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Research paperr

Endocrine Apparatus of the Rat Testes in the Adaptation Dynamics to Low Temperatures

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Abstract

Within the framework of the experimental study, new data were obtained on the endocrine apparatus of rat testicles represented by interstitial endocrinocytes, or Leydig cells (CL), in the dynamics of adaptation of the animal body to low temperatures. Using methods of morphological analysis, including both routine and modern methods, it was found that in the early stages of adaptation the ratio of the main morphofunctional types of CL is violated, the index of CL activity decreases, their sizes decrease, resulting in a decrease in the concentration of testosterone in the blood serum. In the course of further adaptation the relative amount of CL reduces, increasing the number of degenerating CL. In the later stages of adaptation, the relative amount of CL remains reduced, the activity index of endocrinocytes increases, compensatory hypertrophy of CL develops, which leads to an increase in the level of testosterone in the blood serum

Keywords: Leydig cells, rat testes, adaptation.

1. Introduction

The equilibrium state between the environment and living organisms, formed in the process of evolution, ensures their normal functioning and reproduction, as well as effective adaptation to changing environmental conditions. In turn, the ability of animals to reproduce in new conditions is the main criterion for their successful adaptation. Sex hormones, including testosterone, the main producers of which in males are interstitial endocrinocytes of the testicles, or CL, play an important role in both adaptation and reproduction of animals.

Despite the fact that interstitial endocrinocytes were first discovered by Franz Leydig in the XIX century, in the XXI century, scientists continue to show increased interest in their study [19; 24; 27]. The majority of scientific researches, the object of study of which is CL, is of experimental nature [17; 25; 26]. In recent years, the issues of proliferation, differentiation and regeneration of CL [20; 26; 30], age-related changes in CL [13; 17; 18] are actively studied, there are new works on the regulation of CL [25; 26; 30], a number of studies are devoted to the study of CL under the influence of natural and industrial toxicants on the organism[1; 23; 29].

Not enough attention is paid to the study of the impact on the testicles endocrine tissue of natural factors, constantly acting on the body of animals [7; 10], which is explained by the widespread opinion that all living organisms are perfectly adapted to the conditions of their habitat. At the same time, interesting results were obtained in the study of the dependence of functional activity of CL on circadian rhythms [10], which proves high sensitivity of endocrinocytes to the action of natural environmental factors, one of which is low seasonal temperatures.

Previously, we reported on the quantitative changes that the CL population undergoes when animals adapt to low temperatures [7]. Outside our attention there were questions of adaptive CL rearrangements at the ultrastructural level, no correlation between the functional state of endocrinocytes and testosterone levels were established, some pathogenetic aspects of cold adaptation of mammalian organism were not taken into account. Thus, the problem of morphological adaptation of the endocrine apparatus of the testicles to low temperatures is far from being solved.

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The purpose of the present experimental research was to study the endocrine apparatus of the rat testicles represented by CL in the dynamics of adaptation of animals organisms to low temperatures with the use of modern methods of morphological analysis.

2. Methods

The study was conducted on 100 nonlinear Mature white rats-males weighing 200-250 g. All rats were kept in vivarium in compliance with the 12-hour light regime on a standard diet, with free access to food and water. Intact rats kept in standard vivarium temperature conditions formed a control group. Rats of I, II, and III experimental groups were cooled at −15ºC for 3 hours daily for 7, 14 and 28 days, respectively. The day after the end of cooling, the rats were removed from the experiment by decapitation under thiopental anesthesia.

All manipulations with animals were carried out in accordance with the "International recommendations for medical and biological research using animals" and the order of the Ministry of health of the Russian Federation № 267 of 19.06.2003 "on approval of the rules of laboratory practice".

For histological examination, testicular fragments from the Equatorial zone with a thickness of about 10 mm were fixed in 10% neutral formalin, dehydrated and poured into paraffin according to the standard histological scheme, paraffin sections were stained with hematoxylin and eosin.

Quantitative analysis of CL was performed using a hardware-software complex consisting of software for quantitative analysis of video Test – Morphology 5.0. (Videotest, Russia), digital eyepiece cameras DCM 130, the light microscope "Mikromed-1", and a personal computer. The diameters of the nucleus and cytoplasm of CL were measured, the CL per transverse section of one twisted seminiferous tubule was calculated, the % of the number of small, medium and large CL was determined, and the CL activity index was calculated [1].

For electron microscopy, testicular tissue samples were fixed in a 2.5% solution of glutaraldehyde with postfixation in 1% solution of osmic acid, dehydrated in alcohols of ascending concentration, poured into a mixture of EPON and Araldite. Ultrathin sections were contrasted with uranyl acetate solution and lead citrate, studied on transmission electron microscope "Tecnai G2 Spirit TWIN" (FEI Company, Netherlands).

To establish the correlation between morphological and functional criteria of CL activity in blood serum, testosterone concentration was determined by enzyme immunoassay using a standard set for enzyme immunoassay (Vector-best, Russia) according to the manufacturer's Protocol.

Taking into account the important role of oxidative stress in the adaptive process [14], the concentration of lipid peroxidation products (LPO) – diene conjugates (DC), lipid hydroperoxides (GL), malondialdehyde (MDA – in serum) and the main component of the antioxidant system of vitamin E were determined in testicular tissue and serum.

Statistica 6.0 software (Statsoft, USA) was used for statistical processing of quantitative data. To test the hypothesis of normal distribution of values in the samples, the Kolmogorov-Smirnov test was used, after which the samples were compared using the student t-test, the differences were considered significant at $p < 0.05$.

3. Data, Analysis, and Results

Microscopy showed that after 1 week of adaptation a number of structural and functional changes were observed in CL, distinguishing them from CL of the control group rats. In the nuclei of endocrinocytes moderate condensation of chromatin is observed: larger lumps occupy a marginal position, smaller lumps are evenly dispersed throughout the volume of the nucleus (Fig. 1, a). Dividing KL have become an interesting morphological finding: endocrinocytes under metaphase plate can be observed in intatublular connective tissue (Fig. 1, b). In almost every area of the interstitium there are degenerating CL of small size with pyknotic nuclei (Fig. 1) and deep invagination of the karyolemma.

