= РАДІОБІОЛОГІЯ ТА РАДІОЕКОЛОГІЯ =

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ACCUMULATION OF ⁹⁰Sr BY MURINE SKULLS AT CHORNOBYL EXCLUSION ZONE AND VARIABILITY OF THEIR CRANIOMETRIC FEATURES

The questions of specific craniometric features variability of background species of murine rodents (common voles and house mice), which arises as the result of permanent habitation of the animals in ecosystems contaminated after the Chornobyl NPS accident, are studied.

Keywords: radioactive biogeocoenoses, craniometric features, irradiation, accumulation of radionuclides.

Due to the formation of radioactive biogeocenoses the necessity of the detailed studying of features of wild animals' existence in these conditions during several generations and clarification of thr extent of influence of the radioactive contamination of ecosystems on them as well as complex of environmental realities that formed and determined the significance of each of these factors for wildlife, are obvious.

For the generalization of data of the interrelation between radioactive factors determining the adaptability of animals to stress action it is required to apply a systematic approach that makes it possible to find out interactions of adaptive responses, to improve some existing methods and develop the new ones of evaluation extent of the effects of chronic irradiation on the general condition of the faunal components of ecosystems.

Study of peculiarities of intake of radionuclides in wild animals is carried on the most biologically significant Chornobyl fallout isotopes: ⁹⁰Sr and ¹³⁷Cs.

It is known [1 - 8] that these two elements are substantially different by localization in the body and characters. Isotopes of cesium quickly become in the body to a state of equilibrium (T_{σ} to humans is about 110 days), and strontium not – proportion of this isotope from a state of equilibrium for 50 years is 86 % [9].

For the study of the accumulation and distribution of ⁹⁰Sr in terrestrial ecosystems murine rodents were taken as a model group of animals. These animals according to E. Odum [10] take a significant part in the cycles of substances and energy in biogeocoenoses.

That determines the importance of this group of animals as bioindicators in the study of biogeochemical processes in ecosystems, as it is known [6] that, for example, in places of local burial of radioactive waste they accumulate much more isotopes than other mammals. It should be noted that during studying of the patterns of animals accumulation of radionuclides from the environment great importance is in species differences [11] which are determined by physiological features of animals, their morphology [12, 13], specificity of nutrition, features of biotopical allocation [14] and so on. So we know that the various species of animals have different concentration of accumulated 90 Sr of global fallouts [15].

Materials and methods

The biological material in the form of common vole skulls (Microtus arvalis Pall., 1778) and the house mouse (Mus musculus L., 1758), which was collected in the period 1987 - 1990 years in different plots of the exclusion zone (v. Kopachi, Nova Krasnytsia, Cherevach, Chystohalivka, Rozizhdzha and Novoshepelytske forestry; density of radioactive contamination ¹³⁷Cs: 1487, 3237, 389, 4173, 12, 3287 kBq/m² respectively), was analyzed on the content of ⁹⁰Sr.

137 skulls of murine pre-cleaned from soft tissue and brain by Dermestes vulpinus from Chornobyl exclusion zone were analysed on the content of ⁹⁰Sr and measured (in milimeters) using a binocular microscope of MBS 6. The species dependence of voles was determined by analyzing of chromosomes.

Determination of 90 Sr was carried out by 90 Y activity using of radiometer Beta with SBT-10 detector and calibrated by 40 K with using an aluminum filter. To compare the values of the activity the radiochemical separation of 90 Sr by oxalate method with measuring the activity of 90 Y by UMF 1500 was carried.

The comparison showed the differences of specific activity within 16,5 %, which confirmed the possibility of using of Beta radiometer without destroying of craniological material.

The dimensional characteristics of skulls of animals that are traditionally used in murine rodents craniometry were considered as follows [16]: 1) condyle-basal skull length; 2) nasal width; 3) interorbital distance; 4) width of cheekbones; 5) temporal width; 6) width of upper teeth; 7) length of diastema; 8) height of coronary appendix of man-

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dible; 9) length of bottom row of molars; 10) cross length of mandible; 11) length of cutting hole.

