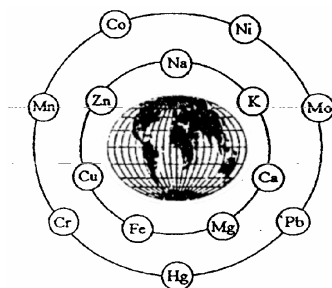


**Metal Elements
in Environment,
Medicine and Biology**

Tome III

ROUMANIAN ACADEMY
BRANCH TIMIȘOARA



GÂRBAN ZENO, DRĂGAN PETRU
EDITORS

Metal Elements in Environment, Medicine and Biology

Tome III

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ZENO GÂRBAN, PETRU DRĂGAN
EDITORS

**METAL ELEMENTS IN ENVIRONMENT
MEDICINE AND BIOLOGY**
Tome III

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FOREWORD

Fundamental and applicative investigations concerning the importance of metals and their role in the environment, medicine and biology constitute a major direction in the contemporary sciences being a domain of great interest.

Nowadays many interdisciplinary studies are dealing with the distribution of metals in lithosphere, hydrosphere and atmosphere as well as with their implications in the biosphere. In this context there are mentioned the researches on biometals (Ca, Mg, Na, K, Cu, Zn, Fe a.o.) with role in the vegetal and animal kingdom and in the human organism as bioconstituents and/or as biochemical effectors (activators / inhibitors) and on metals with toxicogene potential (Pb, Hg, Cd, Al, Sn a.o.).

The International Symposiums with the topics "Metal Elements in Environment, Medicine and Biology" (M.E.E.M.B.) have a large inter- and multidisciplinary addressability by their themes being important for ecology, nutrition, pharmacology, biotechnology, pathology, surgical plasties, toxicology etc.

Roumanian Academy by its vice-president Acad. Prof. Nicolae Cajal, MD, PhD approved and encouraged in 1993 the initiation of the series of M.E.E.M.B. symposiums. Later on his successors, the vice-presidents Acad. Prof. Alexandru Balaban, PhD, Eng., and Acad. Ionel Haiduc, PhD gave valuable suggestions for the continuation of the symposium series. An important role in the orientation of researches and organizing the meetings in this domain was accorded by Acad. Prof. Pius Brânzeu, MD, PhD – member of the Roumanian Academy, Section of Medical Sciences. Also, the interest of Acad. Prof. Ionel Haiduc, PhD – scientific authority with vaste international contribution in the domain of organometallic compounds – the honorary president of the 3rd International Symposium M.E.E.M.B. '98, must be mentioned.

The Biochemical Commission of the Roumanian Academy – having as president Prof. Mihai Șerban, PhD-corresponding member of Roumanian Academy - stimulated also the implementation of these symposium series. The Foreword of the Proceedings Volume of the First M.E.E.M.B. Symposium '93 was written by the president of the Biochemical Commission.

Roumanian Academy - Branch Timișoara had its contribution to the organization of these symposiums, too. The first symposium was organized with the help of the University of Medicine and Pharmacy Timișoara and of the University of Agricultural Sciences and Veterinary Medicine Timișoara. Later on the cooperation was extended also to the University "Politehnica" Timișoara and the West University Timișoara. These symposiums had as participants the teaching staffs of universities from Timișoara and scientists from some research institutes from Timișoara dealing with studies in this domain.

At the symposiums with the topics M.E.E.M.B. participated specialists from various universities and research institutes, both from Roumania and abroad, involved in interdisciplinary studies concerning metal elements

To organize these series of scientific meetings started from the existence of a "Working group for the research of metals in environment, medicine and biology" founded in Timișoara around the years '79 and orientated predilectly to the study of

the interactions metals-proteins (especially nucleic acids), embryopharmacotoxicology (of cis-platinum and various metal ions) and to finding out the role of metals in the biogenesis of uroconcrements being of interest for biochemistry and biochemical pathology. The "Working group for the research of metals" functioned initially in the Institute of Public Health and Medical Research Timișoara-Laboratory of Embryology belonging to the Academy of Medical Sciences Timișoara and in the University Clinic of Urology from the University of Medicine and Pharmacy Timișoara.

Extended and thoroughly interdisciplinary researches in the domain of metals made possible to include this area studies in departmental themes - in the period 1980-1990 - of the National Program for research and technological development of the Academy of Medical Sciences Bucuresti.

Each Symposium with the topics "Metal Elements in Environment, Medicine and Biology" contributed to the enlargement of the international scientific collaboration, to the diversifying and to the profounding of the inter- and multidisciplinary themes bringing new progress in this domain.

