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ULTRATRACE ELEMENT CONTENTS IN RAT TISSUES: COMPARATIVE ANALYSIS OF SERUM AND HAIR AS INDICATIVE MATRICES OF THE TOTAL BODY BURDEN

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Abstract: The aim of this study was to investigate the distribution of ultratrace elements in rat tissues and to perform a comparative analysis of hair and serum as potential bioindicators of the total ultratrace element content. Thirty-six male Wistar rats were fed a standard chow containing 0.006±0.000, 0.001±0.000, 0.017±0.002, 0.382±0.031, 0.168±0.014, 3.211±0.134, 0.095±0.006, 0.000±0.000, 6.675±0.336, 15.327±0.564, 0.002±0.000, and 1.185±0.202 μg/g of silver (Ag), gold (Au), cesium (Cs), gallium (Ga), germanium (Ge), lanthanum (La), niobium (Nb), platinum (Pt), rubidium (Rb), titanium (Ti), thallium (Tl) and zirconium (Zr), respectively, from weaning to 3 months old. The ultratrace element content in the liver, kidney, muscle, heart, serum and hair was assessed using inductively coupled plasma mass spectrometry. The obtained data indicate that the highest concentrations of most of the studied elements (Ti, Zr, Ge, Nb, tungsten (W), La, uranium (U), Ag, Au, Pt) are in hair, whereas the lowest were observed in the serum. Statistical analysis revealed a significant association between concentrations in the hair and other tissues for Cs, Ti, Nb, Tl, La, U and Au. At the same time, serum Cs, Rb, Ti, Ge, Nb, W, Ga, Tl and La concentrations significantly correlated with the tissue content of the respective ultratrace elements. It can be concluded that hair may be used as a potential bioindicator for certain ultratrace element content in the mammalian organism.

Key words: ultratrace elements; inductively coupled plasma mass spectrometry; rats; hair; chow

INTRODUCTION

The intensive development of industry, especially after the Industrial Revolution, has increased the amount of metal emissions into the environment [1]. At the same time, the modern high-technology industry uses a number of rare elements that were not used widely before [2]. In particular, gallium and germanium are used in electronics [3-4], whereas a number of ultratrace elements such as platinum, titanium, and gallium have medical applications [5-7]. The physiological functions and kinetics of ultratrace elements are poorly studied. However, some ultratrace metals exhibit toxic properties and have adverse health effects in the case of overexposure [8-13]. Therefore, humans are at increased risk of exposure to the potential toxic effects of ultratrace elements.

Biomonitoring is widely used for assessment of environmental and occupational exposure to toxicants [14]. Human biomonitoring via the measurement of certain chemicals in human tissues is considered to be a gold standard in exposure assessment [15]. Blood serum has always been used for the analysis of toxic compounds. However, the use of invasive biosamples is unlikely for screening studies involving a high number of examinees [16]. To date, a variety of noninvasive indicative matrices, like saliva, sweat, nails and placenta, has been proposed [17]. Hair is also considered to be a potent indicator for use in human biomonitoring [18]. It has a number of advantages as compared to other biosamples: high mineralization, noninvasive biosampling, irreversible incorporation of minerals into the matrix [19]. At the same time, all noninvasive biomatrices, including hair, should be tested for their ability to indicate the total body burden of certain chemicals [17]. In particular, it is unknown whether hair may reflect the content of ultratrace elements in organs and tissues.

Therefore, the primary objective of the current study was to investigate the distribution of ultratrace elements in rat tissues and to perform a comparative analysis of hair and serum as potential bioindicators of thev total ultratrace element content.

