

Original Research Article **The Silver, Cobalt, Chromium, Iron, Mercury, Rubidium, Antimony, Selenium, and Zinc Contents in Human Bone affected by Ewing's Sarcoma**

ABSTRACT

Aims: To clarify the role of trace elements in etiology and pathogenesis of the Ewing's sarcoma, a non-destructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides were performed.

Methodology: The silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) content, Rb/Co, Rb/Fe, Rb/Se, Cr/Co, Cr/Fe, and Cr/Se mass fractions ratios, Co×Fe, Co×Se, and Fe×Se mass fraction multiplications as well as Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices were estimated in normal bone samples from 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years), who had died from various non bone related causes, mainly unexpected from trauma, and in tumor samples, obtained from open biopsies or after operation of 6 patients with Ewing's sarcoma (6 males, 5 to 24 years old). The reliability of difference in the results between intact bone and Ewing's sarcoma tissues was evaluated by Student's t-test.

Results: In the Ewing's sarcoma tissues the mass fractions of Co, Fe, and Se are significantly higher as well as the mass fraction of Rb is lower than in normal bone tissues. Moreover, significantly higher Rb/Co, Rb/Fe, and Rb/Se mass fraction ratios and values of Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices are typical of the Ewing's sarcoma tissue compared to intact bone. In the Ewing's sarcoma tissue many correlations between trace elements found in the control group was no longer evident.

Conclusion: In malignant transformed bone tissues a trace element homeostasis is significantly disturbed.

Keywords: *Trace elements; human bone; Ewing's sarcoma; neutron activation analysis*

1. INTRODUCTION

The roles of trace elements in the development and inhibition of cancer have a complex character and have risen many questions because of their essential and toxic effects on human health. The effects of trace elements are related to content and recorded

observations range from a deficiency state, to function as biologically essential components, to an unbalance when excess of one element interferes with the function of another, to pharmacologically active doses, and finally to toxic and even life-threatening levels [1,2]. Thus, in normal environmental and health conditions there is a trace element homeostasis in tissues and fluids of human body and an unbalance of trace element contents could be a causative factor for many diseases, including cancer [2].

It is well known that tissues of human body differ greatly in their contents of trace elements. Particularly it concerns the chemical composition of bone tissue investigated by us in details in our previous studies [3-29]. Bone tumors are a heterogeneous group of tumors that all arise from bone tissue, which consists of cartilaginous, osteoid, osseous mineralized and fibrous tissue, and bone marrow elements. Each tissue can be subject to inflammation, benign or malignant tumors.

Ewing's sarcoma, a highly malignant primary bone tumor that is derived from red bone marrow, was first described by James Ewing in 1921 [30,31]. It is one of a group of cancers known collectively as the Ewing's sarcoma family of tumors [32]. The pathologists describe these malignancies as a soft, grayish, poorly differentiated tumor [33]. Ewing's sarcoma affects mainly children and adolescents. It is the second most common pediatric malignant bone tumor. Overall, this malignancy is rare, with an annual incidence of approximately 2.9 cases per 100000 [31,34]. The incidence of Ewing's sarcoma has not increased appreciably over the last 30 years [34]. The median age at diagnosis is 15 years and there is a male predilection of 1.5/1 [35,36] as well as a difference in incidence according to racial origin [37]. Caucasians are affected more frequently [37]. Ewing's tumors are typically found in the long bones of the legs and arms, or bones in the chest, trunk, pelvis, back, or head [31, 32]. It is the most lethal bone tumor [35]. No single morphologic or functional imaging method provides findings for a specific diagnosis of Ewing's sarcoma, but the results do contribute to tumor staging. Therefore, obtaining a histologic specimen of the lesion in all patients is essential in recognizing this tumor and planning therapy.

To our knowledge, no data are available for the trace element contents of Ewing's sarcoma, to permit conclusion about their role in malignant transformation or pathogenesis.

