Comparative Estimation of Cu, Zn, Pb and Cd Contents in Healthy People and in Patients with Blood Oncology Diseases

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Abstract

Inversion voltammetry method was used to test 52 healthy people and 28 patients with Hodgkin's lymphoma (HL) (all Novosibirsk citizens) for the content of microelements such as Cu, Zn, Pb and Cd. In the blood and urine of healthy people zinc, lead and cadmium content was found to fit conventional norm, while Cu content in blood and its fractions was at the lowermost norm level. Copper content in the whole blood of the HL sick people insignificantly differed from that typical for healthy people. However, one of the main homeostasis parameters, namely copper and zinc balance in blood and urine, was disturbed. In particular, there was essential zinc deficit in blood, while in urine zinc was in excess. Moreover, all HL sick patients had insufficient cadmium excretion and blood lead deficit in comparison to the control group. Organic selenium containing, food supplement "Nutricon-Selenium" was clinically tested. Its administration brought zinc excretion to norm, but noticeably increased imbalance between copper and zinc in blood.

INTRODUCTION

It is well known that about 2000 enzymes provide metabolism processes in cells and sub cells, and each of these enzymes catalyses specific chemical reactions [1]. Enzyme catalytic activity is determined by coenzymes of non-protein origin. They are organic compounds containing inorganic elements (ions of metals in macro and micro quantities). Chemical elements, which are the most important catalysts for metabolism processes, play significant role in the organism functioning. Any pathology or health disturbance results from the deficit of vital (essential) elements or from the excess both of essential and of toxic microelements [2–4]. For example, hypofluorose (fluorine deficit) today is one of the most widespread pathologies. Cardiovascular disease as well as oncology prevention and treatment are closely related to selenium deficit detection and correction. Zinc is very important for homeostasis, especially for immune, reproductive and central nervous systems. That is why zinc deficit is a serious problem interfering with the normal development of kids and teen-agers. Copper imbalance causes Westphal–Wilson–Konovalov's disease and brain destruction. Copper deficit provides cardiovascular pathologies and hereditary lung emphysema, and requires profound investigation.

Some microelements (*e.g.* F, Cd, Pb, *etc.*) have osteotropic properties, which are of great importance in pathology formation. As a rule, microelement imbalance disturbs physiological processes. Being in excess, copper is proved to inactivate enzymes and destroy native protein conformation due to its interaction with thiol groups [5]. Lead increases the oxidative stress, when interacts with thiols [6]. Zinc, being physi-

ological antagonist to lead, changes the character of lead distribution between organs and tissues, decreasing its content in the skeleton. Cadmium may be considered as specific zinc antimetabolite [2].

Status of microelements in the human organism is a fundamental problem for research in biology and chemistry. By its significance it is comparable with vitamin discovery. Since metal containing compounds are so important for human metabolism, investigation of their functions in organism under norm and pathology is rather promising with regard to the diagnostics, correction and prognosis of human diseases including professional ones. Unfortunately, in spite of quite a few various physical and chemical analysis methods adapted for biological objects, microelements imbalance or deficit diagnostics is still inaccessible for many practicing physicians [7–17].

Every analysis method has its advantages and disadvantages, and may be applied for some particular clinical task. Thus, X-ray fluorescence [7] and photocolorimetry [8] methods have moderate sensitivity, and require preliminary concentration of the most elements to be detected. Highly sensitive neutron activation analysis [9] is rather laborious and expensive. Emission spectroscopy [10] and mass spectrometry with inductively coupled plasma [11, 12] are available only for large research centres in view of the high equipment costs.

At present atomic absorption spectroscopy (AAS) [3, 8, 13–17] is widely used for biologic analysis. However, AAS analysis is also expensive, and thus is not available for the small clinical laboratories. Moreover, all above-mentioned methods require preliminary sample decomposition, which is an important analysis stage being time consuming and affecting the systematic error and thus final analysis results.

Inversion voltammetry (IVA) is known to be a sensitive, selective, universal and simple method for analysis procedure and data processing. Unlike other methods it is performed with inexpensive portable devices [18].

Applying modified carbon based sensors we managed to develop the techniques for analysing such biological liquids as whole blood and its fractions [19, 20], urine [21] for the content of Cu^{2+}, Zn^{2+}, Pb^{2+} and Cd^{2+}. These techniques require no preliminary sample mineralization, and save labour time, simplify analysis procedure and improve analysis reliability and accuracy. As a unit with portable inversion voltammeter analyser IVA-5 (NPVO IVA-5, Ekaterinburg), this equipment may be used *at site*, *i.e.* at a patient's bed.

In the present study we compare the content of microelements (copper, zinc, lead and cadmium) in the biological liquids of healthy people and of patients suffering from Hodgkin's lymphoma, using the IVA technique with methods described in [19–21].