MATERIALS AND METHODS

Animals and handling

The current investigation was performed in accordance with international ethics regulations and was approved by the local ethics committee. A total of 36 male Wistar rats were used in the study. Animals were maintained in plastic cages in a laboratory with a 12:12 h light-dark cycle (lights on at 8.00 a.m.). Animals were fed a standard laboratory diet ("Laboratorkorm" Ltd., Moscow, Russia) containing 307 kcal/100 g (20% protein, 70% carbohydrate, 10% fat) from weaning to 3 months old. The chow was analyzed for ultratrace element content in our laboratory. The dietary levels of Ag, Au, Cs, Ga, Ge, La, Nb, Pt, Rb, Ti, Tl, and Zr were 0.006±0.000, 0.001±0.000, 0.017±0.002, 0.382±0.031, 0.168±0.014, 3.211±0.134, 0.095±0.006, 0.000±0.000, 6.675±0.336, 15.327±0.564, 0.002±0.000, and 1.185±0.202 (Mean±SD) μg/g, respectively. The diet and bottled drinking water were provided *ad libitum*. At the age of 3 months, the rats were sacrificed in accordance with institutional guidelines. Blood was collected to plastic tubes via venesection of the jugular vein. The obtained blood was centrifuged at 1600g for 10 min to obtain serum. The liver, kidneys, myocardium and skeletal muscle (m. gastrocnemius) were collected for subsequent chemical analysis. The obtained organ samples were separated from connective tissue and rinsed thrice with ice-cold physiological saline in order to remove blood. Rat hair was collected from the cranial part of the spine. All samples were stored at -80°C before analysis.

Sample preparation and chemical analysis

Before analysis, hair samples were washed with acetone and rinsed twice with deionized water to remove exogenous contamination [20] and subsequently air-dried at 60°C. Serum was diluted with an acidified solution (pH=2.0; 1:15, v/v) containing 1% 1-butanol (Merck KGaA, Darmstadt, Germany), 0.1% Triton X-100 (Sigma-Aldrich, Co., St. Louis, USA) and 0.07% HNO_3 (Sigma-Aldrich, Co., St. Louis, USA) in distilled deionized water. Microwave degradation of the obtained samples was performed in concentrated nitric acid in the Berghof speedwave 4 (Berghof, Products Instruments GmbH, Eningen, Germany) system (20 min, 180°C). After decomposition, deionized water was added to a final volume of 15 ml. Tissue ultratrace element content (Ag, Au, Cs, Ga, Ge, La, Nb, Pt, Rb, Ti, Tl and Zr) was assessed by inductively coupled plasma mass spectrometry (ICP-MS) with NexION 300D (PerkinElmer Inc., Shelton, CT 06484, USA) using Dynamic Reaction Cell (DRC) to remove most interference, with little or no loss of analyte sensitivity and equipped with ESI SC-2 DX4 autosampler (Elemental Scientific Inc., Omaha, NE 68122, USA).

The system was calibrated using commercially available multi-element standards. In particular, the

standard trace element solutions (0.5, 5, 10 and 50 μg/L) were prepared from Universal Data Acquisition Standards Kit (PerkinElmer Inc., Shelton, CT 06484, USA) by dilution with acidified (1% HNO_3) distilled deionized water. Internal online standardization using yttrium-89 isotope was performed. The internal standard containing 10 μg/L yttrium was prepared from Yttrium (Y) Pure Single-Element Standard (PerkinElmer Inc., Shelton, CT 06484, USA) on a matrix containing 8% 1-butanol (Merck KGaA), 0.8% Triton X-100 (Sigma-Aldrich, Co.), 0.02% tetramethylammonium hydroxide (Alfa-Aesar, Ward Hill, MA 01835 USA) and 0.02% ethylenediaminetetraacetic acid (Sigma-Aldrich, Co). Commercially available reference materials of hair (GBW09101; Shanghai Institute of Nuclear Research, Academia Sinica, China) and serum (ClinCheck Plasma Control, lot 129, levels 1 and 2; RECIPE Chemicals + Instruments GmbH, Germany) were used for laboratory quality control. The recovery rates of the reference materials for all elements (where available) exceeded 80%.The studied spectrum of chemical elements is rather heterogeneous in the structure of the electron shell and chemical properties. In particular, Rb and Cs (s-elements) are considered very active metals, whereas Ga, Tl, La and U (f-, d- and p-elements), medium activity metals. Ti, Zr, Nb and W are typical transition d-elements, whereas Ge is a p-element. Ag, Au and Pt also belong to the family of d-elements, but, in contrast to the previous elements, their chemical activity is low ("noble" metals). Therefore, the elements should be combined in groups based on the similarity of their chemical properties in order to simplify the discussion.

Statistical analysis

Statistical treatment of the obtained data was performed using Statistica 10.0 (Statsoft). Data distribution was assessed using the Shapiro-Wilk test. As data were not normally distributed, the values were presented as the median with 25 and 75 percentile boundaries. Comparative analysis of tissue element concentration was performed using the Mann-Whitney U-test. Pearson's correlation coefficient was used for correlation analysis. The level of significance was set as $p<0.05$ for all analyses.