The measurements of bilateral structures of common voles skull (6 - 11 signs) right (R) and left (L) were carried out in our research and the dispersion of their difference (RL) was calculated in order to obtain the indicators of fluctuating asymmetry (FA); general phenotypic variability of bilateral features was evaluated by their half-sum dispersion (R+L)/2.

The complex description of the major tendencies of voles variability over the years were studied by using the factor analysis. The factorization was the basic technique that ensured the selection of multiple trends of variability of craniometric signs and was based on analysis of the main components. Own vectors and values were calculated based on the covariance matrix. First main components that together cover more than 90 % of the total dispersions were taken for the analysis.

Results and discussions

Previous data on specific radioactivity of some skulls (4 - 28 kBq/kg) was converted through logarithms that allowed to approximate their distribution to normal, as evidenced by the significant reduction coefficient of variation of specific radioactivity in absolute units from 133.6 to 8.5 % according to logarithm. Average converted figures of specific radioactivity are shown in Table 1.

The obtained results show that there are no differrences in the average data of the specific radioactivity of skull bones for various species of animals there and the small differences occure are statistically unreliable. This undoubtedly indicates an extremely high level of contamination of biogeocenoses by the accident, and it is so high that specific features of animals do not play a leading role (at least in case of studying murine rodents) in accumulation of ⁹⁰Sr.

 Table 1. The average data of the specific radioactivity and coefficients of variation of ⁹⁰Sr contents (log Bq/kg) in the skull bones of various species of murine rodents, M ± SE

| Species and plot | The arithmetic mean | The coefficient of variation |
|--------------------------------|----------------------|------------------------------|
| 1 1 | of converted data | of specific activity (%) |
| Common voles (Kopachi) | 14.9637 ± 0.3269 | 8.46 ± 1.56 |
| Common voles (Nova Krasnytsia) | 14.7452 ± 0.3982 | 7.14 ± 1.92 |
| House mouse (Kopachi) | 14.8644 ± 0.1473 | 4.43 ± 0.70 |

Perhaps only with decreasing of the density of contamination of the territory to the level of global fallout, specific features of the animals will be increasingly mean. In the first approximation, we can assume that a similar process to some extent has already begun, as it is evidenced (despite the absence of significant differences in average data) by variability in content of ⁹⁰Sr in skull bones of different species of murine rodents (see Table. 1).

Such low coefficient of variation of specific radioactivity in house mice allows to assume that most of the animals that make up the population have specific radioactivity of the skull bones and approximate in magnitude to average indicator, while voles are characterized by relatively large number of individuals with both low and high specific radioactivity (Table. 2).

Observed differences are likely to be related to trophic features of voles on the one hand, and house mice - on the other. Some values may also have the biotopical features of their allocation and activity of individual animals.

| Table 2. Allocation of different groups of murine rodents by ⁹⁰ Sr concentra | ation |
|---|-------|
|---|-------|

| | Specific radioactivity of animals (% of individuals) | | |
|--------------------------------|--|----------------------------|---------------|
| Group, plot | low | middle | high |
| | $(\leq 10 \text{ kBq/kg})$ | $(\leq 20 \text{ kBq/kg})$ | (> 20 kBq/kg) |
| Common voles (Kopachi) | 26.7 | 46.7 | 26.6 |
| Common voles (Nova Krasnytsia) | 28.6 | 57.1 | 14.3 |
| House mouse (Kopachi) | 5.0 | 85.0 | 10.0 |

It is known from the literature that essential distinctions in concentrations of accumulated radionuclides are observed in the populations of animals that live in conditions of radioactive contamination of biogeocenoses in different seasons, in particular it is found that the rate of accumulation of radiostrontium by voles is much higher in summer than in winter [6, 11]. We also reviewed the seasonal ⁹⁰Sr accumulation features by given species of murine rodents and found that the absence of statistically significant differences of specific radioactivity of the skull bones in spring, summer and autumn was the feature of the seasonal radionuclide accumulation on plots that were studied (Table. 3).