EXPERIMENTAL

For the purpose four groups of Novosibirsk citizens were examined. Group I included 52 practically healthy volunteers in the age of (32.8±14.2), among them 28 women, 24 men with no hereditary diseases, allergy and chronic infection. Group II was represented by patients in the age of (37.7±13.3) with Hodgkin's lymphoma in a period of steady clinical haematology remission after many times repeated cytostatic therapy. Group III (control) united 23 people from the first group following ordinary diet and water feed (average age 35.4±10.6 years). Control group III was arranged to be examined simultaneously with group II. Group IV united 5 HL patients subjected to polychemotherapy.

Samples of whole blood, blood fractions (serum, plasma and erythrocyte mass) and urine were analysed. Vein blood in amount of 0.5–1.0 ml was put in a cylindrical test tube containing anticoagulant (5 % EDTA or 4 % glugycir solutions in amount of 0.04 and 0.25 ml per blood millilitre, respectively). Blood with anticoagulant was centrifuged (1500 rpm) for plasma and erythrocyte mass separation. Then ruler was used to measure the ratio between the plasma (liquid) and mass (sediment) volumes. If separation was indeed completed, plasma was easily removed. Serum was isolated by sedimentation of blood heated to 37 °C without coagulant or by blood centrifuging (1500 rpm).

Sample volumes of 0.005–0.100 ml (whole blood), 0.050–0.200 ml (plasma or serum), 0.005–0.050 ml (erythrocyte mass diluted with physiological solution by half) are considered as quite representative for securing reliable analysis results. For elements content in whole

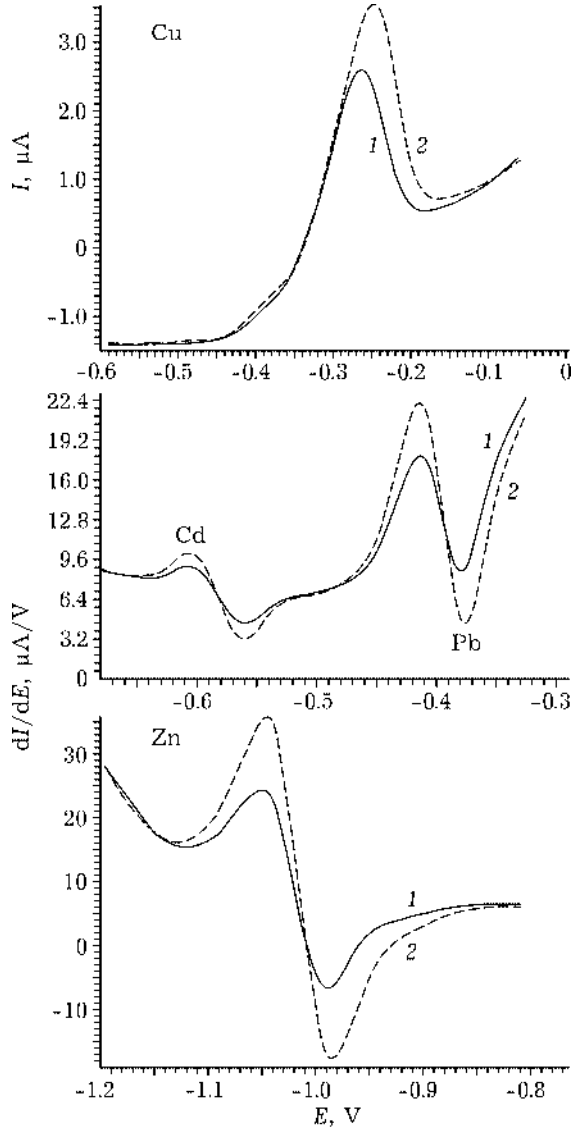

Fig. 1. Typical inversion volt-ampere plots of copper, lead, cadmium and zinc in the whole blood samples (reference electrode Ag–AgCl (3 M KCl); E_{el}, V: −1.0 (Cu), −1.2 (Pb, Cd), −1.4 (Zn); t_{el}, s: 60 (Cu, Pb, Cd), 20 (Zn); v = 0.5 V/s, sample volume 0.05 ml (zinc detection) and 0.10 ml (copper, lead and cadmium detection), background electrolyte volume 10 ml): *1* – no additions, *2* – with additions of ions Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+} in amount of 15.0, 0.2, 1.0, 40.0 μg/l, respectively.