RESULTS

The obtained data indicate that the distribution of ultratrace elements was tissue-specific. In particular, maximal concentrations of Cs were observed in skeletal muscles, exceeding nearly 2-fold the respective values in liver and heart muscle (Table 1). Kidney Cs levels were significantly lower than those in skeletal muscle by 26%. Hair levels of the studied metal were more than 7-fold lower than in muscles. The lowest concentration of Cs was observed in serum. In particular, Cs content in skeletal muscle, kidney, liver, myocardium and hair significantly exceeded the serum values by more than 37-, 27-, 20-, 20- and 2-fold, respectively.

Despite the similarity of chemical properties, Rb distribution was different from Cs (Table 1). In particular, maximal concentrations of Rb were observed in liver. Skeletal muscle and kidney Rb levels significantly exceeded those in the myocardium by 36 and 31%, respectively. Hair Rb concentration was 70, 51, 49 and 33% lower in the liver, muscle, kidney and heart muscle, respectively. As observed in the case of Cs, the lowest concentrations of Rb were detected in serum. Liver, muscle, kidney, myocardium and hair Rb content significantly exceeded serum metal levels by more than 35-, 21-, 21-, 16- and 10-fold, respectively.

The highest content of Ti was detected in the hair, being nearly 2-fold higher than the liver values (Table 2). Kidney and muscle Ti concentration was 26 and

Table 1. Cesium and rubidium in rat tissues (μg/g) and serum (μg/L).

	Cs	Rb
Liver	0.008(0.007-0.009)	11.83(11.35-12.19)
Kidney	0.011(0.010-0.012) [1]	6.96(6.45-7.44) [1]
Heart	0.008(0.007-0.010) [2]	5.32(4.96-5.61) [1,2]
Muscle	0.015(0.013-0.017) [1,2,3]	7.21(6.74-7.50) [1,3]
Serum	0.0004(0.0003-0.0005) [1,2,3,4]	0.33(0.31-0.38) [1,2,3,4]
Hair	0.002(0.002-0.002) [1,2,3,4,.5]	3.54(2.94-4.45) [1,2,3,4,5]

Data are expressed as median (25-75);
[1] – significant difference in comparison to liver;
[2] – significant difference in comparison to kidney;
[3] – significant difference in comparison to heart;
[4] – significant difference in comparison to muscle;
[5] – significant difference in comparison to serum.

34% lower than that in the liver, respectively. At the same time, myocardial Ti content was 443, 180, 107 and 84% lower in comparison to the respective hair, liver, kidney and muscle values. The lowest Ti levels were observed in blood serum. In particular, hair, liver, kidney, muscle and heart Ti levels were more than 73-, 37-, 28-, 24- and 13-fold higher than those in serum, respectively.

Zr distribution in the studied tissues was more linear in comparison to Ti. The maximal Zr content was observed in rat hair, being higher than that in the kidney, liver, muscle and myocardium by more than 3-, 5-, 5- and 10-fold, respectively. As observed for other metals, the lowest Zr concentration was detected in serum. Particularly, hair, kidney, liver, skeletal and heart muscle Zr levels were more than 262-, 75-, 50-, 50- and 25-fold higher than those in serum, respectively.

The highest values of Ge content were observed in hair, exceeding the respective values in kidney, heart, muscle and serum by more than 6-, 2-, 50- and 16-fold. At the same time, the lowest Ge concentration was detected in skeletal muscle, being 30-, 8-, 20- and 3-fold lower than the respective values in liver, kidney, heart and serum.

Heart, skeletal muscle and serum levels of Nb were significantly lower than liver and kidney values by 76, 73 and 80%, respectively. At the same time, the highest concentration of Nb was detected in hair samples, exceeding the respective liver, kidney, heart, muscle and serum values by more than 2-, 2-, 10-, 8- and 11-fold.

The obtained data indicate that the highest content of W was present in animals' hair, exceeding the respective values in liver, kidney, heart and muscle by more than 6-, 10-, 10- and 5-fold. At the same time, the lowest W levels were observed in serum. Particularly, W levels in this tissue were 5-, 3-, 3-, 6- and 33-fold lower in comparison to the liver, kidney, myocardium, muscle, and hair, respectively.

