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Morphophysiological Status of Rats Being on Micronutrient Supplementation

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Aim of the study was structural and functional analysis of the body of laboratory rats on the background of administration of biogenic drugs which include micro- and macroelements and substances of animal and vegetable origin. We have analyzed intergroup difference of morphometric parameters of adrenal glands structures. We revealed particular micromorphological features of adrenal glands structures on the background of biological products administration in animals participating in the subacute experiment. Morphometric changes of the studied gland in rats treated with an aqueous solution of Calcefit-5 were noticed. Morphological changes in the structures of adrenal glands, particularly changes of the beam zone, were objective verification tools of rat's response to experimental conditions.

Key words: Adrenal glands, Biological preparations, Rats.

Actual methods of agricultural management include addition of biologically active substances to the basic diet of animals[1-2]. Earlier studies of the influence of biologically active substances on the growth and development of productive animals show the importance of comprehensive analysis of their effects on the body[3]. Many researchers study morphological and functional characteristics of adrenal body as this is the gland that affects functioning of the organism in all periods of life, especially in maintenance of homeostasis in terms of the adaptation to constantly changing environment conditions[4-5]. The morphological state of the gland allows to assess the degree and adequacy of the organism's response to the effect of various stress factors. All morphological zones of adrenal glands participate in the implementation of adaptive mechanisms to a greater or lesser extent. These facts are confirmed by researches made by O.I. Kirillov, E.I. Hasina, V.B. Durkin (2003) [6]. In this regard, it is necessary to examine the effects of modern biologically active substances on the morphological structure of adrenal glands. A part of our research studies the impact of biological product Calcefit-5 on the adrenal glands of rats.

The aim of the work was to study characteristics of morphological transformations of adrenal glands of outbred albino rats after the intragastric administration of an aqueous solution of Calcefit-5 in terms of subacute experience.

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MATERIALS AND METHODS

In our research we used classical morphological methods.

Animals were enrolled in the experimental group based on the sex, age and weight.

An experiment was performed on 20 outbred male rats aged from 4 to 5 months. Their weight was in the range from 160 to 190 g. These were health, active animals with clean and smooth coat and transparent corneas. All animals were kept under standard conditions with a free access to food and water.

An experiment lasting for 5 weeks was carried out in order to study the effect of substances on morphological and physiological parameters in rats.

Background values of morphofunctional state of animals were registered in Day 1 of the experiment: all animals were checked for such parameters as body temperature, weight, heart rate, and respiratory rate. We performed visual examination of the skin, hair, visible mucous membranes, and lymph nodes. At the beginning and at the end of the study we also measured length of the body and tail.

Further, a visual examination was carried out on a daily basis. Measurements of physiological parameters of the experimental animals were carried out on Day 5, 10, 20, 30 of the experiment and at the time of termination of the experiment - on Day 35. For histological studies we performed decapitation of 3 animals on Day 1 and Day 20 and at the end of the study.

The studied substance - an aqueous solution of Calcefit-5 - was administered to the animals of the experimental group (10 animals – an experimental group) via intragastric way. Administering of the substance previously dissolved in 3 mL of distilled water at the dose of 0.1 g per animal was performed on a daily basis throughout the whole experiment including weekends. Distilled water in equivalent volumes was administered to the animals of the control group (10 animals) via the same scheme.

Calcefit-5 (mineral feed supplement, Russia, Saint-Petersburg). Ingredients: meat and bone flour; calcium lactate; calcium glycerophosphate; citrate; magnesium sulfate; sulfur; iron lactate; potassium silicate; potassium iodide; calcium fluoride; freeze-dried extract of ginseng tincture. St. Petersburg Institute of Veterinary Biology, TU 9219-001-50021486-2002).

Animals were withdrawn from the experiment by decapitation under the ether anesthesia. For the subsequent studies we extracted adrenal glands, determined their absolute mass, and mass ratio. Sampling, fixing, dehydratation and degreasation of the specimen were carried out according to the standard procedures.

On morphometric analysis of histological sections of adrenal glands we determined the thickness of glomerular, beam and mesh zones of the cortex and medulla (μm), measurement of the diameter (μm), area of the nuclei and cell cytoplasm (μm^2) in each of the studied zones of the adrenal glands, and we also calculated nuclear-cytoplasmic ratio (NCR)[7-8].