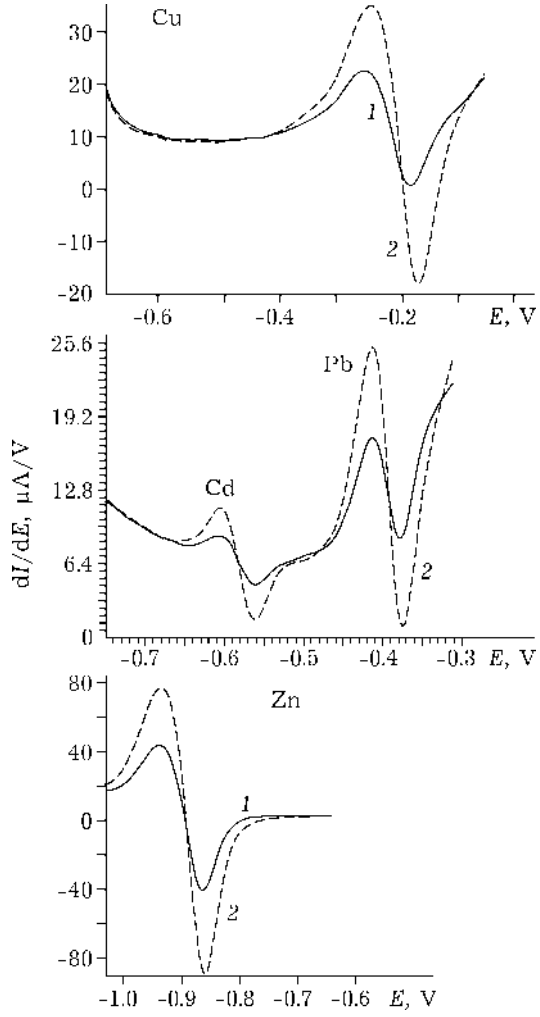

Fig. 2. Typical inversion volt-ampere plots of copper, lead, cadmium and zinc in urine samples (reference electrode Ag–AgCl (3 M KCl); E_{el}, V: −0.8 (Cu), −1.2 (Pb, Cd), −1.4 (Zn); t_{el}, s: 60 (Cu, Pb, Cd), 20 (Zn); v = 0.45 V/s; sample volume 5 ml, background electrolyte volume 0.01 ml at zinc detection, and 0.40 ml at copper, lead and cadmium detection): *1* – no additions, *2* – with additions of ions Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+} in amount of 12.0, 2.0, 10.0 and 100.0 μg/l, respectively.

blood and its fractions to determine, sample was introduced in an electrolytic cell (5 ml) preliminarily filled with a certain electrolyte solution. Average value for three parallel samples was taken as a programmed analysis result. Measurement error was never larger than 5 and 7 rel. % for blood (blood fractions) and urine, respectively. In the case of blood and its fractions, the solution 0.02 M HCl + 0.04 M NaCl was used as a background electrolyte to determine zinc content, and 0.5 M HCl solution served for the copper, lead and cadmium content measurements.

Typical inversion volt-ampere plots obtained at the whole blood analysis for the Zn, Cd, Pb and Cu content are given in Fig. 1. Similar plots were obtained at the blood fractions analysis, though analysis conditions were different.

For urine analysis, the sample alone was operating as a background electrolyte, for which purpose this samples was were simply acidified with the 0.003 M HCl solution (zinc content analysis) or with the 0.7 M HCl (copper,

lead and cadmium analysis). IVA plots resulting from the urine analysis are given in Fig. 2.

Analysis accuracy was checked regarding convergence, reproducibility and analysis error. For error estimation we used independent methods, as well as method of additions together with dilution. Analysis results were statistically processed: average ($\overline{X}$) and mean square deviation (S) were determined. Student t-criterion method was used to compare the

TABLE 1

Concentration of Zn^{2+}, Cu^{2+}, Pb^{2+}, Cd^{2+} in various biological media of healthy people (average age 32.6 years, P = 0.95), μmol/l

