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THE LEVEL OF ABERRANT CELLS IN VARIOUS TISSUES OF BANK VOLE DEPENDING ON DOSES AND RADIONUCLIDE BALANCE IN ORGANISM

A regression analysis shows the direct linear relation between ^{137}Cs accumulation in rodents and the level of aberrant cells. For ^{90}Sr this trend was negative. The dose relationship was the same with ^{137}Cs. The trends were negative 1 month later after feeding of animals with clean food. Correspondingly the dose relationship was also negative. The levels of cells with apoptosis features were different in animals from the control and the radiocontaminated sites. The analysis has revealed the correlation between the ^{90}Sr content in animal body and the number of alveolar macrophages containing micronuclei. The relationship was revealed between the ^{137}Cs content in the animal body and the number of intestinal epithelial cells with micronuclei.

Introduction. In analysing the consequences of chronic radiation effect on natural ecosystems the cytogenetic tests which allow assessment of the DNA damage level in cells of various tissues of wild animals are of great importance [1, 2, 3].

Earlier, basing on the mean population samples we have examined the levels of aberrant cells in different tissues of wild animals in connection with radiocontamination of the territories owing to the Chernobyl catastrophe and radionuclide accumulation in organism [1]. The present paper summerizes the results of the comparative study on the correlation between the level of aberrant cells in various tissues of murine rodents and radionuclide (^{137}Cs, ^{90}Sr) concentration in the bodies of individual animals and the dose loads.

Materials and methods. Adult individuals of bank vole (*Clethrionomys glareolus, Schreber*) were used in the investigations. Animals were caught in Gomel region on the boundary and within Polessky State Radioecological Reserve (PSRER): v. Savichi of Bragin District (^{137}Cs — 740 kBq/m^2; ^{90}Sr — 55,5 kBq/m^2); v.Dronki (^{137}Cs — 1100kBq/m^2; ^{90}Sr — 100,7 kBq/m^2) and v. Lomachi of Khoiniki District (^{137}Cs — 2331 kBq/m^2; ^{90}Sr — 284 kBq/m^2). Animals caught in Berezinsky State Biosphere Reserve (BSBR) were used as the control ones. Radionuclides were determined with radiospectrometric and radiochemical methods [4]. Dose loads were calculated basing on the data of Belarussian Hydrometeorological service on area contamination and information about ^{137}Cs and ^{90}Sr accumulation in animal organism in accordance with ICRU recommendations [5].

Animals were treated with ethanol ether and killed by cervical dislocation. Slides for metaphase analysis were prepared according to conventional technique [6] with colchicine treatment, KCl (0,56 %) hypotonisation and fixation in methanol: acetic acid (3:1). The flame-dried slides were stained by Giemsa. Macrophages were harvested from the lung by bronchoalveolar lavage with warm (37 °C) 0.9 % solution of NaCl. Intestines were scraped and epithelial cells were collected in bovine serum. Thymocytes and bone marrow cells were collected from non-colchicined animals in phosphate — buffered saline (pH 7.2). Cell suspensions were used for slide preparation fixed in ethanol and stained by Giemsa. All the possible microscopic fields of the slides were examined to detect the micronucei, condensation and fragmentation of nuclear chromatin, pyknosis, budding (apoptotic bodies) [7]. Statistical analysis was performed using conventional methods [8].

Results and discussion. When studying the level of aberrant cells in bone marrow of bank vole, it was revealed that insignificant reduction in the aberrant cell level of bone marrow took place within a number of years at considerable decrease in ^{137}Cs accumulation in organism forming basic dose loads (Table 1). As a result, the yield of aberrant cells per dose unit greatly increased that makes an impression of extremely high efficiency of low dose ionizing radiation.

Similar pattern was observed in the experiment when animals were kept under vivarium conditions and were feeded with standard (clean) food for 30 days that approximately corresponds to 10 cycles of ^{137}Cs half-removal from the organism [1]. The results of the experiment allowed supposition of a rather complicated mechanism for the registered level formation of cytogenetic damages in bone marrow cells and a particular role of ^{90}Sr in this process.