| Value | Specific radioactivity of murine rodents (combined sample) log Bq/kg, $M \pm SE$ | | |
|----------------------------------|--|----------------------|----------------------|
| value | Spring | Summer | Autumn |
| Arithmetic mean | 14.1827 ± 0.1483 | 15.8019 ± 0.1597 | 15.1938 ± 0.4541 |
| The coefficient of variation (%) | 1.95 ± 0.69 | 5.61 ± 0.76 | 9.45 ± 2.13 |

Table 3. The seasonal features of accumulation of ⁹⁰Sr and coefficient of variation of specific radioactivity by murine rodents (combined sample)

A slight increase in specific radioactivity of animals in summer can be regarded only as a tendency (compared with spring) as well as statistical differences reaches 90 % of significance level. Perhaps this tendency will obtain sustainable, statistically significant character with the reduction of radioactivity of territory.

The variability analysis of radionuclide content in studied murine rodents in different seasons arises certain interest (see Table 3). Here we see a noticeable and significant increase of variability of specific radioactivity of animals, which reaches the maximum values in autumn.

Individuals that constitute the most diverse populations in autumn and the minimum and maximum levels of specific radioactivity of individual animals at this time have the greatest value in terms of radioactivity. Reduction of variability of specific radioactivity of animals in winter in our opinion speaks for death of the older age animal groups of summer and autumn generations (the most contaminated in the autumn), which causes the fall in maximum level of specific radioactivity about in a half. However, young animals continue to accumulate radiostrontium and minimum levels of their radioactivity increase by about a half too.

There is no doubt that a large content of 90Sr in the bones of common voles cannot but arise corresponding biological effects. For this purpose, population-morphological analysis of craniometric characters of common vole – sufficiently widespread species in Chornobyl exclusion zone – was conducted. Only the adult individuals were investigated; their age was determined by the degree of scaffold of skull [17, 18].

One of the easiest methods to assess the level of developmental disorder is the analysis of intraindividual variability. In this case all differences between homologous structures of the same individuals may be considered as a result of developmental disorder, as they developed on the basis of the same genotype in similar environmental conditions [19, 20]. The most widespread type of variability is nondirectional intraindividual differences between left and right sides of the body in bilaterally symmetrical animals, the so-called fluctuating asymmetry (FA), which can be defined by calculation of variance for correlating samples [21]. Thus, population value of FA is the degree of phenotypic diversity caused by developmental disorders and consistency in change of the value of the total phenotypic diversity; FA vitneses about significant part of developmental disorders in the formation of the first one and about the fact that part of genetic component in the variability that is observed is negligible [22]. Discordance of these values, by contrast, may be an indicator of significant part of genotypic component, as it was shown [23] on the example of variability of number of lateral plates in a threespined stickleback.

As it was mentioned before, there are examples of analysis of phenotypic diversity of populations from places with heightened levels of radioactivity. It was demonstrated that these populations are characterized by higher variability, which was fixed by change in the coefficient of variation (CV). It is interesting that in our case pairwise comparison of variation coefficients features of the studied samples revealed no statistically essential differences between them. The only exception was interorbital distance variability, the coefficient of variation of which in 1987 was 8.38 ± 0.73 , in 1989 decreased to 3.54 ± 0.65 and then remained at that level in 1990.

Were noted significant changes in average indices of studied craniometric characteristics. Comparison of samples from different years has found 7 following features (Table 4).

| Features | M ± SE (1987) | M ± SE (1989) | t - criterion (p < 0.05) |
|----------|-------------------|-------------------|--------------------------|
| 3 | 3.518 ± 0.036 | 3.353 ± 0.031 | 3.47 |
| 6R | 5.918 ± 0.037 | 5.360 ± 0.088 | 5.85 |
| 6L | 5.919 ± 0.036 | 5.367 ± 0.094 | 5.48 |
| 8L | 6.946 ± 0.063 | 6.325 ± 0.101 | 5.22 |
| 9R | 5.724 ± 0.046 | 5.033 ± 0.063 | 8.68 |
| 11R | 4.071 ± 0.038 | 3.663 ± 0.057 | 6.39 |
| 11L | 4.074 ± 0.038 | 3.660 ± 0.063 | 5.63 |