Microstructure of the glands was studied using light-optical microscope "MICMED-6" (JSC "LOMO", St. Petersburg, Russia) with photo coupling and detecting unit. Input and analysis of images was performed using the computer software for morphometric analysis "Micro View" (LLC "LOMO-Microsystems", St. Petersburg, Russia). Statistical analysis of the data was performed using Microsoft Excel 2007. Testing the hypothesis of normality of distribution was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test. Testing the hypotheses about the equality of mean group of quantitative parameters was performed using the nonparametric test of Wilcoxon-Mann-Whitney. Mean values of parameters were given as $M \pm s$ (M - mean, s - standard deviation). An assessment of the statistical significance of differences between mean values was carried out at the critical level of $p = 0.05$.

RESULTS AND DISCUSSION

Daily visual examination of animals of the experimental group showed that during the experiment all rats were in a healthy clinical and physiological state.

On average, at the time of withdrawing from the experimental an average weight of the animal of the control group was 240.2 ± 3.79 g, which was lower by 2.8% than that of the rats in the experimental group at the same period.

The body length of the control rats was 194.8 ± 1.1 at the beginning of the study and 197.4 ± 0.9 cm at the end of the study. At the same time the length of the tail was in the range from 165.4 ± 1.1 to 171.9 ± 1.2 cm. When measuring these morphological parameters in the rats of the experimental group, we received the following information: body length 194.6 ± 0.8 – 197.8 ± 1.3 cm, tail length – 165.0 ± 1.1 - 171.8 ± 1.0 cm.

When analyzing the behavior of rats of the control and experimental groups, we didn't reveal any significant differences: all animals actively resisted any manipulations during the clinical examination and administration of distilled water to the rats of the control group and aqueous solution of the studied biological product via intragastric way.

Body temperature, respiratory rate and heart rate of rats in the control and experimental groups were within the normal range for laboratory animals. Thus, the body temperature was the following: 38.51 ± 39.16 ± 0.44 ^{0}C in the control group and 38.58 ± 0.19 – 38.98 ± 0.48 ^{0}C in experimental group; respiratory rate and heart rate per minute were the following – 80.10 ± 1.20 – 76.20 ± 1.48 and 388.10 ± 1.37 – 372.00 ± 5.44 in the control group; and 80.00 ± 1.15 – 76.40 ± 1.43 and 388.10 ± 1.52 – 373.50 ± 2.59 in the experimental group.

The absolute weight of adrenal glands in the control group was 12.50 ± 0.43 g at the beginning of the study; 12.90 ± 0.48 on Day 20; 13.13 ± 0.02 g at the end of the study. In the experimental group, respectively: 12.68 ± 0.52 g; 13.59 ± 0.08; 13.73 ± 0.04 g.

At the same time the mass ratio of the investigated gland in control animals was 0.06 ± 0.02 g/kg on Day 35 which is higher by 4.3% than this parameter in experimental animals.

During the study of histological sections of the adrenal glands of rats treated additionally with Calcefit-5 there was an increase in the thickness of the cortical substance of the gland at the end of the study by an average of 4.38% ($P < 0.05$) (874.86 ± 7.10 μm) as compared to that in animals of the control group (836.51 ± 8.42 μm). It should be noted that on Day 20 of the experiment this difference was not statistically significant and was equal to 0.4%.

The mean thickness of the glomerular zone of the adrenal glands of rats in the control group on Day 35 was equal to 7.89% from the total thickness of the cortex (66.01 ± 2.54 μm). In this case, it was bigger than in the animals of the experimental group by 6.5% ($P < 0.05$).

We have found that the area parameters of this cellular zone on Day 20 and Day 35 of the study in rats of the experimental group were lower than those in the control group by 3.1 and 4.7% (70.13 ± 1.12 and 71.60 ± 1.25 and μm^2), while the core area, on the contrary, was greater, 14.41 ± 0.21 μm^2 and 14.98 ± 0.18 μm^2 respectively.

The dynamics of the NCR of corticocytes of the glomerular zone naturally reflects the character of changes in the area of nuclei and cells.