Element (n)*	Biological medium	$\overline{X}_{exp} \pm tS$	Reference data	
			X_{ref}	$\overline{X}_{ref} \pm S$
Zn (50)	Whole blood	117.8 ± 18.2	114.6–122.3 [3], 134.6 [13], 116.7 [22], 107.0 [23], 136 [24], 138.4 [25], 110.4 [26], 106.3 ± 16.5 [28], 88.7–125.1 [29], 91.7–122.3 [32]	116.5 ± 15.7
	Plasma	39.2 ± 9.9	45.87 [13], 28.6 [24], 47.4 [25], 45.87 [31], 17.14 ± 1.86 [32]	37.0 ± 13.4
	Erythrocyte mass	201.4 ± 18.6	184–198 [8], 194.2 [24], 124–192 [32]	178.4 ± 30.9
	Serum	18.4 ± 2.1	10.7–22.9 [8], 10.79 ± 1.96 [30], 17.95 [32], 13.8–32.6 [33], 14.7 [34], 18.3–35.1 [37]	19.6 ± 8.9
	Urine	6.1 ± 1.5	<12.2 [8], 3.1–6.1 [13], 5.7 [32], 6.73 ± 0.8 [36]	7.7 ± 3.0
Cu (47)	Whole blood	12.9 ± 2.0	11.2–19.8 [3], 11.0–23.6 [8], 14.5–15.3 [13], 15.6 [22], 15.9 [23], 15.4 [24], 14.8 [25], 19.5 [26], 11.3–15.07 [27], 16.85 ± 1.89 [28], 12.6 [29], 16.53 ± 3.67 [30], 15.7–21.4 [37], 12.92 [38], 17.5 [39], 13.6 [49]	15.1 ± 3.0
	Plasma	12.4 ± 2.4	18.3 [29], 15.7 ± 0.6 [36], 8.2–18.9 [37]	15.3 ± 4.9
	Erythrocyte mass	13.9 ± 1.6	14.13–23.55 [8], 15.75 [24], 18.9 [25], 15.7–23.6 [37]	18.6 ± 4.1
	Serum	15.3 ± 2.3	11.0–23.6 [3], 11.8–23.1 [8], 18.1 [29], 12.2–23.2 [32], 17.53 [34], 15.09 [35], 11.0–22.0 [40], 11.0–24.4 [41]	17.2 ± 5.5
	Urine	0.38 ± 0.09	0.16–0.31 [8], 0.05–0.36 [13], 0.16–0.53 [32], 0.31 ± 0.05 [36], 0.66 [42]	0.32 ± 0.20
Pb (52)	Whole blood	0.78 ± 0.31	1.21–1.45 [3], 0.96–1.45 [13], 1.01 [23], 0.42–0.70 [27], 0.23 ± 0.14 [28], 0.20 [29], 0.34–0.92, 1.46–1.93 [32], 0.57 [38], 1.06 [43], 1.35 [44], 0.67 [48], 0.56 [49], 0.77 ± 0.05 [50]	0.94 ± 0.52
	Plasma	0.13 ± 0.05	0.23 [44]	0.23
	Erythrocyte mass	1.47 ± 0.25	2.6 [44]	2.6
	Serum	0.04 ± 0.02	No evidence	–
	Urine	0.075 ± 0.040	0.05–0.36 [13], 0.11 ± 0.07 [43], 0.14 [36], 0.04–0.11 [45]	0.13 ± 0.12
Cd (49)	Whole blood	0.037 ± 0.022	0.013–0.036 [3], 0.0088 ± 0.0084 [28], 0.0063 [29], up to?? 0.107 [34], 0.017 [38], <0.045 [39], 0.038–0.12 [46], 0071 [48], 0.019 [49], 0.014 ± 0.001 [50]	0.041 ± 0.038
	Plasma	0.008 ± 0.004	No evidence	–
	Erythrocyte mass	0.069 ± 0.012	« «	–
	Serum	$<1 \cdot 10^{-3}$	« «	–
	Urine	0.067 ± 0.042	Up to 0.36 [13], 0.060 ± 0.021 [43], 0.039–0.11 [45], 0.082 – 0.20 [46]	0.14 ± 0.12

Note. Average volume fraction of erythrocyte mass α = 0.48 units (α = V_{er}/V_b, where V_b is blood volume, ml; V_{er} is volume occupied by erythrocyte mass after blood centrifuging, ml).

*In parentheses we show the number of people, whose analyses were taken for statistical estimates.

average values and reliability of data obtained in different groups.

RESULTS AND DISCUSSION

Microelements and helthy people

Table 1 presents the concentrations of copper, zinc, lead and cadmium in the blood, blood fractions and urine of practically healthy people (group I).

We have compared these values with the reference ones. Average reference values ($\overline{X}_{ref}$) were considered as norm. Apparently, zinc content in blood, blood fractions and urine is normal for the 96 % of donors. However, in some cases we observed essential deviation. For example, zinc content in the blood of donor No. 4 is at the lowermost norm limit ((102.4±5.1) μmol/l, n = 6), but exceeds norm in urine ((11.2±0.2) μmol/l, n = 6). This discrepancy may be related to some pathology, which requires supervision and diagnostics. Essential excess of zinc in the whole blood ((204.06±8.2) μmol/l, n = 6) is found in donor No. 16, but such content is typical solely for the erythrocyte mass. According to particulars for the whole month this donor (18 year old man) took zinc containing vitamin complex each time before blood analysis. Analysis (repeated three weeks after vitamins intake was completed) indicated that zinc content in whole blood was at a level of (121.6±4.9) μmol/l (n = 6, P = 0.95), which is quite normal. As for copper, average copper content in the whole blood and its fractions was at the lowermost norm limit for 90 % of the inspected patients (see Table 1).