The method of regression analysis was applied to ascertain the character of the correlation between radionuclide accumulation in animal organism and the yield of chromosome aberrations in bone marrow cells. For this purpose the animals were selected for which the maximum number (100 and more) of counted metaphase plates was observed and the data on the individual radionuclide (^{137}Cs; ^{90}Sr) content in their bodies were determined. The total number of such animals caught in different years and in diverse biotopes was 110 individuals, including 57 ones analysed immediately after capture and 53 ones analysed after feeding with clean food. In 1990 the ^{90}Sr content was not determined. The task consisted in revealing the main tendencies rather than in selecting potential mathematical models.

The linear relation between ^{137}Cs accumulation in the body and the number of aberrant cells in bone marrow ($R = 0,32$; $P < 0,01$) was observed in the total group of animals (Fig. 1). The relationship between ^{90}Sr accumulation in the body and the yield of aberrant cells was inverse: $R = -0,22$; $P < 0,05$. After feeding of animals with clean food the linear relationship between the level of aberrant cells and radionuclide accumulation was of inverse character in both cases: ^{137}Cs accumulation in the body ($R = -0,22$; $P < 0,05$), ^{90}Sr accumulation in the body ($R = -0,24$; $P < 0,05$).

The correlation between the level of cytogenetic damages and the dose load in animals analysed immediately after capture was the same as that one between the level of cytogenetic damages and ^{137}Cs concentration in the body ($R = 0,27$; $P < 0,05$). On removal of a greater portion of caesium from the organism the relationship acquired the inverse character ($R = -0,22$; $P < 0,05$). Practically in the whole dose range the studied trend was parallel to the X axis, deviation took place with dose increase *by a factor of* 10^2 and the regression coefficient itself pointed to the absence of sharp variations in this range. That is why the biological dosimetry applied to small animals under natural conditions is extremely difficult and the results of cytogenetic investigations can be used for bioindication of radiation exposure.

The cause of the observed phenomena may be the balance between the survived aberrant cells that completed mitosis and the cells eliminated by apoptosis as a result of unrepaired DNA damages [9]. Reduction of the number of bone marrow cells and

Table 1

Radionuclide content in animal body and chromosome aberration yield per absorbed dose unit

Site	Year	Radionuclide content, Bq/kg		Absorbed dose, mSv/day	Aberrations per, mSv/day
		^{137}Cs	^{90}Sr		
v. Veprin	1990	105000	—	0,294	0,024
v. Savichi	1990	162000	—	0,454	0,029
	1993	3200	187,0	0,010	1,100
v. Lomachi	1990	140000	—	0,392	0,036
	1991	75600	190	0,227	0,101
	1993	4000	351	0,013	0,769
	1991*	5600	155	0,009	1,440
v. Dronky	1994*	270	37	0,001	21,00