Table 4. Craniometric characters of common vole, according to which differences between samples of 1987 and 1989 were detected (mm)

| Features | M ± SE (1989) | M ± SE (1990) | t – criterion ($p < 0.05$) |
|----------|-------------------|-------------------|------------------------------|
| 2 | 4.833 ± 0.071 | 5.258 ± 0.072 | 4.20 |
| 3 | 3.353 ± 0.031 | 3.517 ± 0.034 | 3.60 |
| 8R | 6.300 ± 0.113 | 6.658 ± 0.110 | 2.33 |
| 8L | 6.320 ± 0.110 | 6.667 ± 0.097 | 2.37 |
| 9R | 5.033 ± 0.065 | 5.317 ± 0.097 | 2.43 |

Table 5. Craniometric characters of common vole, according to which differences between samples of 1989 and 1990 were detected (mm)

It should be noted the decrease in the average of every last craniometric character, which revealed differences between the consideration samples of voles; this decrease of features reaches, for example, in length of the bottom row of molars (9R) approximately 14 %. Between samples of 1989 and 1990 there were found significant differences in 5 features (Table 5). As it can be seen in this table, changing of average has the opposite character and a definite increase in the size of features in the greatest extent of tangent of nasal width (feature 2) is observed – an increase of about 8 %. However, overall population keeps declining trend in morphological features of the skull, which is known from a comparative analysis of samples (Table 6).

Table 6. Craniometric characters of Commion vole in which the differences between samples of 1987 and 1990 were detected (mm)

| Features | M ± SE (1987) | M ± SE (1990) | t - criterion (p < 0.05) |
|----------|--------------------|--------------------|--------------------------|
| 6R | 5.918 ± 0.037 | 5.458 ± 0.087 | 4.87 |
| 6L | 5.919 ± 0.036 | 5.508 ± 0.087 | 4.37 |
| 9R | 5.724 ± 0.046 | 5.317 ± 0.097 | 3.79 |
| 10R | 10.167 ± 0.066 | 10.490 ± 0.128 | 2.24 |
| 11R | 4.071 ± 0.038 | 3.564 ± 0.092 | 5.09 |
| 11L | 4.074 ± 0.038 | 3.527 ± 0.102 | 4.35 |

Analyzing the Tables 5 - 7 we can conclude not only about the average figures changes over time but also about asymmetrical change of the part of bilateral features, that is resizing on one side of the body is not always followed by appropriate changes on the other one. It leads to the fact that in some cases the average difference of right and left features significantly differs from zero. In a sample of 1987 there were two of such features - the length of the bottom row of teeth and cross length of the mandible; in the sample of 1989 there were three of themthe height of coronary appendix of the mandible is added to the two previous ones; and the sample of 1990 had one - the length of the lower teeth row. It is interesting that all observed features are related to mandible.

During comparison of the samples by the level of fluctuating asymmetry significantly different data was found (Table 7). Thus between samples of 1987 and 1989 there are detected significant differences by F-criterion in four bilateral featured (6, 8, 9, 10), between samples of 1989 and 1990 also in four (7, 8, 9, 11) and between samples of 1987 and 1990 - in five features (6, 8, 9, 10, 11).

The fact that in all cases there where observed differences in FA value between samples of different years, its increasing in comparison with indexes of previous years has a particulat interest. The most contrast pattern of increasing values of FA is naturally shown during comparison of samples of 1987 and 1990. In particular, dispersion of asymmetry of cross length of the mandible and length of incisive holes (features 10 and 11) increases approximately 2 times, the length of the upper teeth row (feature 6) – 4 times, the height of coronary appendix of the mandible (feature 8) - 6 times, and the length of the lower teeth row (feature 9) – is almost 20 times increased!

| <i>Table 7.</i> The value of asymmetry dispersion (FA) |
|--|
| of six bilateral craniometric characters |
| of common vole population in different years |

| Features | Years | | |
|----------|--------|--------|--------|
| (number) | 1987 | 1989 | 1990 |
| 6 | 0.0051 | 0.0107 | 0.0191 |
| 7 | 0.0059 | 0.0031 | 0.0099 |
| 8 | 0.0133 | 0.0263 | 0.0853 |
| 9 | 0.0068 | 0.0258 | 0.1329 |
| 10 | 0.0053 | 0.0124 | 0.0107 |
| 11 | 0.0138 | 0.0064 | 0.0325 |

To our opinion it is the most interesting to represent the results that characterize the dynamics of value of fluctuating asymmetry (FA). As it is noted above, since the FA value shows the level of disorders and therefore could be used as an indicator of development stability, the data indicates about the disorders in development stability and the amplitude of these disorders increases from year to year.