This work had three aims. The first was to assess the silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), selenium (Se), and zinc (Zn) contents in two groups of bone tissue samples – intact bone and Ewing's sarcoma using non-destructive instrumental neutron activation analysis (INAA) with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to calculate some combinations (ratios and multiplications) of these trace element contents in intact bone and Ewing's sarcoma tissues. The third aim was to compare the contents and selected combinations of trace element contents in two groups of samples and the final was to evaluate the inter-correlations of trace element contents in normal and Ewing's sarcoma tissues.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

2. MATERIAL AND METHODS

Thirty-three children, adolescents and adults were included in this study. The subjects were divided into two groups: reference (1) and Ewing's sarcoma (2). The reference group consisted of 27 patients with intact bone (12 females and 15 males, aged from 16 to 49 years) who had died from various non bone related causes, mainly unexpected from trauma. The intact cortical bone samples of femur, femoral neck, tibia and iliac crest were collected

at the Department of Pathology, Obninsk City Hospital. Samples from 6 patients with Ewing's sarcoma (6 males, 5 to 24 years old) were obtained from open biopsies or after operation from resected specimens. All patients with bone diseases were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis was confirmed by clinical and histological data.

A titanium tool was used to cut and to scrub samples [38,39]. All bone and tumor tissue samples were freeze dried, until constant mass was obtained, and homogenized. Then samples weighing about 100 mg were wrapped separately in high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins and aliquots of commercial, chemically pure compounds were used. Corrected certified values of BSS element contents were reported by us before [40]. Ten certified reference material (CRM) IAEA H-5 (Animal Bone) and standard reference material (SRM) NIST 1486 (Bone Meal) sub-samples weighing about 100 mg were analyzed in the same conditions as bone and tumor samples to estimate the precision and accuracy of results.

A vertical channel of the WWR-c research nuclear reactor was applied to determine the mass fraction of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn by INAA-LLR. The quartz ampoule with bone samples, tumor samples, standards, CRM, and SRM was soldered, positioned in a transport aluminum container and exposed to a 100-hour neutron irradiation in a vertical channel with a thermal neutron flux about $10^{13} \text{ n}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$. Two months after irradiation samples were reweighed and repacked. The duration of each measurement was from 1 to 10 hours. To reduce the high intensity of ^{32}P β -particles ($T_{1/2}=14.3 \text{ d}$) background, a berillium filter was used. A coaxial $98 \text{ cm}^3 \text{ Ge (Li)}$ detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported by us before [22,24,25,41].

A dedicated computer program of INAA mode optimization was used [42]. Using the Microsoft Office Excel programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for different trace element mass fractions and their selected combinations. The reliability of difference in the results between intact bone and Ewing's sarcoma tissue was evaluated by Student's t-test. For the estimation of the Pearson correlation coefficient between different pairs of the trace element mass fractions in intact bone and Ewing's sarcoma tissue the Microsoft Office Excel program was also used.

3. RESULTS

Fig. 1 show individual data sets for Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in all samples of intact bone (1) and Ewing's sarcoma tissue (2).