* after feeding with clean food; — no data

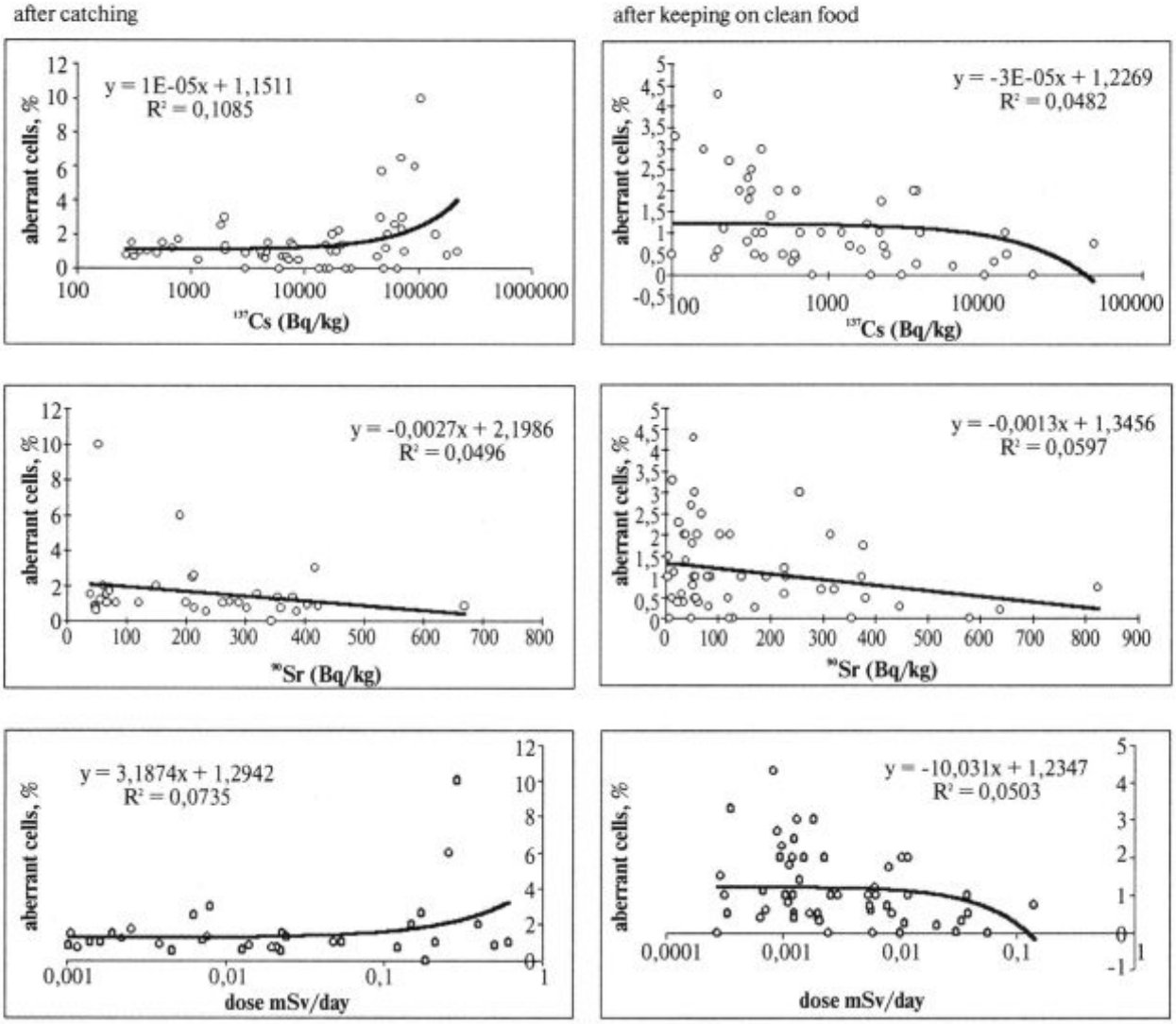

Fig. 1. The level of bone marrow aberrant cells depending on radionuclide balance in the organism and the dose load on bone marrow

the drop in the neutrophil level in peripheral blood occur under chronic ^{90}Sr penetration into organism, with the relationship being of a inverse character [10]. Even the fact itself of radiation exposure after single irradiation or ^{90}Sr *administration* into organism becomes the cause of peculiar «flight» of some bone marrow cells into peripheral blood [11, 12]. Under dose load reduction repopulation of bone marrow prevails [10, 13] and the number of cells with cytogenetic damages that completed the cell cycle increases [14].

There are diverse elimination mechanisms of cells with DNA damages emerged as a result of irradiation. They are «rapid interphase» death, «delayed interphase» death after arrest of the cell cycle in G2-phase or «mitotic/delayed mitotic» death after one or several mitoses [9, 15, 16]. The pattern observed in bone marrow and thymus of animals caught in radiocontaminated areas seems to be manifestation of the latter along with «flight» of some cells with potential DNA damages (Table 2).