After 2 weeks of adaptation in the nuclei of CL moderate condensation of nuclear chromatin retain, the interstitial tissue depletion of the testicles of CL draws attention. Compared with the previous period of adaptation, the number of degenerating CL is progressively increasing, they are in the amount of three to four are found in all interstitial triangles. Degenerating CL are visualized by small hyperchromatic nuclei with deep intussusception of karyolemma that on the longitudinal section gives the kernels the form of the coffee bean.

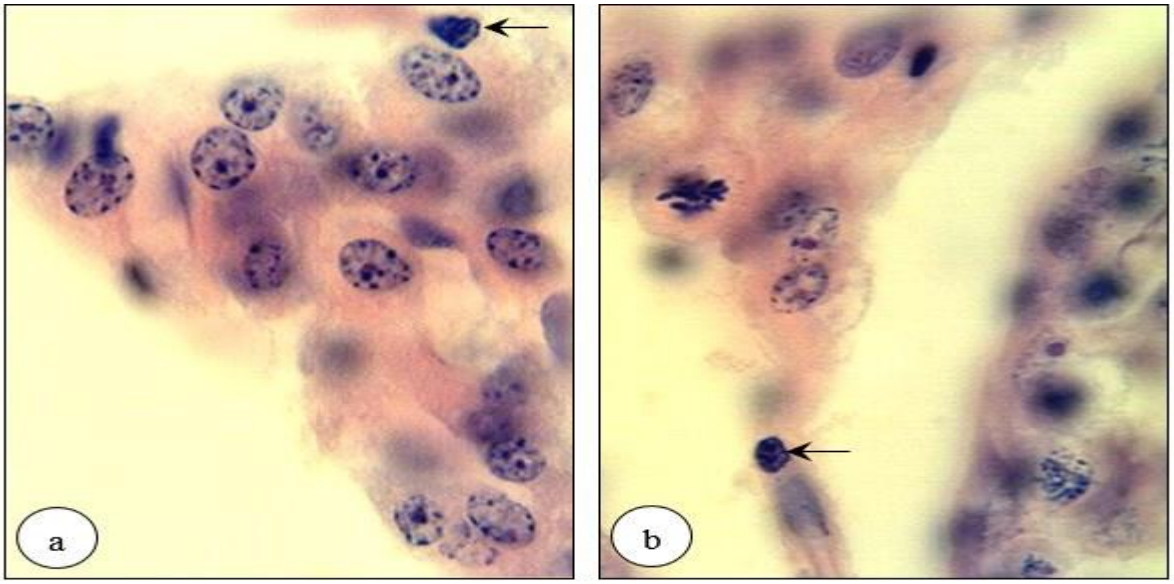

Figure 1. The testicle after the 1st week of adaptation a) chromatin condensation in the CL nuclei; b) Pattern of mitosis in CL (stage of metaphase plate).
↑ degenerating CL. Staining with hematoxilin and eosin.
Increase: 10×100.

After 4 weeks of adaptation, the poorer interstitial of the testes of CL increases. The predominant morphofunctional type in the population are medium and large round or oval CL with light vacuolated cytoplasm and light nuclei, virtually devoid of condensed chromatin.

Quantitative analysis showed (Fig. 2, 3), that after the 1st week of adaptation in the population the representation of small cells increases, at the same time, medium and large CL becomes smaller, which leads to a decrease in the CL activity index ($p < 0.05$), the diameter of the nucleus and cytoplasm of CL decreases ($p < 0.05$). The relative amount of CL after the 1st week of adaptation does not change.

After 2 weeks of adaptation, the diameter of the CL nuclei has no statistically significant differences with the control group, the cytoplasm diameter is still smaller ($p < 0.05$). The relative amount of CL is reduced by 17 % ($p < 0.05$). Analysis of the population composition of CL showed that small forms of 10 % more than in the control, the number of medium-sized cells is reduced by 8%, and large cells by 2%. Decrease in the population of medium-and large-size CL endocrinocytes leads to a decrease in the activity index by 1.5 times ($p < 0.05$).

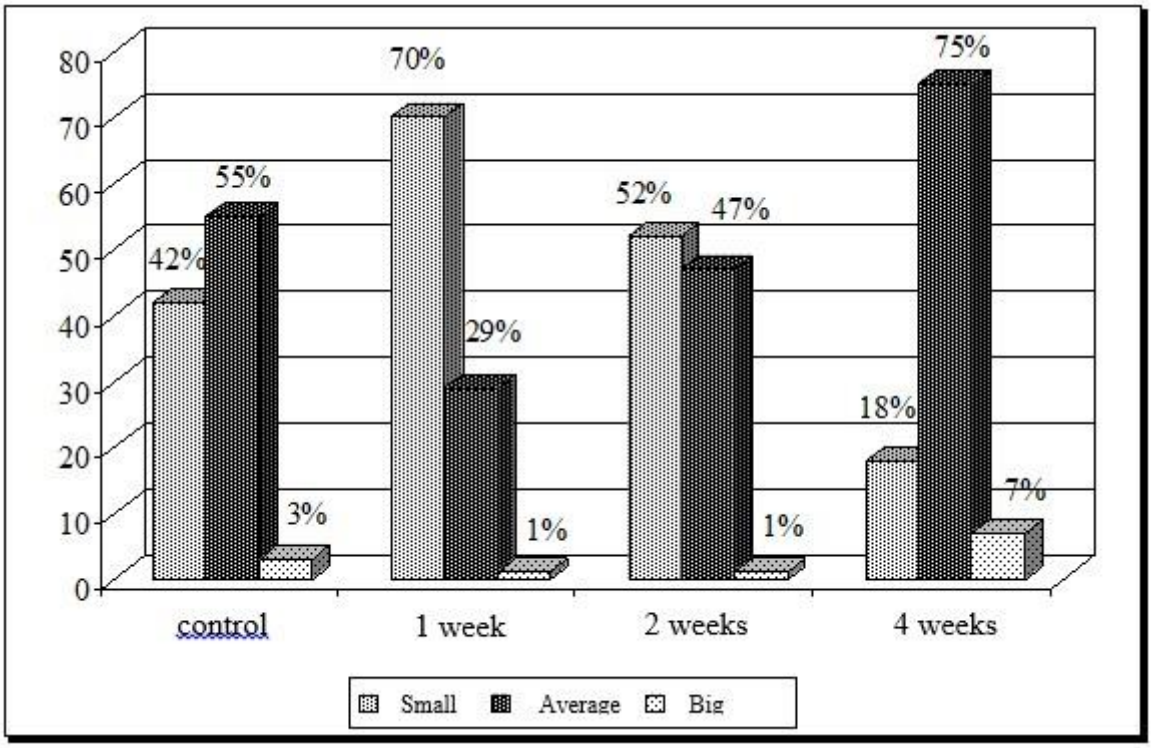

Figure 2. Distribution of the population of CL on three morphofunctional types at the stages of adaptation to low temperatures (according to the results of quantitative analysis).