It has been demonstrated (Table 3) that maximal Ga content was noted in liver samples. In particular, the values were significantly higher than that in the kidney, heart and muscle by 24, 57, and 63%, respectively. Serum and hair Ga concentrations were significantly lower than those in the other studied organs. At the same time, hair Ga levels exceeded the respective values in serum by 40%.

Kidney Tl levels exceeded by more than 10-fold those observed in the heart and skeletal muscles. Moreover, the kidney Tl concentration was 15- and 5-fold higher in comparison to liver and hair values. As observed for the other studied elements, the minimal Tl content was observed in rat serum.

The La concentration in hair was the highest in comparison to the other organs by 28-, 143-, 286-,

Table 2. Titanium, zirconium, germanium, niobium and tungsten levels in rat tissues (μg/g) and serum (μg/L).

	Ti	**Zr**	**Ge**	**Nb**	**W**
Liver	0.227 (0.144-0.834)	0.004 (0.002-0.013)	0.003 (0.001-0.005)	0.0003 (0.0002-0.0010)	0.0003 (0.0002-0.0006)
Kidney	0.168 [1] (0.122-0.265)	0.006 (0.002-0.011)	0.0008 [1] (0.0000-0.0019)	0.0003 (0.0002-0.0004)	0.0002 [1] (0.0002-0.0004)
Heart	0.081 [1,2] (0.066-0.174)	0.002 [1,2] (0.001-0.005)	0.002 [1] (0.000-0.003)	0.00007 [1,2] (0.00000-0.00018)	0.0002 [1,2] (0.0001-0.0003)
Muscle	0.149 [1,3] (0.084-0.294)	0.004 [3] (0.003-0.009)	0.0001 [1,3] (0.0000-0.0015)	0.00008 [1,2] (0.00000-0.00042)	0.0004 [2,3] (0.0002-0.0006)
Serum	0.006 [1,2,3,4] (0.005-0.009)	0.00008 [1,2,3,4] (0.00006-0.00011)	0.0003 [1,3] (0.0001-0.0006)	0.00006 [1,2] (0.00005-0.00006)	0.00006 [1,2,3,4] (0.00003-0.00008)
Hair	0.440 [1,2,3,4,5] (0.350-0.588)	0.021 [1,2,3,4,5] (0.015-0.033)	0.005 [1,2,3,4,5] (0.004-0.006)	0.0007 [1,2,3,4,5] (0.0005-0.0012)	0.002 [1,2,3,4,5] (0.001-0.002)

Data are expressed as median (25-75); [1] – significant difference in comparison to liver; [2] – significant difference in comparison to kidney; [3] – significant difference in comparison to heart; [4] – significant difference in comparison to muscle; [5] – significant difference in comparison to serum.

Table 3. Gallium, thallium, lanthanum and uranium content in rat tissues (μg/g) and serum (μg/L).

	Ga	Tl	La	U
Liver	0.083 (0.077-0.090) [1]	0.0002 (0.0002-0.0003)	0.0003 (0.0002-0.0004)	0.00006 (0.00003-0.00011)
Kidney	0.067 (0.064-0.072) [1,2]	0.003 (0.002-0.004) [1,2]	0.00006 (0.00001-0.00011) [1]	0.0005 (0.0003-0.0006) [1,2]
Heart	0.053 (0.048-0.057) [1,2]	0.0003 (0.0003-0.0004) [1,2]	0.00003 (0.00000-0.00009) [1]	0.00003 (0.00000-0.00005) [1,2]
Muscle	0.051 (0.048-0.056) [1,2]	0.0003 (0.0003-0.0004) [1,2]	0.0002 (0.0001-0.0004) [2,3]	0.00005 (0.00002-0.00008) [2,3]
Serum	0.005 (0.004-0.005) [1,2,3,4]	0.00001 (0.00001-0.00002) [1,2,3,4]	0.000007 (0.000000-0.000019) [1,2,4]	0.00002 (0.00001-0.00005) [1,2,4]
Hair	0.007 (0.007-0.009) [1,2,3,4,5]	0.0006 (0.0004-0.0006) [1,2,3,4,5]	0.0086 (0.0065-0.0164) [1,2,3,4,5]	0.0005 (0.0003-0.0007) [1,3,4,5]

Data are expressed as median (25-75); [1] – significant difference in comparison to liver; [2] – significant difference in comparison to kidney; [3] – significant difference in comparison to heart; [4] – significant difference in comparison to muscle; [5] – significant difference in comparison to serum.