Urine is a more complex object for investigation. Therefore, there are a few methods for determining the content of microelements. According to the reference data copper and zinc concentration in the urine of healthy people varies within 0.16–0.66 and 3.1–12.2 μmol/l, respectively (see Table 1). Our results ((0.38±0.09) and (6.1±1.5) μmol/l, for copper and zinc, respectively) fit well into the context of data cited. Excretion efficiency (kidney excretory function factor), calculated as the ratio of average element content in urine to that in blood ($\overline{X}_u/\overline{X}_b$), for group I is 0.052 and 0.031 for zinc and copper, respectively. In ref. [2, p. 42] this factor is reported to be 0.045. Excretion efficiency, calculated according to other data, is 0.013–0.015 [8] and 0.034–0.047 [13] for copper and 0.034–0.068 [13] and 0.047–0.062 [32] for zinc.

As for the reference data related to lead and cadmium concentrations in healthy people whole blood, they are widely spread ((0.94±0.52) and (0.11±0.08) μmol/l for lead and cadmium, respectively (see Table 1). Our investigations show that in the blood of non-smoking healthy people (group I) lead and cadmium concentrations vary within 0.47–1.09 and 0.005–0.059 μmol/l, respectively. Higher cadmium concentration in the blood of donors Nos. 9, 23, 24 (0.14, 0.15, and 0.21 μmol/l, respectively) is related to the number of cigarettes smoked daily: donor No. 24 has a long smoking history (two packs of cigarettes daily), others smoke two packs of cigarettes in three days. Let us note that cadmium content, which we consider to be above the norm, was approved as normal by the USSR Ministry of Health [43]. We think that the norm presented by the Ministry of Health is not enough reliable, since the cited cadmium norm for blood (27 μg/l or 0.24 μmol/l) is given with no metrological performance. We have similar situation with lead: its normal content in blood (220 μg/l or 1.06 μmol/l) is cited without confidence interval, sex and age differentiation, *etc*. Lead and cadmium concentrations in the urine of non-smoking donors of group I do not exit reliability range of norms and are (0.075±0.040) and (0.067±0.042) μmol/l, respectively (see Table 1). Excretion efficiency for lead and cadmium is 0.09 and 1.81, respectively, and so agrees with the values calculated from the data of other researchers: 0.031–0.16 [13], 0.104 [43] for lead and 2.0 [3], 1.67–2.15 [46] for cadmium. A wide spread of data (50–60 %) related to lead and cadmium in blood and urine in comparison to the data for copper and zinc (15–25 %) is most likely caused by the insufficient organism ability to maintain the stable homeostasis of lead and cadmium [2, 3]. Additional complex investigation by analysts and clinic physicians is required to confirm this assumption.

Same method application with the same measurement error applied for the fractional analysis of blood may help a lot in providing

very important information related to elements distribution over blood fractions at pathology. Moreover, fractional analysis allows a simultaneous estimation of results reliability. Results are taken as reliable, if they follow equation $\overline{X}_b = \alpha\overline{X}_e + (1 - \alpha)\overline{X}_p$, where $\overline{X}_b$, $\overline{X}_e$, $\overline{X}_p$ are the calculated average values of element concentration in the whole blood, erythrocyte mass and plasma, and α is the volume fraction of erythrocyte mass. This equation, derived from the data of Table 1, is fulfilled for all microelements. For example, for zinc $\overline{X}_b = 117.1$ μmol/l. This value corresponds to zinc content in the whole blood with an error less than 2 % (see Table 1).

According to the blood fractional analysis, 82 % of zinc and 90 % of lead and cadmium present in the whole blood are located in erythrocytes (see Table 1). Copper distribution over fractions is rather uniform (52 % in erythrocytes). Unfortunately, there are few publications related to the elements distribution over blood fractions, since there are no analysis methods providing such information. Monographs [2, 3] only mention that more than 90 % of lead and cadmium are located in erythrocytes.

We have found some imbalance between copper and zinc due to the noticeable copper deficit in blood. Ratio Cu/Zn equals 0.109 and 0.83 for the whole blood and serum, respectively. According to the average values of copper and zinc concentrations in the whole blood and serum, calculated from the reference data (see Table 1), ratio Cu/Zn equals 0.130 and 0.88, respectively. However, specialized research related to healthy people evidences that this ratio is 0.108–0.114 for the whole blood [13], and 0.95 [35] and 0.83 [47] for serum.