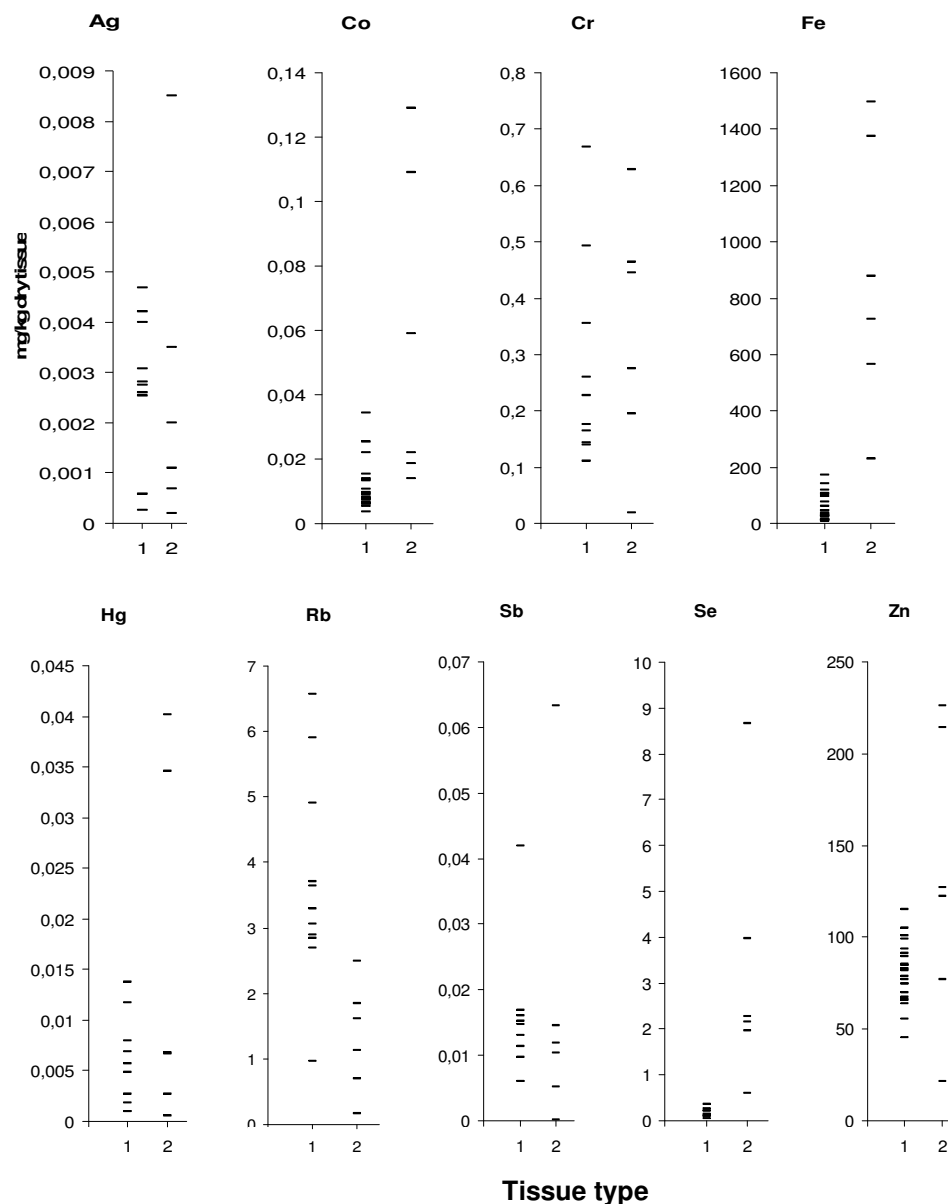


Fig.1. Individual data sets for Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg dry tissue) in intact bone (1) and Ewing's sarcoma (2).

Table 1 depicts the basic statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fraction in intact bone and Ewing's sarcoma tissue.

The ratio of means and the reliability of difference between mean values of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in tissue of intact bone and Ewing's sarcoma are presented in Table 2.

143 **Table 1. Basic statistical parameters for Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass**
 144 **fractions (mg/kg, dry mass basis) in tissue of intact bone and Ewing's sarcoma**
 145

E	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone, n=27								
Ag	0.0027	0.0015	0.0005	0.00026	0.0047	0.0028	0.00032	0.0046
Co	0.0107	0.0070	0.0014	0.00370	0.0345	0.0079	0.00464	0.0288
Cr	0.274	0.182	0.057	0.110	0.669	0.202	0.117	0.629
Fe	51.2	46.3	9.3	9.20	173	30.2	9.68	155
Hg	0.0057	0.0044	0.0014	0.00100	0.0138	0.0052	0.0010	0.0133
Rb	3.68	1.58	0.48	0.970	6.57	3.30	1.40	6.41
Sb	0.0151	0.0102	0.0032	0.00600	0.0420	0.0139	0.00600	0.0364
Se	0.176	0.092	0.029	0.0550	0.358	0.169	0.0633	0.336
Zn	80.6	15.4	3.0	45.4	115	82.1	51.7	109
Ewing's sarcoma, n=6								
Ag	0.0027	0.0031	0.0013	0.00020	0.0085	0.0016	0.00026	0.0079
Co	0.059	0.050	0.020	0.0140	0.129	0.0405	0.0146	0.127
Cr	0.338	0.218	0.089	0.0200	0.628	0.360	0.0419	0.608
Fe	878	484	198	228	1497	803	270	1482
Hg	0.015	0.017	0.007	0.00056	0.0402	0.0067	0.00082	0.0395
Rb	1.32	0.83	0.34	0.170	2.49	1.38	0.235	2.41
Sb	0.0176	0.0230	0.0094	0.00010	0.0634	0.0111	0.00073	0.0573
Se	3.27	2.85	1.16	0.608	8.66	2.22	0.776	8.07
Zn	131	79	32	21.3	226	125	28.2	225

146 *E* element *M* arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum
 147 value, *Max* maximum value, *Med* median, *P0.025* percentile with 0.025 level, *P0.975* percentile with
 148 0.975 level.
 149
 150