Here we can remind that the sample of this population of voles was collected at different phases of these rodents population dynamics in Chornobyl exclusion zone. In 1987 the material was selected at the peak of populations, and in 1989 - 1990 decline was observed. In a similar situation V. M. Zaharov and others [24] studied the instability of development of another mammal species - ordinary shrew Sorex araneus. Authors have shown the distortion stability of young individuals in the year of population peak which kept for next year. In the year of the minimum quantity of population the development passes naturally, and the next generation is characterized by lower values of FA. In our situation, there is a significant increase in indicators of FA, in spite of decreasing of animals population approximately from 750 individuals per ha to 45 - 60 individuals per ha, which is less than about 15 times. It is possible that the observed dynamics of FA value may be the result of radioactive contamination and accumulation of mutations that destabilize the gene pool of the population that were studied.

A comparison of value of total phenotypic diversity with FA value can show an approximate information about which part of the total phenotypic diversity is caused by disabilities of development. Unfortunately, three samples were insufficient for accurate quantitative assessment (using, for example, the correlation coefficient) of the degree of consistency changes in indicators of total phenotypic dispersion and FA. However, the relative height of coronary appendix of the lower mandible (feature 8), and especially the length of the lower teeth row (feature 9) shows that the value of dispersion of asymmetry is more than a half or even exceeds the amount of total phenotypic diversity, which indicates that disorders of development is crucial in total variability of these features.

In general, developmental disorders and related bilateral asymmetric development of some features of the voles skull, and possibly also the decrease trend of the craniometric features part may indicate the deteriorating of population habitats conditions. It is also indicated by factor analysis of data, when deteriorating conditions of the habitats and the structure of factorial matrices is changed, in particular, the proportion (part) of the first factor are decreasing in them[16].

This phenomenon can be considered to be a general decline of the individuals' sustainability from specific populations in presence of a specific inhibition by environment [25]. It is likely that increased radioactivity of the environment is that factor that leads to destabilization of morphogenetic processes and disruption of correlations between different features of the organism.

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НАКОПИЧЕННЯ ⁹⁰Sr ЧЕРЕПАМИ МИШОПОДІБНИХ ГРИЗУНІВ ЗОНИ ВІДЧУЖЕННЯ ЧАЕС ТА МІНЛИВІСТЬ ЇХНІХ КРАНІОМЕТРИЧНИХ ОЗНАК

Розглянуто питання мінливості окремих краніометричних ознак фонових видів мишоподібних гризунів (сірої нориці та хатньої миші), яка виникає внаслідок постійного мешкання тварин в екосистемах, забруднених радіонуклідами після аварії на Чорнобильській АЕС.

Ключові слова: радіаційні біогеоценози, краніометричні ознаки, опромінення, накопичення радіонуклідів.

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НАКОПЛЕНИЕ ⁹⁰Sr ЧЕРЕПАМИ МЫШЕВИДНЫХ ГРЫЗУНОВ ЗОНЫ ОТЧУЖДЕНИЯ ЧАЭС И ИЗМЕНЧИВОСТЬ ИХ КРАНИОМЕТРИЧЕСКИХ ПРИЗНАКОВ

Рассмотрены вопросы изменчивости отдельных краниометрических признаков фоновых видов мышевидных грызунов (обыкновенной полевки и домовой мыши), возникшей в результате обитания животных в экосистемах, загрязненных вследствие Чернобыльской катастрофы.

Ключевые слова: радиационные биогеоценозы, краниометрические признаки, облучение, накопление радионуклидов.

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