After 4 weeks of adaptation, the relative amount of CL decreases by 19% ($p < 0.05$), the average size of the nucleus and cytoplasm of cells increases ($p < 0.05$). The population composition of CL is changed: the share of medium and large CL in the population increases by 20% and 4%, respectively, the share of small CL decreases by 24% (Fig. 2). As a result, the number of active CL in the population increases to 82%, and the CL activity index increases by 3.2 times ($p < 0.05$).

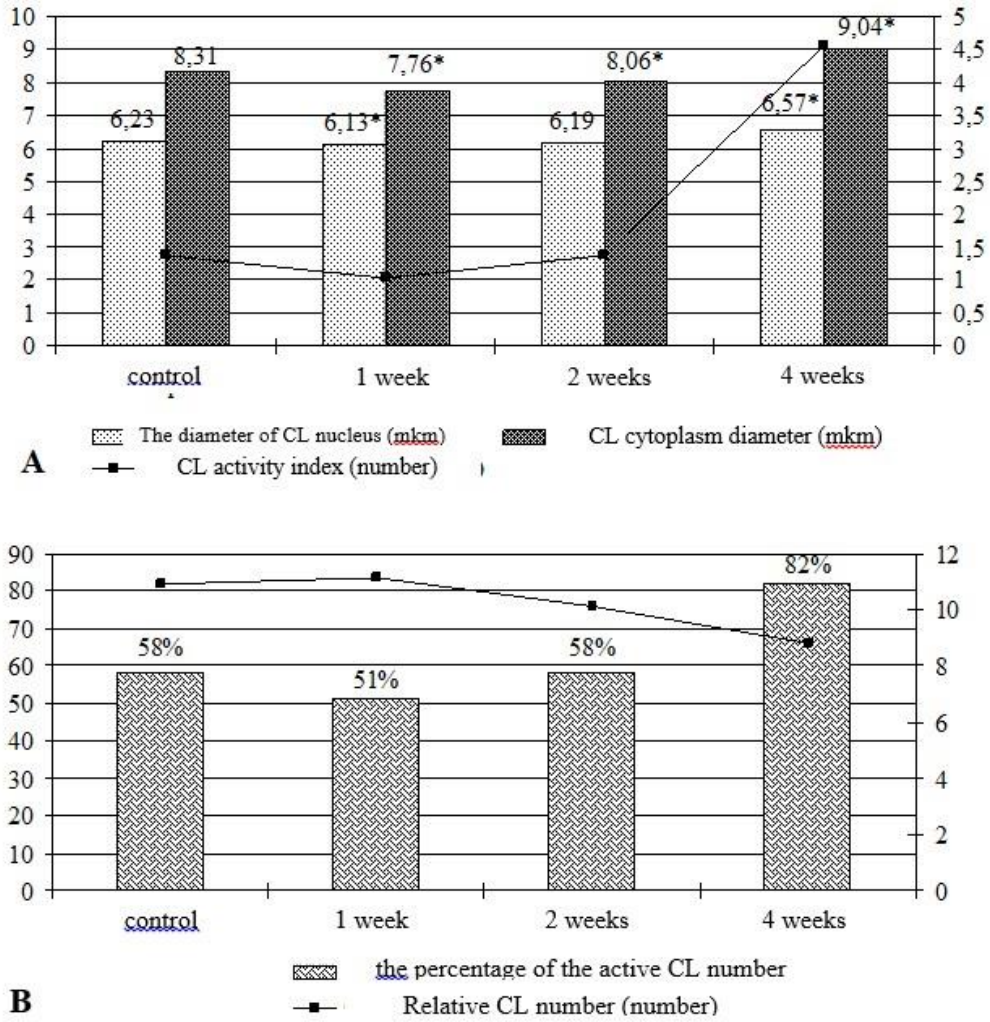

Figure 3. A, B. Quantitative indicators of endocrine activity of the testicle at the stages of adaptation to low temperatures. Note: * – differences are statistically significant compared to Control at $p < 0,05$.

According to ultrastructural analysis, after 1 week of ahdaptation in the cytoplasm of CL, there is an accumulation of lipid drops, which are clearly visible against the background of moderately enlightened cell matrix (Fig. 4). The thickened strip of the marginal chromatin is detected in the CL nuclei . In the cytoplasm of some CL moderate expansion of the vesicles of smooth endoplasmic reticulum is observed.

After 2 weeks of adaptation degenerating CL are detected in the cytoplasm of which there is expansion of vesicles of the agranular endoplasmic reticulum, lysosomes accumulate, the inner membrane of mitochondria become indistinct, resulting in the internal contents of most of the mitochondria becomes structureless (Fig. 5). There is a close contact of degenerating endocrinocytes and testicular macrophages (Fig. 5, a).