Thickness of the beam area in all animals from the beginning to the end of the experiment increased from 552.58 ± 5.69 to 657.99 ± 6.56 μm with an advantage in this indicator by 6.9% ($P < 0.05$) in favor of the experimental animals group as compared to intact peers. At this time thickness of the beam zone from the start to completion of the study in the control animals increased from 71.80 to 73.3% in rats of the experimental group - from 71.82 to 74.67%.

Parameters of the corticocytes and their nuclei in the beam zone of the studied gland in rats at the time of the end of the study were, respectively, in the control group: 126.53 ± 1.53 and 18.90 ± 0.68 μm^2 and in the experimental group – 120.25 ± 1.22 and 19.40 ± 0.90 μm^2.

At the end of the observation beam NCR value of corticocytes of the beam zone of the second group of animals was higher than that in the control group of peers by 7.4% ($P < 0.05$).

On Day 20 of the experiment thickness of the mesh zone of adrenal glands of rats of the control and experimental groups did not differ, while on Day 35 this parameter was higher in intact animals (158.07 ± 1.62 μm) by 1.6%. At the same time the proportion of the mesh area in the thickness of the cortex in animals of the control group was 18.6%, while in rats of the experimental group - 17.8%.

Indicators of the corticocytes area in the mesh zone of adrenal glands of rats in the control group at the end of the study were higher as compared with that of the animals of the experimental group by 4.2%. At the same time the area of nuclei on the contrary was lower by 1.4%

which resulted in a slight increase in NCR in rats of the experimental group as compared to the control ones by 0.9%.

Thickness of the medulla at the end of the study was higher in the experimental group by 5.2% ($p < 0.05$) (685.94 ± 21.93 μm) as compared to the intact peers. Besides it was noticed that the area of the cells of adrenal medulla zone in the second group of rats was smaller than that in the control peers by 3.5%, and area of the cytoplasm - by 4.7% ($P < 0.05$) as compared to the intact animals.

NCR indicators on Day 20 and Day 35 of the observations were slightly higher in the animals of the second group as compared with that of the control group of peers by 2.1%.

Thus, the above-mentioned changes in morphometric parameters of the gland after administration of Calcefit-5 to the standard circuit reflects increasing signs of its functional activity when compared with animals treated with distilled water. Based on the obtained morphometric data, more significant changes in activity occurred in the beam area of the adrenal cortex. Micromorphological indicators of glomerular and mesh zones of the cortex and medulla of the adrenal glands in rats of experimental group didn't differ so much from the same parameters in the rats of the control group.

Caltcefit-5, due to its constituent elements and microelements as well as biologically active substances of plant origin affected morphophysiological status of adrenal glands. All adrenal zones were involved in the implementation of the adaptive mechanisms to a greater or lesser extent. These facts are also supported by the studies of O.A. Solodkova *et al*. (2006), I.I. Buzueva *et al*. (2010) and others[9-10].

We know the fact that adrenal mass increases under the influence of stress. We can also see the reduction of testosterone and body mass, as well as elevated levels of corticosteroid hormones. It is noted that the increase in weight is caused by cortical hypertrophy of adrenal glands[11]. In our opinion structural and functional parameters obtained in the experiment are characterized by an adequate response of the neuroendocrine system of the body to the administration of the drug Calcsefit-5 in the nutrition and do not define it as stress-induced. So in the modeling of different types of stress in laboratory rats we can see significant changes in the macro- and microstructural organization of the adrenal glands, particularly in the cortex[12-15].

CONCLUSION

It was found that administration of the dietary supplement Calcefit-5 in the basic diet of male outbred rats had no appreciable effect on the overall weight and growth, clinical and physiological parameters.

The study of adrenal glands in rats of the experimental groups showed that the cortex and medulla preserve their normal histological structure.

We revealed that the thickness of adrenal cortex at the end of the study was significantly greater in the experimental group of animals. At the same time it was noted that the greatest contribution to this morphometric parameters is made by the beam area.

The observed changes of micromorphological indicators of the medulla indicate activation of the sympathetic-adrenal system aimed at adaptation to the new conditions.

Areas of cells and nuclei of the studied areas objectively reflect morphophysiological state of adrenal glands in terms of introduction of Calcefit-5 to the basic diet. This fact was naturally reflected in the corresponding values of NCR.

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