Table 4. Silver, gold and platinum levels in rat tissues (μg/g) and serum (μg/L).

	Ag	Au	Pt
Liver	0.0003 (0.0002-0.0005)	0.0007 (0.0005-0.0012)	0.00000 (0.00000-0.00001)
Kidney	0.0002 (0.0002-0.0004)	0.0004 [1] (0.0003-0.0007)	0.0000000 (000000-0.000007)
Heart	0.0002 [1] (0.0001-0.0003)	0.0004 [1] (0.0003-0.0006)	0.000000 (0.000000-0.000004)
Muscle	0.0016 [1,2,3] (0.0003-0.0028)	0.0014 [2,3] (0.0004-0.0168)	0.00000 (0.00000-0.00001)
Serum	0.00005 [1,2,3,4] (0.00002-0.00007)	0.000004 [1,2,3,4] (0.000001-0.000006)	0.000000 (0.000000-0.000001)
Hair	0.0022 [1,2,3,4,5] (0.0018-0.0034)	0.0021 [2,3] (0.0004-0.0116)	0.00003 [1,2,3,4,5] (0.00002-0.00004)

Data are expressed as median (25-75); [1] – significant difference in comparison to liver; [2] – significant difference in comparison to kidney; [3] – significant difference in comparison to heart; [4] – significant difference in comparison to muscle; [5] – significant difference in comparison to serum.

43, and 1228-fold as compared to liver, kidney, heart, muscle and serum values, respectively. At the same time, no significant difference in La content was observed between liver and skeletal muscle as well as between kidney and heart.

The highest levels of U were detected in the kidney and hair of the experimental animals. The obtained values exceeded those for the liver, heart, muscles and serum by more than 8-, 16-, 10- and 25-fold, respectively.

Au and Ag levels were also maximal in the rats' hair and skeletal muscles (Table 4). In contrast, the lowest values of Au and Ag concentration were observed in blood serum. Pt levels in the rats' tissues were nearly undetectable. However, the obtained data indicate that the highest Pt concentration was detected in the hair.

In order to find possible biomarkers for the estimation of ultratrace element content in the organism, we performed correlation analysis between the animals' serum and tissue contents of the studied elements (Table 5). The obtained data indicate that serum Cs content significantly correlated with metal content in all studied organs. Serum Rb levels were significantly related to its concentration in the myocardium. Ti concentration in the serum was directly

Table 5. Correlation of serum ultratrace elements with their tissue content.

	Liver	Kidney	Heart	Muscle	Hair
Cs	r=0.849 p<0.001*	r=0.618 p<0.001*	r=0.842 p<0.001*	r=0.658 p<0.001*	r=0.545 p=0.001*
Rb	r=0.051 p= 0.769	r=0.186 p=0.278	r=0.506 p=0.002*	r=0.096 p=0.579	r=-0.051 p=0.768
Ti	r=0.379 p=0.023*	r=-0.030 p=0.863	r=0.350 p=0.037*	r=0.145 p=0.399	r=0.098 p=0.569
Zr	r=0.081 p=0.640	r=-0.106 p=0.540	r=-0.029 p=0.869	r=-0.081 p=0.639	r=-0.085 p=0.624
Ge	r=-0.055 p=0.750	r=-0.141 p=0.412	r=-0.219 p=0.199	r=-0.370 p=0.026*	r=0.020 p=0.907
Nb	r=-0.348 p=0.038*	r=-0.185 p=0.279	r=-0.103 p=0.549	r=-0.293 p=0.083	r=-0.098 p=0.571
W	r=0.515 p=0.001*	r=-0.275 p=0.105	r=-0.189 p=0.269	r=-0.247 p=0.147	r=-0.124 p=0.470
Ga	r=0.348 p=0.037*	r=0.560 p<0.001*	r=0.538 p=0.001*	r=0.447 p=0.006*	r=0.158 p=0.358
Tl	r=0.238 p=0.163	r=0.272 p=0.109	r=0.404 p=0.015*	r=0.377 p=0.023*	r=0.030 p=0.863
La	r=0.012 p=0.944	r=0.037 p=0.831	r=-0.004 p=0.982	r=-0.075 p=0.663	r=0.592 p<0.001*
U	r=-0.231 p=0.174	r=-0.273 p=0.108	r=-0.041 p=0.811	r=-0.212 p=0.215	r=-0.263 p=0.121
Ag	r=-0.098 p=0.570	r=-0.229 p=0.180	r=-0.224 p=0.189	r=0.005 p=0.977	r=-0.042 p=0.809
Au	r=-0.061 p=0.723	r=-0.055 p=0.752	r=-0.053 p=0.758	r=-0.008 p=0.965	r=-0.064 p=0.711
Pt	r=-0.094 p=0.596	r=-0.138 p=0.436	r=-0.166 p=0.350	r=-0.145 p=0.414	r=-0.192 p=0.277