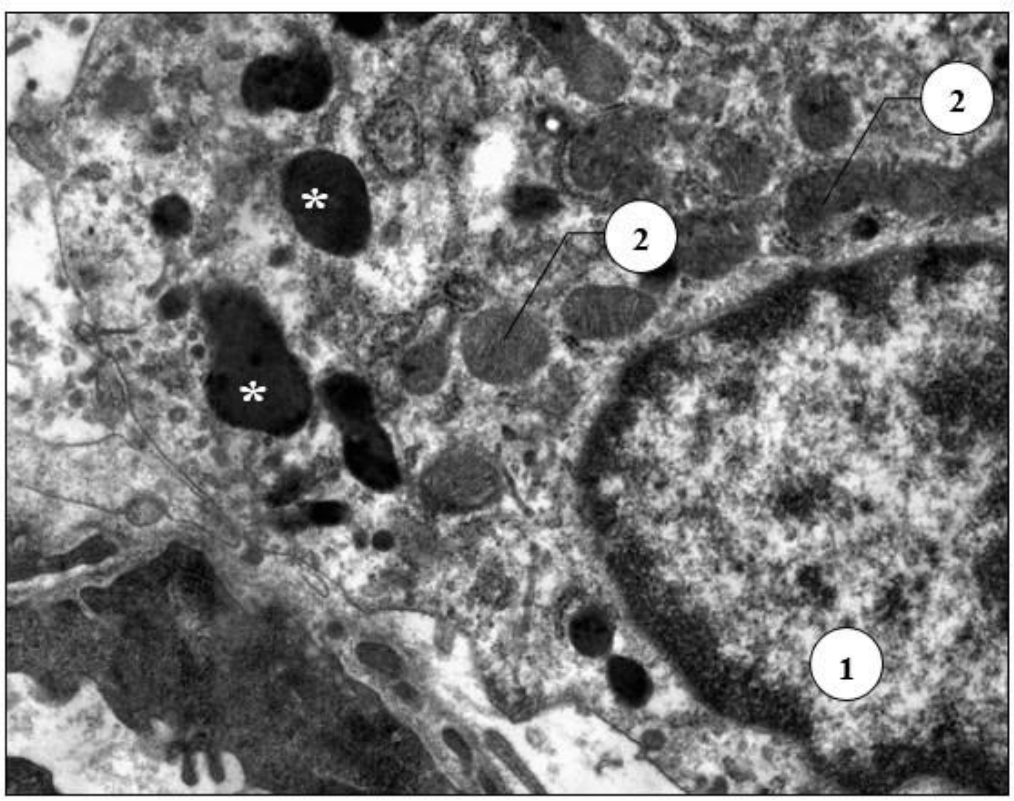

Figure 4. The testicle after the 1st week of adaptation. Chromatin condensation iIn the core (1) CL. 2 – mitochondria; * – lipid drops; Staining with uranyl acetate and lead citrate. Increase: 23000.

According to electronic microscopy, after 4 weeks of adaptation CL with enlightened matrix and advanced elements of the agranular endoplasmic reticulum are dominated. Mitochondria in such cells have fuzzy, blurred crosses, lipid drops in the cytoplasm are absent (Fig. 6, a). In parts of CL cytoplasmic matrix preserves the electronic density. In the interstices of the testes representation of testicular macrophages is increased. In the cytoplasm of some of them giant phagosomes, containing fragments of cytoplasm of CL are found (Fig. 6, b). Close contact of macrophages with hypertrophied, vacuolated CL, and with dark CL is observed (Fig. 7, a, b).

According to the enzyme immunoassay, the concentration of testosterone in the blood serum of rats from the control group is 33.72±2.03 nmol/l, after the 1st week of adaptation, the concentration of testosterone in the blood serum decreases to 28.06±1.80 nmol/l, after 2 weeks of adaptation 28.76±2.15 nmol / l ($p < 0.05$). After 4 weeks of adaptation, the level of serum testosterone rises to 32.17±0.83 nmol/l, and has no statistically significant differences with the control ($p > 0.05$).

The data of biochemical research showed that already in the early stages of adaptation of the body to low temperatures oxidative stress in the testicles develops (Fig. 8). GL content in the testicular tissues increased by 81%, and the content of vitamin E decreased by 28% ($p < 0.05$). In the blood serum after the 1st week of adaptation, the DC content increased by 61%, MDA became more by 25% ($p < 0.05$), there was a clear trend towards an increase in the level of GL and a decrease in the concentration of vitamin E (Fig. 9).

After 2 weeks of adaptation, biochemical manifestations of oxidative stress are preserved (Fig. 8, 9). The content of GL in the testicular tissues exceeds the control group by 48%, the content of DC increases by 16%, vitamin E becomes less by 31% ($p < 0.05$). The content of DC in blood serum increased by 86%, MDA by 15% ($p < 0.05$), the level of vitamin e continues to decrease ($p > 0.05$).

