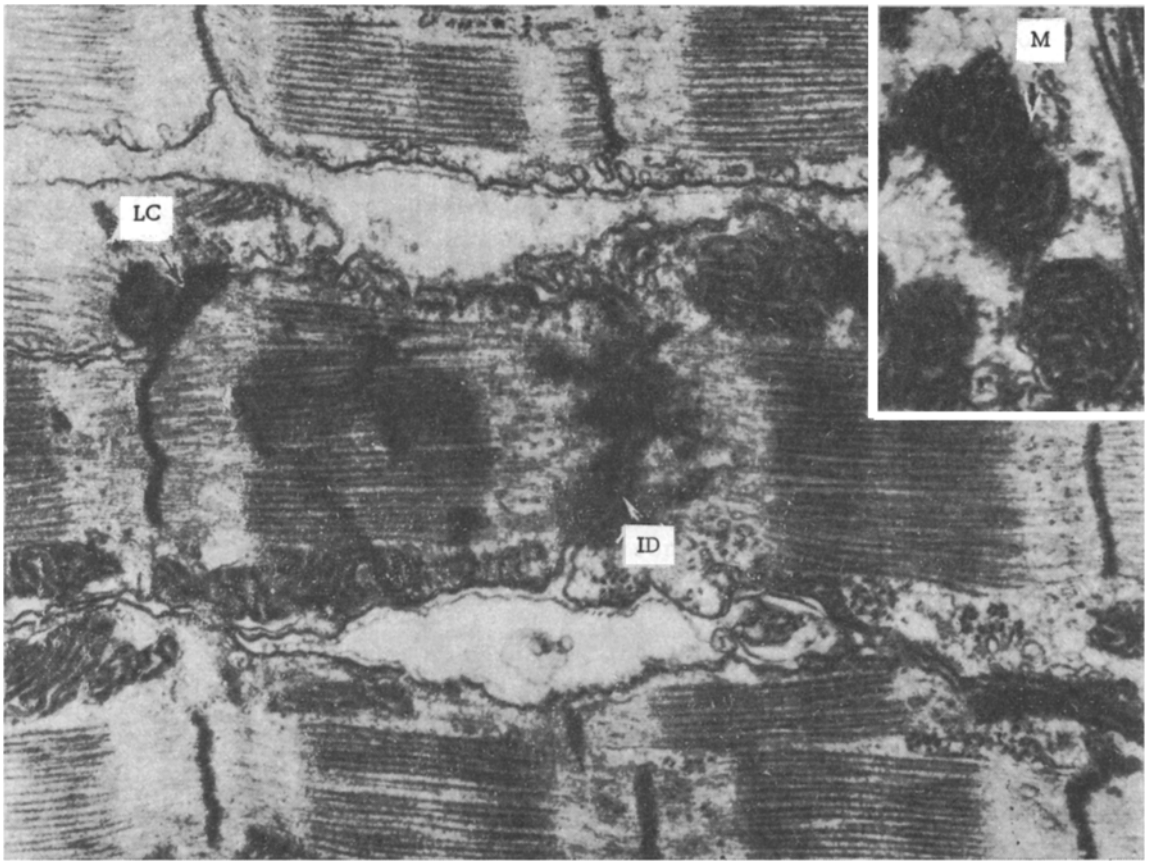

Fig. 3. Muscle cell of ventricular myocardium of autumn frog, 38,800 ×.

where P_i is the number of test points lying on the cross section of the i-th structure and P the number of points on the photograph. The surface areas of the outer mitochondrial membranes, the lateral contacts, and the intercalated disks were determined in μ^2/μ^3 tissue by the equation of Lound et al. [7],

$$S_{vi} = \frac{2P_{Li}}{L}\left(\frac{M}{1000}\right),$$

where S_{vi} is the area/volume fraction of the i-th structure, P the number of intersections of tracks and measuring lines on the section, L the total number of measuring lines passing through the section, the M the magnification of the microscope. These definitions were adopted at the Second International Congress of Stereology (1967).

The widths of the spaces of the lateral contacts and intercalated disks were measured from the negatives by means of an ocular measuring scale (division 0.1 mm) and calculated by Borovyagin's equation [2],

$$W_s = \frac{D \times 10^6}{M},$$

where D is the width of the space in mm, M the magnification of the microscope, and W_s the width of the space in nanometers.

Altogether 40 negatives were used. The photographic prints for the morphometric investigation were made to a size of 12 × 18 cm.

Statistical analysis of the results was carried out on a Promin'-2 computer by means of Plokhinskii's [4] algorithm.

EXPERIMENTAL RESULTS

Comparison of the ultrastructure of the ventricular cardiomyocytes of animals killed in the spring and fall showed differences in the electron-microscopic picture as regards both the configuration of the muscle elements and the organization of their organoids.

The study of the intercellular contacts revealed two types of these formations: side to side and end to end. Lateral contacts between cardiomyocytes had a structure resembling that of desmosomes: Fixation plaques and the intercellular space could be distinguished in them.

In the myocardium of the autumn frogs compared with the myocytes of the spring frogs the width of the space between the fixation plaques was increased (28.85 ± 2.085 nm in spring, 37.79 ± 2.8 nm in the fall; $P < 0.05$). Morphometric analysis of the lateral contacts showed (Fig. 1) that the surface area of the contact, its volume fraction, and the ratio of these parameters per unit volume of tissue were significantly ($P = 0.05$) greater in autumn than in spring frogs.

Contacts between myocytes formed in the end-to-end pattern were represented by intercalated disks. These are complex and highly specialized derivatives of the sarcolemma of the adjacent cells, and in their composition it is possible to distinguish an intersarcoplasmic region, an interfibrillary zone, and a long connecting surface. It can be concluded from the electron-microscopic findings that there is no difference in the organization of the intercalated disks of the myocytes in the two seasons, but much osmiophilic material was observed in the autumn frogs in the region of the intercalated disks. The results of the morphometric analysis indicated no significant difference between any of the parameters of the intercalated disks (Fig. 1).

Turning to the characteristics of the mitochondria in cardiomyocytes of the autumn and spring frogs, these organelles were polymorphic. However, many small mitochondria containing a few thick, densely packed cristae, arranged transversely to the long axis of the mitochondria, were found in the myocytes of the spring frogs. As regards the large mitochondria, they were fewer in number. They were organized in the same way. The pattern described is incomplete without mention of the presence of mitochondria in which cristae were almost completely absent, despite the integrity of the outer membrane, in the myocytes of the spring frogs (Fig. 2).

In the ventricular myocytes of the autumn frogs several categories of mitochondria also were noted. However, the majority of the mitochondria were large in size. Irregularly arranged cristae were frequently observed in them. In addition, many mitochondria with signs of destruction were discovered in the cardiomyocytes (Fig. 3).

The following parameters of the mitochondria were calculated by morphometric analysis: volume fraction, surface area of the structure, and the ratio between them per unit volume of tissue. The quantitative results indicate that the percentage of altered mitochondria per unit volume of tissue was higher in the fall than in the spring (28.28 ± 5.15% and 16.87± 4.75%, respectively; $P < 0.1$). The ratio Vv/Sv for the unchanged ($P < 0.02$) and altered ($P < 0.02$) mitochondria was significantly increased.

The results of these experiments confirm that the ultrastructural characteristics of the cardiomyocytes of autumn and spring frogs differ in several parameters. Hence it follows that, when the ultrastructure of the cardiomyocytes of the amphibian ventricle is analyzed, the dependence of their morphological indices on the season of the year must be taken into account.

The stereological approach to the analysis of electron-microscopic data ensures their most objective evaluation.

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