r – Pearson's correlation coefficient; p – p value for the respective correlation coefficient; * – significant correlation at p<0.05

Table 6. Correlation of hair ultratrace elements with their tissue content.

	Liver	Kidney	Heart	Muscle	Serum
Cs	r=0.686 p<0.001*	r=0.430 p=0.009*	r=0.634 p<0.001*	r=0.584 p<0.001*	r=0.545 p=0.001*
Rb	r=0.072 p=0.677	r=0.267 p=0.115	r=-0.018 p=0.919	r=0.102 p=0.556	r=-0.051 p=0.768
Ti	r=0.169 p=0.324	r=0.150 p=0.384	r=0.661 p<0.001*	r=0.520 p=0.001*	r=0.098 p=0.569
Zr	r=0.206 p=0.228	r=0.158 p=0.357	r=0.291 p=0.085	r=0.302 p=0.074	r=-0.085 p=0.624
Ge	r=0.200 p=0.243	r=-0.106 p=0.537	r=0.240 p=0.159	r=0.003 p=0.988	r=0.020 p=0.907
Nb	r=0.281 p=0.098*	r=-0.094 p=0.585	r=0.332 p=0.048*	r=0.308 p=0.068	r=-0.098 p=0.571
W	r=0.027 p=0.874	r=0.046 p=0.792	r=-0.068 p=0.693	r=0.128 p=0.457	r=-0.124 p=0.470
Ga	r=0.105; p=0.541	r=-0.005; p=0.976	r=0.077; p=0.655	r=0.094; p=0.586	r=0.158; p=0.358
Tl	r=0.375 p=0.024*	r=0.295 p=0.080	r=0.074 p=0.669	r=0.338 p=0.044*	r=0.028 p=0.863
La	r=-0.118 p=0.495	r=-0.167 p=0.331	r=-0.056 p=0.744	r=0.239 p=0.161	r=0.592 p<0.001*
U	r=0.463 p=0.004*	r=-0.100 p=0.560	r=-0.054 p=0.757	r=0.511 p=0.001*	r=-0.263 p=0.121
Ag	r=0.024 p=0.891	r=0.031 p=0.856	r=-0.079 p=0.648	r=0.052 p=0.766	r=-0.042 p=0.809
Au	r=-0.120 p=0.484	r=0.485 p=0.003*	r=0.148 p=0.389	r=0.314 p=0.062	r=-0.064 p=0.711
Pt	r=-0.132 p=0.457	r=-0.120 p=0.500	r=-0.004 p=0.981	r=-0.163 p=0.357	r=-0.192 p=0.277

r – Pearson's correlation coefficient; p – p value for the respective correlation coefficient; * – significant correlation at p<0.05

associated with liver and heart metal levels. No significant correlation was observed between serum Zr and its concentration in the other studied tissues. It has been noted that Ge was characterized by a significant negative correlation between its serum and muscle levels. Similarly, serum Nb was indirectly associated with liver Nb content. Serum W significantly correlated only with liver element content. Ga concentrations in the animals' serum were significantly interrelated with liver, kidney, heart and skeletal muscle element content. At the same time, serum Tl directly correlated with its concentrations in the heart and muscle. Serum La levels did not correlate significantly with its content in the studied organs, but were directly interrelated with hair La values. Serum values of U, Ag, Au and Pt were not associated with organ element content.