The level of lymphoid cells of bone marrow with micronuclei is higher than that of cells with apoptosis characters. The role of apoptosis processes in formation of the aberrant cell level is verified by the fact that there is a direct correlation ($r = 63$; $P < 0,01$) between the number of lymphocytes with micronuclei, i.e. with cells that completed mitosis, and leucocytes that

die in the course of nuclear fragmentation in animals within PSRER per ^{137}Cs accumulation up to 20 kBq/kg body weight. On the contrary, the pattern is of the opposite character in thymus, where the cells originated from bone marrow divide, differentiate and die (preferably in interphase). This is the difference between thymus and bone marrow where cells of myeloid and lymphoid series have a different rate of renewal.

The possibility of realization of potential DNA damages in cells, which directly originate from bone marrow or which are descendants of such cells, was studied in alveolar macrophages. No relation between ^{137}Cs content in animal body and the number of macrophages with micronuclei was observed but such relation was noted in connection with ^{90}Sr accumulation ($r = 0,66$; $P < 0,02$). Tissue macrophages originate from bone marrow cells, in other tissues their mitotic index is small and, on the whole, for instance in alveoli, it corresponds to daily fraction recruited from peripheral blood and repopulation of the whole pool occurs during a month [17, 18].

The absence of the correlation between the level of macrophages with micronuclei and ^{137}Cs content may be accounted for by high mobility of the latter in the body of bank vole. The period of biological ^{137}Cs semiremoval in voles caught in radiocontaminated areas was 27 h [19]. As we have showed before, the strontium content in animal body changed little even after monthly feeding of animals with «clean» food [1]. So the revealed relationship in connection with ^{90}Sr accumulation displays the role of this osteotropic radionuclide in induction of potential DNA damages which were realized later as micronuclei at subsequent division of monocytes escaped from the bone marrow due to their final tissue differentiation [18].

Information on the level of intestinal epithelium cells with cytogenetic damages dates back to 1990 when the major dose load on animal body was caused by incorporated ^{137}Cs. The time of cell transit from the base to the tip of intestinal villus does not exceed 3

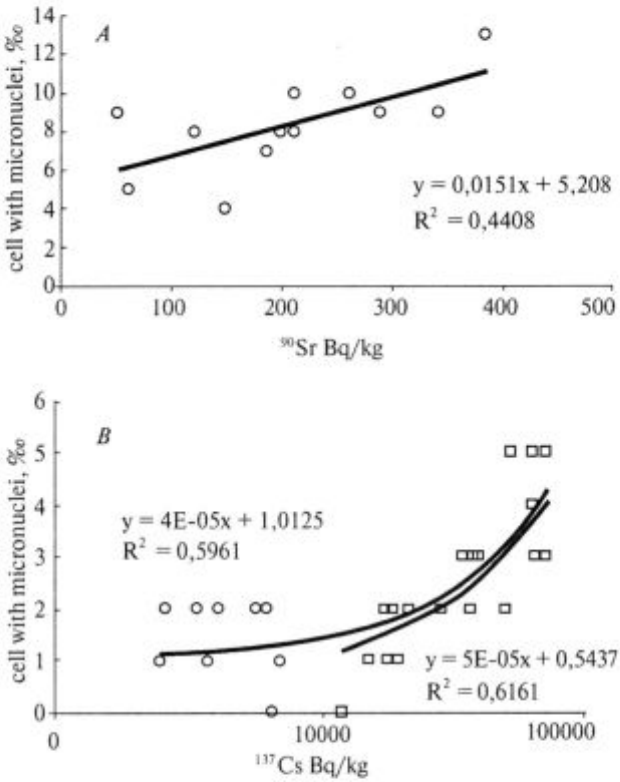

Fig. 2. *A* — The relation between the level of macrophages with micronuclei and ^{90}Sr content in the organism; *B* — The relation between the level of intestinal epithelium cells with micronuclei and ^{137}Cs content in the organism, ^{137}Cs accumulation: o — up Bq/kg, □ — more 10000 Bq/kg

days. Therefore it may be supposed that the level of intestinal epithelium cells with cytogenetic damages in wild animals of bank vole population from radiocontaminated areas should display quite closely the value of dose loads at the time of animal capture.