151 **Table 2. Means (M±SEM, mg/kg on dry mass basis), ratio of means and the reliability**
 152 **of difference between mean values of Al, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass**
 153 **fractions in tissue of intact bone and Ewing's sarcoma**
 154

Element	Intact bone M_1	Ewing's sarcoma M_2	$M_2(R) / M_1(R)$ Ratio	Student's t-test
Ag	0.0027±0.0005	0.0027±0.0013	1.00	N.S.
Co	0.0107±0.0014	0.059±0.020	5.51	$p \leq 0.05$
Cr	0.274±0.057	0.338±0.089	1.23	N.S.
Fe	51.2±9.3	878±198	17.1	$p \leq 0.001$
Hg	0.0057±0.0014	0.0152±0.0071	2.67	N.S.
Rb	3.68±0.48	1.32±0.34	0.36	$p \leq 0.001$
Sb	0.0151±0.0032	0.0176±0.0094	1.17	N.S.
Se	0.176±0.029	3.27±1.16	18.6	$p \leq 0.05$
Zn	80.6±3.0	131±32	1.63	N.S.

155 *M* arithmetic mean, *SEM* standard error of mean, *N.S.* not significant.
 156
 157

158 Table 3 represents the basic statistical parameters for Rb/Co, Rb/Fe, Rb/Se, Cr/Co, Cr/Fe,
 159 and Cr/Se mass fractions ratios, for Co×Fe, Co×Se, and Fe×Se mass fractions
 160 multiplications as well as for Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices in the
 161 samples of intact bone and Ewing's sarcoma.

162 The ratio of means and the reliability of difference between mean values of Rb/Co, Rb/Fe,
 163 Rb/Se, Cr/Co, Cr/Fe, and Cr/Se mass fractions ratios, for Co×Fe, Co×Se, and Fe×Se mass

fractions multiplications as well as for Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices in tissue of intact bone and Ewing's sarcoma are presented in Table 4.

Table 3. Basic statistical parameters for Rb/Co, Rb/Fe, Rb/Se, Cr/Co, Cr/Fe, and Cr/Se mass fractions ratios, for Co×Fe, Co×Se, and Fe×Se mass fractions multiplications as well as for Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices in tissue of intact bone and Ewing's sarcoma

Combination of mass fractions	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact bone, n=27								
Rb/Co	368	201	61	28.1	784	397	51.7	716
(Rb/Fe)×10 ⁴	1442	1308	394	56.1	4022	921	119	3726
(Rb/Se)×10 ²	2662	1235	391	271	4909	2878	496	4529
(Co×Fe)×10 ²	72	123	25	6.1	597	25.6	6.34	371
(Co×Se)×10 ⁴	30	36	11	3.40	124	19.8	4.46	107
Fe×Se	13.7	18.5	5.9	1.22	61.9	7.38	1.31	54.0
[Rb/(Co×Fe)]×10 ²	1146	1109	351	16.3	3313	859	48.9	3228
Rb/(Co×Se)	2738	2307	730	78.5	8519	2131	347	7454
[Rb/(Fe×Se)]×10 ⁴	9375	9755	3085	157	30239	6380	399	28571
Ewing's sarcoma, n=6								
Rb/Co	46	44	18	1.56	116	28.0	3.76	112
(Rb/Fe)×10 ⁴	23	29	12	2.34	80.7	12.5	3.52	72.7
(Rb/Se)×10 ²	83	110	45	4.29	303	45.0	7.35	274
(Co×Fe)×10 ²	5984	7110	2902	502	19311	3552	572	17888
(Co×Se)×10 ⁴	2916	4336	1770	134	11171	778	157	10315
Fe×Se	3673	4682	1911	139	12964	2296	274	11733
[Rb/(Co×Fe)]×10 ²	9.21	13.8	5.6	0.215	36.7	4.34	0.349	33.2
Rb/(Co×Se)	36	53	22	0.394	138	13.4	0.623	127
[Rb/(Fe×Se)]×10 ⁴	25.4	52.6	21.5	0.59	133	5.42	0.76	117

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Med* median, *P0.025* percentile with 0.025 level, *P0.975* percentile with 0.975 level.