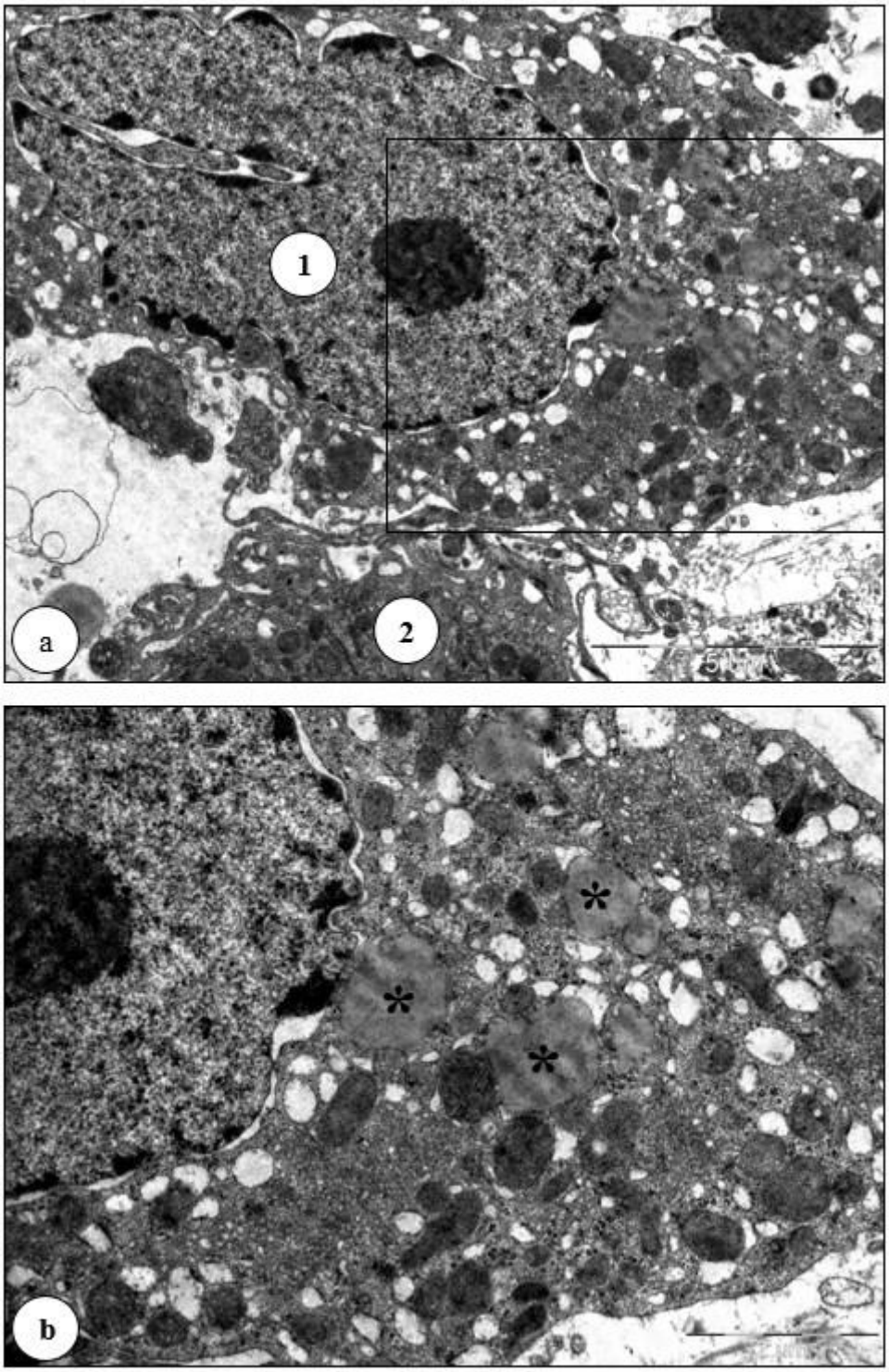

Figure 5. The testicle after 2 weeks of adaptation. Staining with uranyl acetate and lead citrated. a) Degenerative CL contact (1) with testicular macrophage (2). Increase: 13500. b) The fragment from the frame. In the cytoplasm of CL the number of lysosomes (*) and lypids is increased, smooth EPS is vacuolated. Increase: 23000.

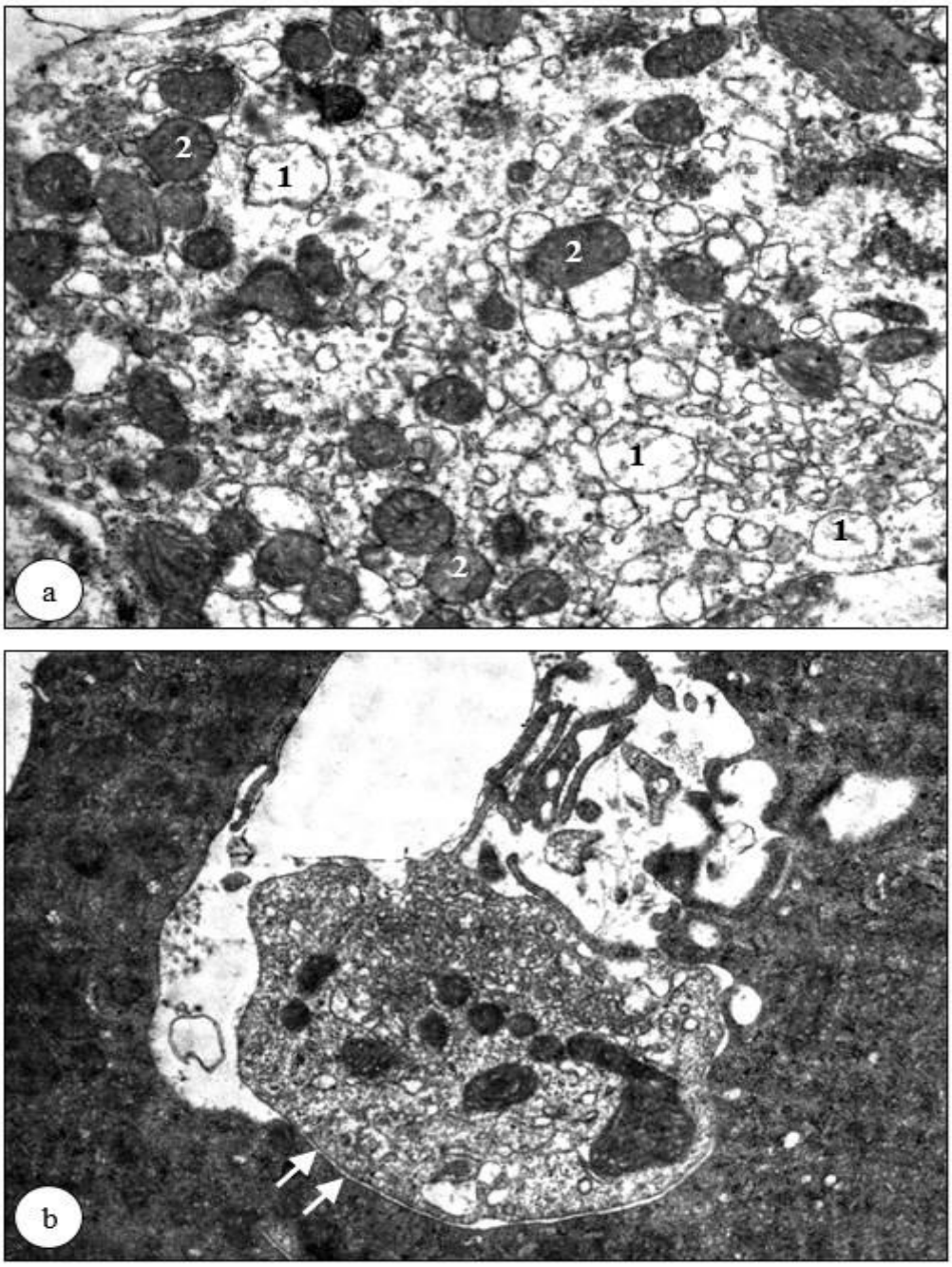

Figure 6. The testicle after 4 weeks of adaptation. Staining with uranyl acetate and lead citrated. a) The fragment of the cytoplasm of light CL. 1 – advanced vesicles of agranuljarnaja EPS; 2 – mitochondria with blurred crosses. Increase: 23000. b) Giant phagosome
In the cytoplasm of the testicular macrophage (↑↑), containing a fragment of CL.
Increase: 25000.