The levels of intestinal epithelium cells with micronuclei in three studied populations were rather close and exceeded the control values 2–2,5-times [1]. The number of cells with micronuclei per 1000 cells studied varied from 0 to 5. In the animals with known cytogenetic data individual ^{137}Cs concentra-

Table 2

Apoptosis and cytogenetic damages in mice bone marrow and thymus

Site	^{137}Cs, kBq/kg	Animals examined	% of cells with micronuclei		% of apoptotic cells	
			bone marrow	thymus	bone marrow	thymus
PSRER	2–20	20	0,24 ± 0,05*	0,10 ± 0,02	0,26 ± 0,05*	1,41 ± 0,09*
BSBR	0,01	12	0,09 ± 0,03	0,08 ± 0,02	0,51 ± 0,10	0,79 ± 0,06

* $P < 0,05$

tions varied from 2 to 80 kBq/kg (Fig. 2) though it exceeds 100 kBq/kg when all animals were simultaneously measured (Table 1). These values are apparently caused by contribution of some individuals which were not subjected to the cytogenetic analysis.

The number of cells with micronuclei was established to correlate directly with ^{137}Cs concentration in animal body: $r = 0,77$; $P < 0,01$. It is quite evident that this relationship, as in the analysis of chromosome aberrations in bone marrow cells, becomes distinct with caesium concentration exceeding 10 kBq/kg. With isolation of the animal group with caesium accumulation more than the stated value, the correlation coefficient practically does not change ($r = 0,78$; $P < 0.05$). This fact displays the peculiarity of the regression analysis taking into account extreme values. The revealed relationship between the aberrant cell level of intestinal epithelium and ^{137}Cs concentration in animal organism is more distinct than in the case of bone marrow cell analysis where the correlation coefficient was twice less. Probably, this is associated with the death of some cells of bone marrow since it is impossible to distinguish diverse cell subpopulations with the metaphase analysis. Under radiation exposure the stem cells are more subjected to apoptosis and in bone marrow their sensitivity is several times as high as in intestine [20]. In mucosal epithelium of the intestine the cells die in intestinal crypts [21, 22]. We observe cytogenetic damages in terminal cells, reaching intestine lumen, after their last division on villus.

Thus, in animals inhabiting radiocontaminated areas the aberrant cell level of bone marrow does not depend only on dose loads. In many respects it is defined by radionuclide balance in the organism, by redistribution of cell populations and by cell apoptosis.

РЕЗЮМЕ. Регресивний аналіз показав пряму лінійну залежність між накопиченням в тілі гризунів ^{137}Cs і рівнем аберантних клітин. Для ^{90}Sr цей тренд був від'ємним. Залежність від дози була такою ж, як і одного ^{137}Cs. Залежність в обох випадках мала зворотний характер 1 місяць по тому утримання тварин на чистих кормах. Відповідно і дозова залежність носила зворотний характер. Рівні клітин з ознаками апоптоза у тварин контрольної і забруднених радіонуклідами ділянок були різні. Встановлено зв'язок між вмістом ^{90}Sr в тілі тварин і числом альвеолярних макрофагів з мікроядрами. Виявлено зв'язок між вмістом ^{137}Cs в тілі тварин і рівнем клітин кишкового епітелію з мікроядрами.

РЕЗЮМЕ. Регрессионный анализ показал прямую линейную зависимость между накоплением в теле грызунов ^{137}Cs и уровнем аберрантных клеток. Для ^{90}Sr этот тренд был отрицательным. Зависимость от дозы была такой же, как и одного ^{137}Cs. Зависимость в обоих случаях носила обратный характер 1 месяц спустя после содержания животных на чистых кормах. Соответственно и дозовая зависимость носила обратный характер. Уровни клеток с признаками апоптоза у животных контрольного и загрязненных радионуклидами участков были различны. Установлена связь между содержанием ^{90}Sr в теле животных и числом альвеолярных макрофагов с микроядрами. Выявлена связь между содержанием ^{137}Cs в теле животных и уровнем клеток кишечного эпителия с микроядрами.

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