Microelements and Hodgkin's lymphoma

Charts, characterizing the content of copper, lead, zinc, cadmium in the blood and urine of patients from groups II and III, are shown in Fig. 3. Table 2 lists the corresponding average values, calculated from the results presented in Fig. 3. Apparently, a set of microelements is like a "visiting card" for every patient as well as for every biological liquid under study. According to the data of Fig. 3, 69 % of HL patients and 74 % of healthy people have blood with copper content ranging within 9.0–15.5 μmol/l. At the same time there are some deviations. For example, in the blood of volunteer No. 3 copper content (23.6±1.2) μmol/l essentially exceeds the average value for healthy people (see Tables 1, 2). In the blood of volunteer No. 18 copper content is the lowest (7.1 μmol/l). Most likely these anomalies are caused by some pathology, which requires further investigation for the more precise diagnosis. The lowest copper concentration in the blood of HL patients (6.05 μmol/l) as well as the lowest organism adaptation reserves are observed for patient No. 3. In the blood of patients Nos. 5, 16 and 18 copper content exceeds 17 μmol/l. Moreover, we observe the buffer capacity of antioxidant organism protection system to decrease and thioldisulphide coefficient to lower to 2.2–2.6. These facts evidence the exhaustion of organism reserves. Average copper concentration in the blood of HL patients [(12.6±3.1) μmol/l] insignificantly differs from that for healthy people (see Tables 1, 2). Results deviation does not exceed 24 % for group II (HL patients) and 18.5 % for groups I and III. At the same time for 91 % of HL patients the copper excretion efficiency is 0.014, which is reliably lower ($p < 0.05$) than that for the healthy people from group III (see Table 2).

After "Nutricon-Selenium" administration some 50 % of HL patients show the sharp growth of copper content in blood: average value is 15.9 μmol/l (see Table 2). Simultaneously we observe the decrease of excretion efficiency (average value 0.010), which most likely is the reason for the copper content growth related to the "Nutricon" administration during four weeks.

With the exception of patient No. 20 (see Fig. 3, *c*), whose blood showed abnormally high zinc concentration, for all HL patients average zinc content in blood is reliably lower and in urine reliably higher ($p < 0.05$) than in the blood and urine of patients from group III (see Table 2). Therefore, calculated zinc excretion efficiency is also more than twice higher than similar index for healthy people.

After "Nutricon" receiving for one month the spread of zinc content in blood increased from 12.7 to 22 % (see Fig. 3, *c*). For most patients