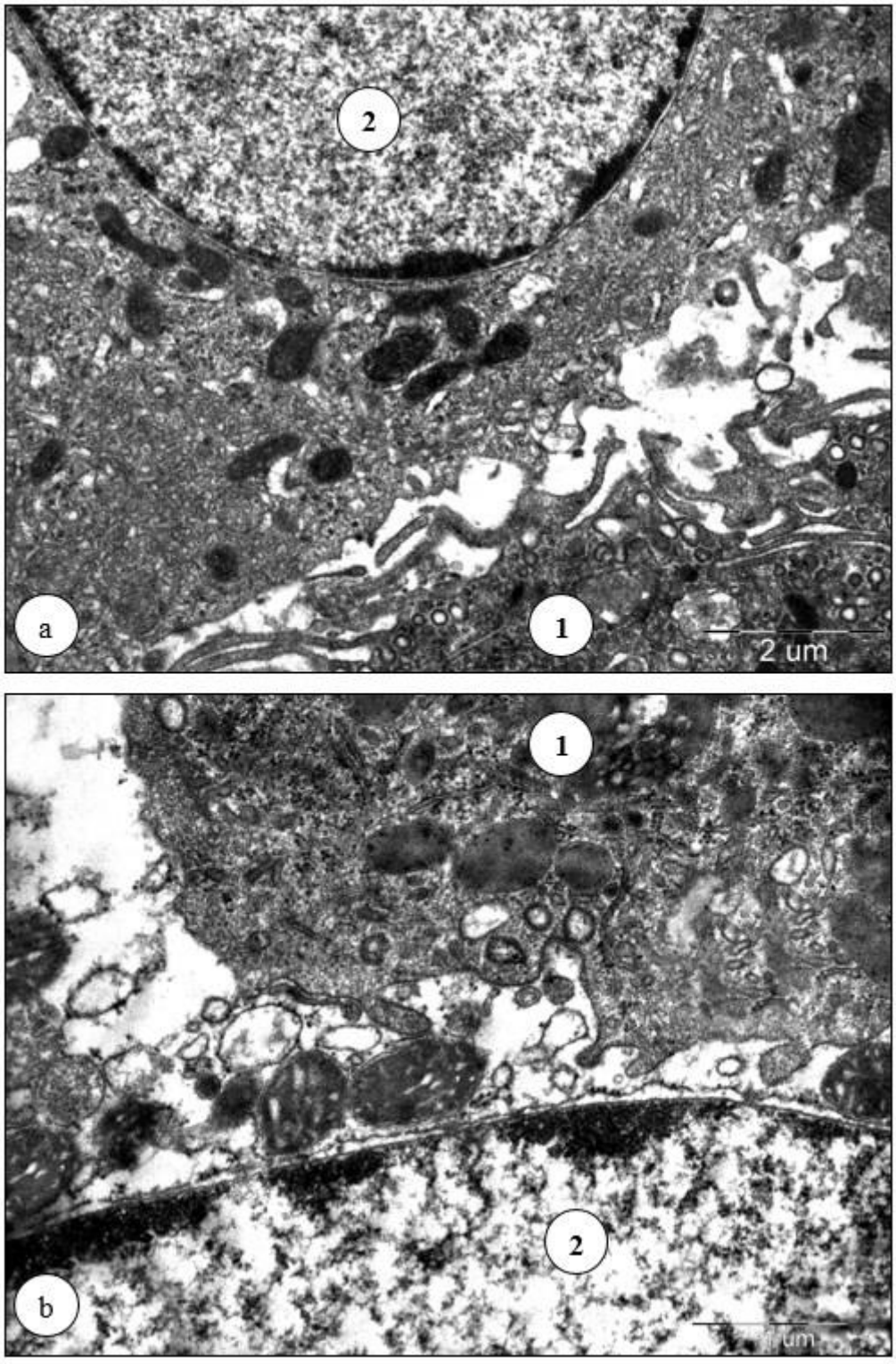

Figure 7. The testicle after 4 weeks of adaptation. Staining with uranyl acetate and lead citrated. a) contact of activated macrophage (1) and dark CL; 2 – the core of CL. Increase: 23000. b) contact of the macrophage (1) and the light CL. 2 – the core of CL. Increase: 46000.

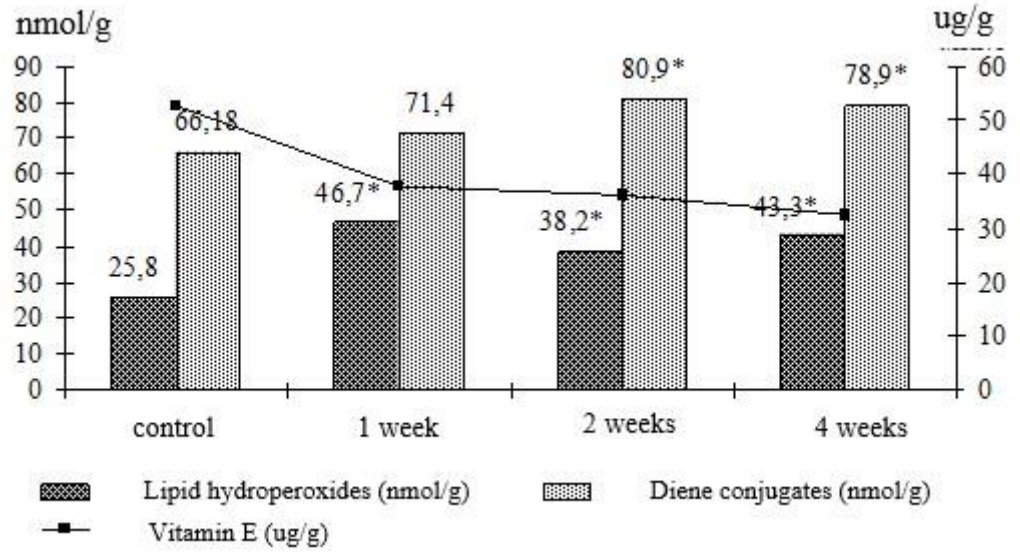

Figure 8. Content of POL and vitamin E products at the stage of adaptation in the testicular tissue.
Note: * – differences are statistically significant with control at $p < 0,05$.