Table 4. Means (M±SEM), ratio of means and the reliability of difference between mean values of Rb/Co, Rb/Fe, Rb/Se, Cr/Co, Cr/Fe, and Cr/Se mass fractions ratios, for Co×Fe, Co×Se, and Fe×Se mass fractions multiplications as well as for Rb/(Co×Fe), Rb/(Co×Se), and Rb/(Fe×Se) indices in tissue of intact bone and Ewing's sarcoma

Combination of mass fractions	Intact bone M ₁	Ewing's sarcoma M ₂	M ₂ (R) / M ₁ (R) Ratio	Student's t-test
Rb/Co	368±61	46±18	0.125	<i>p</i> ≤0.001
(Rb/Fe)×10 ⁴	1442±394	23±12	0.0160	<i>p</i> ≤0.01
(Rb/Se)×10 ²	2662±391	83±45	0.0312	<i>p</i> ≤0.001
(Co×Fe)×10 ²	72±25	5984±2902	83.1	<i>p</i> ≤0.05
(Co×Se)×10 ⁴	30±11	2916±1770	97.2	N.S.
Fe×Se	13.7±5.9	3673±1911	268	N.S.
[Rb/(Co×Fe)]×10 ²	1146±351	9.21±5.6	0.00804	<i>p</i> ≤0.01
Rb/(Co×Se)	2738±730	36±22	0.0131	<i>p</i> ≤0.01
[Rb/(Fe×Se)]×10 ⁴	9375±3085	25.4±21.5	0.00271	<i>p</i> ≤0.01

M arithmetic mean, *SEM* standard error of mean, *N.S.* not significant.

The data of inter-correlation calculations (values of r – coefficient of correlation) including all pairs of the chemical elements identified by us in intact bone and Ewing's sarcoma tissue are shown in Table 5.

Table 5. Intercorrelations of pairs of the trace element mass fractions in tissue of intact bone and Ewing's sarcoma

Tissue	E	Co	Cr	Fe	Hg	Rb	Sb	Se	Zn
Intact bone n=27	Ag	-0.23	0.51	-0.80 ^b	-0.02	0.62 ^a	0.31	-0.45	0.38
	Co	zzzz	0.16	0.55 ^b	0.79 ^b	-0.10	0.08	0.52	0.17
	Cr	0.16	zzzz	-0.48	0.51	0.56 ^a	-0.31	-0.08	0.46
	Fe	0.55 ^b	-0.48	zzzz	0.09	-0.54	-0.25	0.60 ^a	-0.17
	Hg	0.79 ^b	0.51	0.09	zzzz	0.18	-0.13	0.35	-0.14
	Rb	-0.10	0.56 ^a	-0.54	0.18	zzzz	-0.05	-0.06	0.34
	Sb	0.08	-0.31	-0.25	-0.13	-0.05	zzzz	0.04	0.22
	Se	0.52	-0.08	0.60 ^a	0.35	-0.06	0.04	zzzz	0.24
	Zn	0.46	0.46	-0.17	-0.14	0.34	0.22	0.24	zzzz
Ewing's sarcoma n=6	Ag	-0.31	-0.71	-0.54	0.67	0.44	-0.03	-0.85 ^a	-0.20
	Co	zzzz	-0.06	0.42	0.23	0.08	0.81 ^a	0.80 ^a	0.39
	Cr	-0.06	zzzz	0.52	-0.42	0.01	0.12	0.24	0.46
	Fe	0.42	0.52	zzzz	-0.09	0.45	0.50	0.43	0.07
	Hg	0.23	-0.42	-0.09	zzzz	0.74	0.59	-0.65	0.42
	Rb	0.08	0.01	0.45	0.74	zzzz	0.60	-0.85 ^a	0.22
	Sb	0.81 ^a	0.12	0.50	0.59	0.60	zzzz	0.17	0.56
	Se	0.80 ^a	0.24	0.43	-0.65	-0.85 ^a	0.17	zzzz	0.03
	Zn	0.39	0.46	0.07	0.42	0.22	0.56	0.03	zzzz

E element, statistically significant difference: ^a - $p \leq 0.05$, ^b - $p \leq 0.01$.