Hair Cs levels also correlated with its content in the liver, kidney, heart, muscle and serum (Table 6). In contrast to serum, statistical analysis failed to detect a significant relationship between hair Rb and its tissue concentrations. Hair Ti content significantly correlated with metal levels in the heart and skeletal muscles. Despite the absence of a significant correlation between hair Zr and its tissue concentrations, a nearly significant direct association was revealed for Zr concentrations between hair on the one hand, and the myocardium and skeletal muscle on the other. In contrast to serum, hair Ge was not associated with its content with other studied organs. The same situation was observed in the case of W, Ga, Ag and Pt. In accordance with statistical analysis, hair Nb was significantly associated with liver and heart element content. Moreover, a nearly signifi-

Table 7. Comparison of ultratrace elements content in the studied organs.

Element	Tissues and organs *
Cs	Muscle > Kidney > Liver=Heart > Hair > Serum
Rb	Liver > Muscle > Kidney > Heart > Hair > Serum
Ti	Hair > Liver > Kidney > Muscle > Heart > Serum
Zr	Hair > Kidney > Liver=Muscle > Heart > Serum
Ge	Hair > Liver > Heart > Kidney > Serum > Muscle
Nb	Hair > Liver=Kidney > Muscle > Heart > Serum
W	Hair > Muscle > Liver > Kidney=Heart > Serum
Ga	Liver > Kidney > Heart > Muscle > Hair > Serum
Tl	Kidney > Hair > Heart=Muscle > Liver > Serum
La	Hair > Liver > Muscle > Kidney > Heart > Serum
U	Hair=Kidney > Liver > Muscle > Heart > Serum
Ag	Hair > Muscle > Liver > Kidney=Heart > Serum
Au	Hair > Muscle > Liver > Kidney=Heart > Serum
Pt	Hair > Muscle=Liver > Kidney > Heart > Serum

*Concentrations are indicated in the descending order

cant direct correlation was detected between hair and skeletal muscle Nb concentrations. A similar situation was observed in the case of Tl. In particular, hair Tl levels correlated with liver and muscle metal concentrations, whereas its association with kidney content was nearly significant. The U concentration in rat liver and muscle was directly interrelated with hair content. The hair Au concentration was significantly associated with kidney metal levels, whereas its correlation with muscle Au content was nearly significant.

The distribution of the studied metals in the rats' organs and tissues may be presented as given in Table 7.

DISCUSSION

The obtained data on ultratrace element distribution are partially in agreement with previous studies. The present data indicate that the highest concentrations of Cs was in muscles and that the lowest was in the serum [21]. At the same time, a more recent study has found liver Cs levels to be higher than in the kidney [22], unlike our data.

The results on Rb distribution are in a good agreement with a detailed autoradiographic study indicating that the highest Rb levels were observed in the liver followed by skeletal muscle. At the same time, the authors indicate that hair and blood serum concentrations of radiorubidium were among the lowest in the body [23].

As is seen from the results, the distribution of Rb and Cs in the examined organs and tissues was unequal. This fact can be explained on the basis of the similarity in chemical properties of Rb and potassium (atomic radius, radius of the hydrated ions, electronegativity, first ionization potentials and standard reduction potentials) [24], as well the content of these elements in the environment [25].

The observed Ti tissue distribution was in agreement with previously published data indicating the highest Ti levels in liver [26-27]. At the same time, the obtained data on tissue Zr and Nb distribution did not confirm previous observations [28]. Previously published data on Ge deposition in rat organs are in contrast to the present observations. A number of studies have demonstrated that Ge is concentrated in the kidneys [29-31]. Moreover, it has been shown that blood Ge concentrations were significantly higher in comparison to other organs [31]. A previous study using radiolabeled W has demonstrated that the kidney is the main organ of W deposition. Serum concentrations were also reported to be higher than in the liver and heart [32], being in contrast to the present research data. Moreover, only trace quantities of W were observed in rat muscles in an earlier study [33], whereas the present work has shown that skeletal muscles contain nearly maximal concentrations of this element.

Comparison of the tissue content of Ti, Zr, Ge, Nb and W in rats has indicated that the Ti concentration is nearly 10-fold higher than in the case of the other elements, regardless of the type of studied tissue.