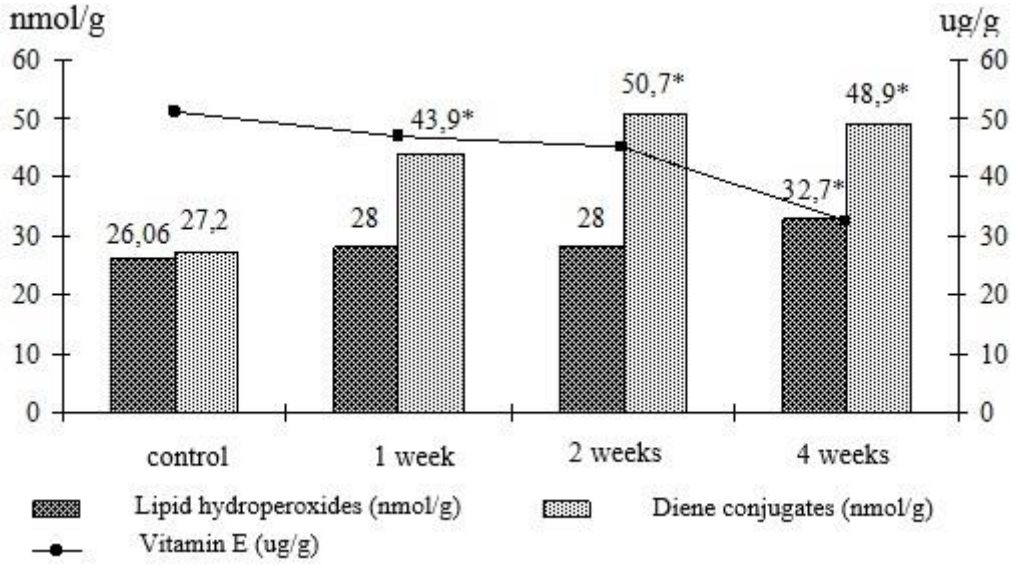

Figure 9. Content of POL and vitamin E products at the stage of adaptation in the blood serum.
Note: * – differences are statistically significant with control at $p < 0,05$.

After 4 weeks of adaptation, GL content in the testicular tissues remains increased by 68%, DC by 13%, and vitamin e content is reduced by 18% ($p < 0.05$). Serum levels of DC are increased by 76%, GL and MDA by 25%, while vitamin e is reduced by 34% ($p<0.05$).

4. Discussions

A review of the printed works showed that under stress of different etiology changes in CL of rat testicles have a pronounced depressive character and consist in a steady decrease in the number of endocrinocytes and a decrease in the level of testosterone in the blood of animals [6; 13; 19; 29].

Changes in the endocrine apparatus of the testicle, found after the 1st week of adaptation to low temperatures, also have a depressive orientation. Quantitative analysis showed a change in the population composition of CL in favor of small forms that are inactive in relation to steroidogenesis [1; 13], a decrease in the diameter of the nucleus and cytoplasm of endocrinocytes. The representation in the population of medium and large CLS, actively involved in the synthesis of steroid hormones [1; 13], decreases. The found changes are consistent with the results of other authors who noted a decrease in the proportion of functionally active CL in the testicles of rats under various types of stress [6; 19; 24; 27; 29]. In this study, the relative amount of CL after 1 week of adaptation does not change, due to the physiological nature of acute cold stress.

Normally, the cytoplasm of CL of rats does not contain lipid inclusions due to their active utilization in the process of steroidogenesis [8], therefore, the accumulation of lipids in the cytoplasm of CR after the 1st week of adaptation, discovered by electron microscopy, in conjunction with a moderate condensation of nuclear chromatin, also points to inhibition of the functional activity of CL.

The appearance after the 1st week of adaptation of mitotically dividing CL, in our opinion, is a compensatory-adaptive reaction of the endocrine apparatus of the testicle, aimed at the formation of polyploid cells. In the testicles of Mature rats, CL represent a highly specialized cellular population with a very low mitotic index, which remains constant throughout the life of the animal [13; 26; 27; 30]. A number of authors admit the

presence of the phenomenon of polyploidy in the CL population. Indirect evidence of the ability of replicative DNA synthesis in CL is the presence of endocrinocytes with the amount of cores that differ by a multiple number of times [27; 30].

Consider the possible reasons for the decrease in the functional activity of CL and testosterone concentration in the blood serum of rats at the early stages of adaptation to low temperatures. The complex of protective and adaptive mechanisms of the body, accompanied by changes in physiological and biochemical parameters, and ensuring the maintenance of constancy of the internal environment at low ambient temperature, is called cold stress. The initial stage of adaptation of animals to low seasonal temperatures corresponds to acute cold stress [2; 8; 14] and is characterized by the development of a General adaptation syndrome, the basis of which is the activation of the Central stress-implementing system of the hypothalamus – pituitary – adrenal glands [3; 9; 22]. Activation of the hypothalamus-pituitary-adrenal glands is accompanied by increased secretion of corticotropin-releasing factor by the nuclei of the hypothalamus and adrenocorticotropic hormone adenohypophysis, which leads to excessive secretion of glucocorticoids by the adrenal glands [9; 12; 22]. Increase of cortisol level is a reliable indicator of stress-reaction recorded in cold stress in rats [14].

Studies show that an increase in the level of glucocorticoids under stress of different etiologies is accompanied by a decrease in the concentration of testosterone in the blood serum of rats [19; 22; 23; 28]. The excess of corticosterone in Mature male rats reduces the production of gonadotropin releasing factor, luteinizing and follicle stimulating hormones, as well as the secretion of testosterone CL [19; 22].