4. Discussion

The non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides was used in this research work because this method has many definite advantages over other analytical methods, particularly, in the clinical chemistry. For example, after non-destructive INAA-LLR there is a possibility to check the results for some trace elements and to receive additional information about other trace element contents by destructive analytical methods such as AAS, ICP-AES, ICP-MS and so on, using the same bone samples. Moreover, if a deep-cooled channel of nuclear reactor is available, the non-destructive INAA-LLR allows determining trace element contents in the fresh bone/tumor samples and combining trace element study with histological investigation. It is also necessary to keep in mind that the non-destructive methods are the current gold-standard solution to control destructive analytical techniques [2]. The destructive analytical methods are based on measurements of processed tissue. In such studies tissue samples are ashed and/or acid digested before analysis. There is evidence that certain quantities of chemical elements are lost as a result of such treatment [2,39,43]. There is no doubt that every method available for the measurement of trace element contents in bone and tumor samples can be used. However, when using destructive analytical methods it is necessary to control for the losses of trace elements, for complete acid digestion of the sample, and for the contaminations by trace elements during sample decomposition, which needs adding some chemicals.

214 In our previous study it was shown that the results of mean values for all representative
215 elements of CRM IAEA H-5 (Animal Bone) and SRM NIST1486 (Bone Meal) were in the
216 range of 95% confidence interval ($M \pm 2SD$) of the certificates' values [22,24,25,41]. Good
217 agreement with the certified data of CRM and SRM indicate an acceptable accuracy for the
218 trace element mass fractions obtained in the study of intact bone and Ewing's sarcoma
219 tissue presented in Tables 1-5.

220
221 The mean values and all selected statistical parameters were calculated for 9 trace element
222 (Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn) mass fractions (Table 1), for Rb/Co, Rb/Fe, Rb/Se,
223 Cr/Co, Cr/Fe, and Cr/Se mass fractions ratios, for $Co \times Fe$, $Co \times Se$, and $Fe \times Se$ mass
224 fractions multiplications as well as for $Rb/(Co \times Fe)$, $Rb/(Co \times Se)$, and $Rb/(Fe \times Se)$ indices
225 (Table 3). In control group the mass fractions of Co, Fe and Zn were measured in all
226 samples, but the mass fraction of Rb – in 11 samples and mass fractions of Ag, Cr, Hg, Sb,
227 and Se – in 10 samples (Fig. 1). In the Ewing's sarcoma group the mass fraction of all nine
228 trace elements were determined in all samples (Fig. 1).

229
230 From Table 2 it is observed that in the Ewing's sarcoma tissue the mean mass fraction of
231 Co, Cr, Fe, Hg, Sb, Se, and Zn is higher as well as the mean mass fraction of Rb is lower
232 than in normal bone tissues. However, in Ewing's sarcoma only the mean mass fractions of
233 Co ($p \leq 0.05$), Fe ($p \leq 0.001$), and Se ($p \leq 0.05$) are significantly increased and the mean mass
234 fraction of Rb ($p \leq 0.001$) is significantly decreased when compared with those in normal
235 bone. Different directions of mass fraction changes suggest potential use of mass fraction
236 ratios of these trace elements as Ewing's sarcoma markers. A simple multiplication of two or
237 more trace element mass fractions, which change in one direction, can improve the
238 difference between such characteristics of intact bone and Ewing's sarcoma tissues. These
239 conclusions were the main reason for calculating Rb/Co, Rb/Fe, and Rb/Se mass fraction
240 ratios, $Co \times Fe$, $Co \times Se$, and $Fe \times Se$ mass fractions multiplications, and also $Rb/(Co \times Fe)$,
241 $Rb/(Co \times Se)$, and $Rb/(Fe \times Se)$ indices (Table 3). It was found that significant lower ($p \leq 0.01$
242 or ≤ 0.001) mean values of all selected mass fraction ratios and indices were typical of
243 Ewing's sarcoma tissue compared with intact bone (Tables 4).

244
245 In control group a statistically significant direct correlation was found, for example, between
246 the Fe and Se ($r = 0.60$, $p \leq 0.05$), Fe and Co ($r = 0.55$, $p \leq 0.01$), Co and Hg ($r = 0.79$, p
247 ≤ 0.01), Rb and Ag ($r = 0.62$, $p \leq 0.05$), and between Rb and Cr ($r = 0.56$, $p \leq 0.05$) mass
248 fractions (Table 5). In the same group a pronounced ($p \leq 0.01$) inverse correlation was
249 observed between the Fe and Ag ($r = -0.80$, $p \leq 0.05$). If some positive correlations between
250 the trace elements were predictable (e.g., Fe–Co), the interpretation of other observed
251 relationships requires further study for a more complete understanding.