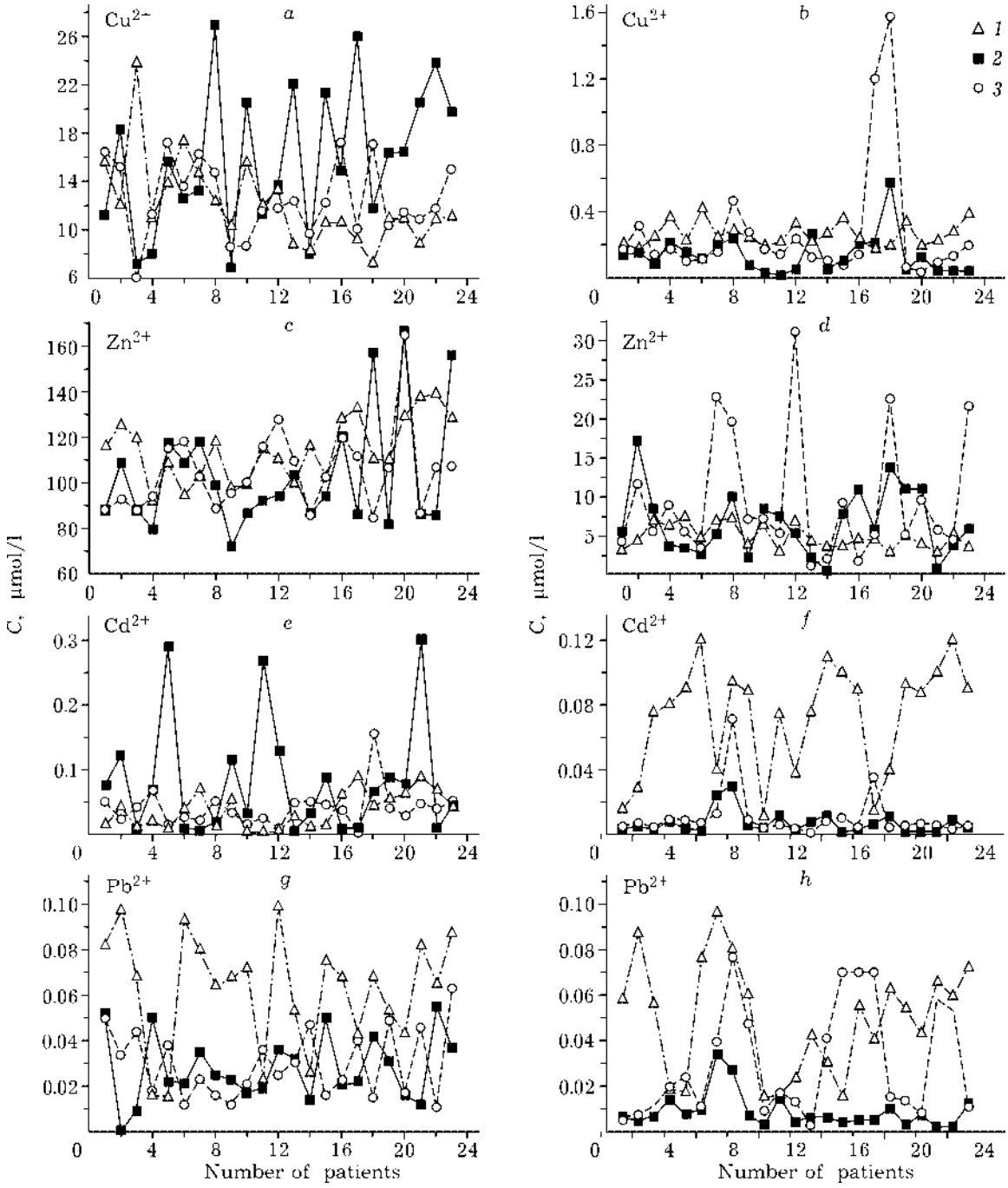

Fig. 3. Microelements content in the samples of blood (*a*, *c*, *e*, *g*) and urine (*b*, *d*, *f*, *h*) of healthy people (*1*) and Hodgkin's lymphoma patients before (*2*) and after (*3*) receiving of "Nutricon-Selenium".

(87 %) average zinc content in blood decreased to 100.7 μmol/l. The only exception was patient No. 20, whose blood zinc content remained unchanged. Blood of patients Nos. 18, 23 was also analysed, and analysis results were included into calculations. After these patients received "Nutricon", zinc content in their blood became close to zinc concentration in erythrocyte mass and attained 157 and 156.2 μmol/l, respectively [39]. Let us note that patients Nos. 18 and 23 are the eldest in group II (44 and 46 year old, respectively). They suffer from hypercholesterolemia index and elevated ESR (erythrocyte sedimentation rate). Besides, patient No. 23 suffers from ischemia and high blood pressure. Patient No. 20 was found to have pancytosis (leukocytosis – up to $20.3 \cdot 10^9$ corp./l and erythrocytosis – $9.17 \cdot 10^{12}$ corp./l), and statistically doubt-

TABLE 2

Average values of microelement status (Zn, Cu, Pb, Cd) in the blood and urea of HL deceased patients in the phase of steady clinical-haematology remission before and after receiving "Nutricon" *versus* relatively healthy people ($n = 23$, $P = 0.95$), μmol/l

Index	Sample	HL patients				Control group	
		before "Nutricon" receiving		after "Nutricon" receiving			
		$\overline{X} \pm S$	$\overline{X}_u/\overline{X}_b$	$\overline{X} \pm S$	$\overline{X}_u/\overline{X}_b$	$\overline{X} \pm S$	$\overline{X}_u/\overline{X}_b$
Cu	Blood	12.6 ± 3.1	0.014	15.9 ± 6.0	0.010	12.1 ± 3.5	0.022 0.013–0.015 [8]
	Urine	0.17 ± 0.09		0.14 ± 0.11		0.27 ± 0.05	0.034–0.047 [13]
Zn	Blood	102.7 ± 13.1	0.094	100.7 ± 22.3	0.066	117.6 ± 11.9	0.041 (0.045 [3])
	Urine	9.7 ± 8.1		6.7 ± 4.3		4.8 ± 1.5	0.034–0.068 [13], 0.046–0.062 [32]
Cu/Zn (bl.)		0.120		0.153		0.103 0.108–0.114 [13]	
Pb	Blood	0.30 ± 0.15	0.10	0.29 ± 0.14	0.028	12.1 ± 0.25	0.078 0.031–0.17 [13]
	Urine	0.030 ± 0.025		0.008 ± 0.007		0.27 ± 0.025	0.104 [43]
Cd	Blood	0.041 ± 0.029	0.27	0.091 ± 0.082	0.08	117.6 ± 0.027	1.95
	Urine	0.011 ± 0.009		0.007 ± 0.009		4.8 ± 0.034	2.0 [3], 1.67–2.15–3.17 [46]

ful drop in these indices against a background of "Nutricon" administration. Average zinc excretion also decreased (from 0.094 to 0.066) but not to the level of control group III (see Table 2). Owing to the pronounced deficit of zinc in the blood of HL patients, average ratio Cu/Zn reliably increased ($p < 0.05$) in comparison to that for groups III and I (0.120 against 0.103 and 0.109, respectively, see Tables 1, 2).