The inhibitory effect of excessive corticosterone concentration on steroidogenesis in CL is realized through reduction of glucose oxidation and violation of NADP production [24]. Stress-induced increase of corticosterone concentration disrupts the work of key enzymes necessary for NADP synthesis [22; 28], and inhibits the activity of key enzyme of steroidogenesis 11β-hydroxysteroid dehydrogenase in rats CL [23].

Adaptation of mammals to low temperatures is one of the complex compensatory-adaptive reactions of the organism, including physiological and chemical mechanisms of thermoregulation [14]. An important role in maintaining the temperature homeostasis is played by the sympathoadrenal system, which regulates energy processes through the activation of lipolysis and glycogenolysis [14]. Catabolism of lipids and strengthening of redox processes in cells inevitably leads to activation of LPO reactions.

The data of biochemical research showed that already in the early stages of adaptation of the body to low temperatures in the tissues of the testicles oxidative stress develops. The accumulation of POL products in the simulation of cold stress in rats is noted by many authors [3; 8; 14], and studies [4] showed that in winter, in testicular tissues of male rats the content of POL products is higher than in summer.

It is known that induction of LPO processes is an integral part of steroidogenesis [11; 15; 16]. The activity of LPO processes necessary for normal steroid Genesis is regulated by luteinizing hormone, which simultaneously supports high activity of enzymes inactivating lipid peroxidation products [16]. Oxidative stress in rat testicles induced by adaptation to low temperatures can inhibit testosterone synthesis through inhibition of CL enzyme systems. For example, one of the reasons that cause a decrease in the production of testosterone CL in aging rats is the active forms of oxygen produced in the process of steroidogenesis, while reducing the activity of antioxidant enzymes [17; 18]. It is established that accumulation of peroxidation products in the tissue of the testes of rats depresses the activity of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase, catalyzes the synthesis of testosterone in microsomal fractions of CL [18].

After 2 weeks of adaptation, there is a tendency to restore the ratio of the three main morphofunctional types of CL, at the same time, the relative amount of CL decreases, and the level of serum testosterone remains reduced. The decrease in the relative amount of CL is the result of the elimination of degenerative forms of endocrinocytes, which were found in large numbers after the 1st week of adaptation.

We believe that the inducer of CL apoptosis, which leads to a decrease in their population, is oxidative stress, which is considered as the main cause of programmed cell death [5, 16, 19]. The data of the biochemical study showed that after 2 weeks of adaptation in the tissues of the testis and blood serum there is an escalation of oxidative stress.

The data obtained are consistent with the results of studies by other authors, which show that oxidative stress in rat testicles caused by various causes leads to the development of degenerative changes and apoptosis of CL [16; 19].

Consequently, oxidative stress, developing in the adaptation of the body of animals to low temperatures, can inhibit the production of testosterone by CL in two ways: through the suppression of enzyme systems involved in steroidogenesis, and by induction of apoptosis of endocrinocytes.

After 4 weeks of adaptation, there is a change in the population composition of CL in favor of active forms, but the relative amount of CL is reduced and reaches minimum values. Despite the persistence of biochemical manifestations of oxidative stress, serum testosterone concentration increases. Transmission microscopy is dominated by CL with ultrastructural signs of functional stress, indicating their active participation in the synthesis of steroids [13], but also by endocrinocytes, whose cytoplasm indicates the depletion of adaptive reactions of these cells.

The question of whether the surviving CL with signs of compensatory hypertrophy and functional exhaustion are able to provide an increase in serum testosterone level remains unresolved. The level of serum testosterone does not fully reflect its concentration in the testicular tissues. It is known that in rats in the physiological norm 90% of serum testosterone is formed by CL of the testicular and 10% is synthesized by endocrinocytes of the adrenal cortex. Consequently, an increase in serum testosterone levels can be provided by the adrenal cortex, stress hypertrophy of which reaches its maximum by the end of the first month of adaptation to cold [3].

Accelerated elimination of CL is accompanied by a natural increase in the representation of testicular macrophages involved in the phagocytosis of endocrinocytes. To date, the role of testicular macrophages in the regulation of steroidogenesis of CL has been established, as well as their stimulating effect on the proliferation of poorly differentiated predecessors of CL [21]. Based on this, the increase in the number of testicular macrophages at the stages of adaptation to low temperatures may be due to their important regulatory potentials aimed at restoring the population of CL.

Thus, the study of the dynamics of quantitative parameters of CL and testosterone concentration in the blood serum of rats at the stages of adaptation of the animal organism to low temperatures showed that depressive changes in the CL population, together with a decrease in serum testosterone levels, are more typical for early adaptation. The decrease in the relative amount of CL begins with the 2nd week of adaptation, and the minimum number of cells is noted after 4 weeks of adaptation to low temperatures. The concentration of serum testosterone after 4 weeks of adaptation, on the contrary, increases to the level of intact animals. The structural basis of this phenomenon, in our opinion, is compensatory hypertrophy of CL, confirmed by the results of morphological, ultrastructural and quantitative analysis. However, as a source

of serum testosterone it is impossible to exclude the adrenal cortex, which stress hypertrophy according to the literature reaches its maximum by the end of the first month of adaptation [3].

5. Conclusion

After the 1st week of adaptation, there is inhibition of the endocrine apparatus of the testis, as indicated by the condensation of chromatin in the nuclei of CL, a decrease in the linear size of the nucleus and the cytoplasm of CL, an increase in the population of small forms of CL, a decrease in the activity index of CL, which together leads to a decrease in the concentration of testosterone in serum;
After 2 weeks of adaptation, the relative amount of CL decreases, the number of endocrinocytes with signs of involution increases progressively, the concentration of testosterone in the blood serum is reduced;
After 4 weeks of adaptation, which corresponds to the completion of the first phase of adaptation to low temperatures, the relative amount of CL remains reduced, compensatory hypertrophy of CL develops, the activity index of CL increases, the concentration of testosterone in the blood serum increases;
The detected remodeling of the testicular endocrine apparatus is aimed at the production of CL steroids in an amount sufficient to ensure reproductive function in the conditions of adaptation of the animal organism to extreme environmental factors.

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