Distribution in the Earth's crust may be a reason for the high difference in the content of these elements in the rats' tissues. In particular, Ti is the most common d-metal element along with iron [34]. Therefore, the high Ti content in tissues and organs can be hypothetically explained by its intake. Moreover, Nb, Zr, Ge and W compounds have low solubility and are prone to

hydrolysis, which leads to the formation of insoluble hydroxides in nearly physiological pH. This fact may result in poor absorption of these elements in the gastrointestinal tract [25]. The observed distribution of Nb, Ge, Zr and W in the studied tissues was relatively similar. These elements tended to accumulate in the skeletal muscle, liver, kidney and hair. The relatively high level of these elements in blood serum can be explained by metal binding by the mechanism of protein adsorption. Ti distribution in the organism was irregular, being indicative of its affinity for a particular type of tissue and organs. The low content of Ti ions in serum may occur due to the fact that Ti (IV) compounds are prone to hydrolysis [25,34]. The concentration of this metal in the organs and tissues could be explained by the formation of stable complexes with certain bioligands.

The obtained data only partially confirm the results of Chilton et al. [35] who demonstrated that the liver and kidney contain similar quantities of Ga, whereas the blood Ga content exceeded the respective value for muscles.

The presented results are in agreement with the results of a previous study indicating the highest concentration of Tl is in rats' kidneys [36]. Moreover, the character of the Tl distribution in tissues also corresponds to earlier observations [37]

The observed tissue distribution of La was not in agreement with previous studies. In particular, earlier data indicate that the kidney contains higher quantities of La in comparison to the liver [38].

It has been shown that maximal U levels were observed in the kidney after implantation with depleted U pellets [39]. Moreover, it has also been noted that heart and serum U content was significantly lower than that in the liver [40].

The low abundance of Tl, U and La in the environment and the absence of biological functions may explain the obtained data regarding their low content in the rats' tissues. At the same time, the relatively high Ga content does not fit into the overall picture. Possibly the similarity of Ga to iron(III) ions (ionic radius, tendency to hydrolyse, complexes formation) may determine its rather high content in tissues. In addition to the ionic radius, the triple-charged Ga and iron cations also have similar values of electronegativity and third ionization potential [24]. Therefore, Ga (like iron(III)) easily forms stable complexes with oxygen and nitrogen-containing bioligands [41]. In particular, it has demonstrated that Ga(III) competes with iron for ferritin binding [42].

The obtained data confirm previous observations on Ag distribution in rat organs, with the liver being the main organ of metal deposition [43]. At the same time, a more recent publication has demonstrated the highest quantities of Ag in kidneys [44].

In contrast to our findings, a previously performed study has demonstrated the highest levels of Au [45] and Pt [46] in the liver but not in the skeletal muscles.

Pt, Ag and Au are d-elements. Complete d- and f-orbitals in atoms of Au, Pt and Ag weakly shield the external s-electrons. Consequently, the s-electron of these elements is strongly associated with the nucleus [25]. The latter results in a reduction in atomic radius and a sharp decrease in chemical activity. Ag^{+} and Au^{3+} ions are referred to soft bases, explaining the high affinity of these ions to the nitrogen- and sulfur-containing ligands [47]. In particular, Ag is capable of forming chelate complexes with nucleic acid nitrogenous bases [48]. Thus, one can suppose that the soft sulfur atoms and the aromatic nitrogen in protein molecules are able to bind the ions of Au and Ag. Due to a low affinity of Ag and Au to oxygen-donor ligands, these elements do not form stable aqua complexes [49]. Therefore, the low concentration of Ag and Au compounds in the serum may be due to the dissociation of labile Ag(I) and Au(III) complexes in the plasma and subsequent binding of metal ions to the thiol groups of proteins and rapid transport to the tissues and organs.

At the same time, the obtained data should be critically compared to previous findings. In particular, most of the reviewed manuscripts deal with acute exposure (overexposure) to the studied elements. In contrast, in our investigation the studied elements were of dietary origin and did not reach toxic quantities.

CONCLUSION

The results of our study reveal a high heterogeneity of ultratrace element content in the rat organism. For most of the ultratrace elements, hair was the tissue with the highest content, whereas serum was characterized by extremely low values of element content. It has been demonstrated that serum concentrations of Cs, Ti, Nb, W, Ga and Tl significantly correlate with organ content. At the same time, the tissue content of Cs, Ti, Nb, Tl, U and Au was significantly associated with its levels in hair. From the presented results, it can be concluded that hair could be used as a potential bioindicator for certain ultratrace element content in the mammalian organism.

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