Copper *versus* zinc imbalance is even more pronounced in the serum of HL patients (Table 3). With this regard our results agree with the reference data. Thus, copper *versus* zinc imbalance towards increasing Cu/Zn in the blood serum of patients suffering from larynx cancer was observed in [35]. It has been found that Cu/Zn ratio changes from 1.34 to 1.42 depending on cancer stage, while for the control group it was 0.95. According to [47], Cu/Zn ratio in the blood serum of children (40 patients) suffering from progressing lymphoma is higher in comparison to that for healthy people (2.46 and 0.83, respectively). Although exact mechanisms of this phenomenon remain unstudied yet, authors [47] have come to conclusion that copper and zinc concentrations in blood serum as well as their ratio are easily measurable biochemical indices that may be used for diagno-

TABLE 3

Copper and zinc content in blood serum (μmol/l) and ratio Cu/Zn for relatively healthy people ($n = 6$) and Hodgkin's lymphoma patients ($n = 5$, $P = 0.95$)

Index	Norm	HL patients		Control group	
		$\overline{X}$	S	$\overline{X}$	S
Cu	17.4	18.6	3.5	15.8	1.3
Zn	20.7	11.2	2.6	18.5	1.1
Cu/Zn	0.84	1.66 (1.42 [35], 2.46 [47])		0.85 (0.95 [35], 0.83 [47]	

TABLE 4

Dynamics of copper and zinc content in the blood and ratio Cu/Zn for Hodgkin's lymphoma patients subjected to polychemotherapy before and after treatment with enterosorbents against the background of polyvitamins ($n = 5$, $P = 0.95$)

Index	$\bar{X} \pm S$, μmol/l		
	Donors	HL patients	
		before treatment	after treatment
Cu	12.6±1.2	13.6±1.8	12.4±1.4
Zn	118.3±11.4	92.9±8.2	112.6±13.1
Cu/Zn	0.106	0.146	0.110

sis prediction and microelements imbalance correction for patients with lymphomas. Obviously, copper and zinc content in blood may also be used for diagnosis prediction and effective treatment of patients with blood oncology. This is confirmed by investigation of copper and zinc content in the blood of HL patients subjected to polychemotherapy (group IV) before and after treatment with modified carbon adsorbent (SUMS-1) against a background of polyvitamins administration (Table 4).

Average concentration of cadmium in blood is slightly higher ($p > 0.05$) than that for the control group, and cadmium urination ($\bar{X}_u/\bar{X}_b = 0.27$) is more than 7 times less efficient for sick people than for healthy people (1.95) (see Fig. 3, *c*, and Table 2).

Quite suddenly, we observe lead deficit in blood, and its lower renal excretion for HL patients (see Fig. 3, *d*, and Table 2). Up to now, this element attracts interest in medicine and biology uniquely as a cumulative poison, though its vital necessity for the living organism was proved in 1974 and confirmed in a set of investigations in 1984 [2]. However, mechanisms of the toxic and physiological effects of lead are still unclear, and remain worse studied than those of other vital microelements. Nevertheless, there are reference data that such diseases as hepatitis, rheumatism and leucosis, are accompanied by the lead deficit in the blood plasma [32]. It is quite probable that a similar statement is true for the HL patients as well.

In order to elucidate the action of disease on the distribution of microelements between plasma and erythrocytes we have compared the blood fractional analysis data of two women (sick and healthy) of the same age (31 years). It has been found that erythrocytes of the healthy woman contain 52 % of copper, 80 % of zinc, 89 % of lead and 91 % of cadmium, which is similar to the same indices of other healthy people (group I). Erythrocytes of HL patient reliably ($p < 0.05$) contain more copper (58.6 %), lead (94 %), cadmium (98 %) and less zinc (70.5 %, $p < 0.02$). The following regularity is observed: a decrease in the localization of the element in erythrocytes is accompanied by an increase in its excretion efficiency, and *vice versa*.

CONCLUSION

Inversion voltammetry method appears to be a highly sensitive and informative way for the quantitative investigation of microelements pathways in human organism. It allows the real time analysis (30–120 min) of such biological liquids as whole blood, plasma, erythrocyte mass, serum and urine. It has been found that zinc, lead and cadmium content in the blood, blood fractions and urine of healthy people is comparable with standard norms, while copper content in blood and its fractions are at the lowermost norm limit. In comparison to healthy people, patients with Hodgkin's lymphoma at the stage of clinical-hematological remission are characterized by the following microelement indices: zinc deficit, cadmium excess, reliably higher ratio Cu/Zn, zinc excretion is higher, but cadmium renal excretion is lower.

Low zinc content and cadmium accumulation in erythrocytes may serve as diagnostic criterion for blood oncology. Conceivably the more pronounced study of microelements "portrait" might help in formulating criteria for the prognosis of diseases at the different stages of their development and revealing the prospects for prevention and correction of metabolism imbalance.

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