252
253 In the Ewing's sarcoma tissue many significant correlations between trace elements found in
254 the control group are no longer evident, for example, direct correlation between Fe and Se,
255 etc. (Table 5). However, direct correlations between Co and Se ($r = 0.81$, $p \leq 0.05$) and Co
256 and Sb ($r = 0.80$, $p \leq 0.05$), as well as inverse correlations between Se and Ag ($r = -0.85$, p
257 ≤ 0.05) and Se and Rb ($r = -0.85$, $p \leq 0.05$) were arisen (Table 5). Thus, if we accept the
258 levels and relationships of trace element mass fraction in the intact bone samples of control
259 group as a norm, we have to conclude that with a malignant transformation the levels and
260 relationships of trace elements in bone significantly changed. No published data referring to
261 correlations between trace elements mass fractions in Ewing's sarcoma tissue were found.

262
263 The changes in trace element contents of cancerous tissues in comparison with non-
264 cancerous tissues may be attributed to a cause or effect of malignant transformation. Bone
265 is a mineralized connective tissue. It is formed by osteoblasts, that deposit collagen and
266 release Ca, Mg, and phosphate ions that combine chemically within the collagenous matrix

into a crystalline mineral, known as bone hydroxyapatite. On average, bone tissue contains about 10-25% water, 25% protein fibers like collagen, and 50% hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Many trace elements are bone-seeking elements and closely associated with hydroxyapatite [24-28]. Ewing's sarcoma is classified as a bone tumor. Our previous findings showed that the means of the Ca and P mass fraction in the Ewing's sarcoma tissue are lower than in normal bone, but the mean of Ca/P ratio is similar [44]. It suggested that Ewing's sarcoma continue to form bone hydroxyapatite but in a less degree than normal bone. Our findings show that the mean of the Fe mass fraction in Ewing's sarcoma tissue samples was 17.1 times greater than in normal bone tissues (Table 2). It is well known that blood and red bone marrow are rich in iron [45]. Ewing sarcoma is derived from red bone marrow [30,31]. Thus, it is possible to speculate that a histological origin of Ewing sarcoma is a cause of elevated Fe mass fraction in tumor tissue. As was found by us, there is a direct correlation between Fe and Co mass fractions (Table 5). Therefore an increased level of Co in the Ewing sarcoma may be closely connected with a high Fe content in tumor tissue (Table 2).

In the Ewing sarcoma tissue the mean Se mass fractions is 18.6 times higher ($p \leq 0.05$) than in normal tissues (Table 2). The high Se level was reported in malignant tumors of ovary [46], lung [47], prostate [48], breast [49,50], intestine [51], and in gastric cancer tissue [52]. The role played by Se in those tumors remains unknown, but in general it is accepted that certain proteins containing Se can mediate in the protective effects against oxidative stress. The literature-based analysis found the association of malignant tissue transformation with local oxidative stress. Studies have shown that oxidative stress conditions play an important role in both the initiation and the progression of cancer by regulating molecules such as DNA, enhancers, transcription factors, and cell cycle regulators [53]. However the cause of increased Se in cancerous tissue and particularly in the Ewing sarcoma is not completely understood and requires further studies.

5. CONCLUSION

Instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides is a satisfactory analytical tool to determine non-destructively the elemental content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn in human bone samples and samples of intraosseous lesions weighing about 100 mg. In the Ewing's sarcoma tissue the mass fractions of Co, Fe, and Se are significantly higher as well as the mass fraction of Rb is lower than in normal bone tissues. Moreover, significantly higher Rb/Co, Rb/Fe, and Rb/Se mass fraction ratios and values of $\text{Rb}/(\text{Co} \times \text{Fe})$, $\text{Rb}/(\text{Co} \times \text{Se})$, and $\text{Rb}/(\text{Fe} \times \text{Se})$ indices are typical of the Ewing's sarcoma tissue compared to intact bone. In the Ewing's sarcoma tissue many correlations between trace elements found in the control group are no longer evident. Thus, if we accept the levels and relationships of trace element mass fraction in the intact bone as a norm, we have to conclude that in malignant transformed bone tissues a trace element homeostasis is significantly disturbed.

ETHICAL APPROVAL

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

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