The remarked aspects make us to believe that the perpetuation of symposiums with the topics M.E.E.M.B. will focus the interest of an increasing number of well known scientists as well as of young researchers in this domain. With these sentiments, we wish future success for these series of scientific symposiums.

*Acad. Prof. Toma Dordea, PhD, Eng.
President
Roumanian Academy-Branch Timișoara*

PREFACE

The Symposium "Metal Elements in Environment, Medicine and Biology" - being at the third edition - continues the arise the interest of the scientific community from at least two distinct reasons of joining the desideration of fundamental and applicative researches.

A first aspect is represented by the inter- and multidisciplinary horizon of the moderne coceptions reffering to the mechanisms of metals translocation which stay at the base of the so-called trophic chain soil-plants-animals-humans. In this context the problems have in view biochemical and pathobiochemical, physiological and pathophysiological as well as morphological and morphopathological aspects.

The second aspect is related also to the specific inter- and multidisciplinary character of the theme but in the acceptance of the applicative sphere with practical problems involving the habitual environment of humans (i.e. soil, water, air), macro- and micronutrients, chemotherapeutical products (for human and veterinary use), pathological processes, materials utilized in prothesis and implantology, different residues with metal content discharged in the environment etc.

Research on metals permit to approach the problem of bioconstituents represented by various metal compounds, the metals represented by biochemical effectors (activator / inhibitors), as well as the metabolic processes involving metals. Such an approach make possible to investigate the chemical structure-biological activity relationship in the homeostatic context of the blood and tissular metallograms as well as of the pathological implications.

There is also a reverse side of this problem: situation in which the metallic compounds (in facto the metal ions) are of interest for using them as food supplements and as chemotherapeutical products. Such situations may appear in nutritional and metabolic diseases (dysmineralosis), in cytostatic chemotherapy (e.g.: use of cis-platinum), in endocrinopathies (e.g.: use of restriction of metallic compounds as drugs and nutrients), in cardiovascular diseases (e.g.: use of magnesium containg compounds) etc. One can mention also iatropathies in the case of an excess of metal containing chemotherapeutical products.

The editors express their gratitude to all prestigious personaities for their support during the series of symposiums with the topics "Metal Elements in Environment, Medicine and Biology" (M.E.E.M.B.). In this context they thank to Acad. Prof. Nicolae Cajal MD, PhD, vice-president of Roumanian Academy in 1993, for his helpful indications beginning on the first symposium and the suggestion of continuity and to Acad. Prof. Pius Brânzeu MD, PhD – member of the Roumanian Academy, Section of Medical Sciences, for continuous help in the development of this research domain.

Also, they are indepted to Acad. Prof. Aelxandru Balaban PhD, Eng. and Acad. Prof. Ionel Haiduc PhD, who successively – as vice-presidents of the Roumanian Academy – who encouraged and supported this manifestation.

The 3rd symposium benefited by the participation of the vice-president of the Roumanian Academy – Acad. Prof. Ionel Haiduc PhD as honorary president of this symposium. We express the whole gratitude to Acad. Prof. Ionel Haiduc PhD - recognized personality of the international scientific life with remarkable contributions in the domain of organometallics study – for the support given to this scientific meeting, for the approach to the topics of bioinorganic chemistry (inorganic biochemistry) and of the interrelations from this remarkable domain by the large thematic area and multidisciplinary relations.

The symposium benefited by the help of Acad. Prof. Toma Dordea PhD, Eng. – President of the Roumanian Academy-Branch Timișoara, Prof. Păun Ion Otiman PhD, Eng., corresponding member of the Roumanian Academy – rector of University of Agricultural Sciences and Veterinary Medicine Timișoara, Prof. Ioan Carțiș PhD, Eng., member of the Academy of Technical Sciences – rector of University “Politehnica” Timișoara, personalities with solicitude and opening for cooperation ascertaining the location of symposium and pertinent suggestions in the scientific and organizing problems. The fruitful collaboration with the rectorate of University “Politehnica” assured the efficiency and the prestige of this Symposium. Also, we would like to express our gratitude for technical help to Prof. Gheorghe Ciuhandu PhD, Eng. – Mayor of town Timișoara. For constant encouraging of the series of M.E.E.M.B. symposiums we express our thank also to Acad. Prof. Emanuel V. Sahini PhD and to Acad. Prof. Gheorghe Zarnea PhD.

In this preface we will reiterate the contribution of prestigious schools with international recognition in the study of metals – in time and space – as reference scientific forum for such researches. In this context we emphasize the contribution of the following schools: G. Eichhorn et al. (Bethesda-U.S.A); J.E. Underwood (Nedland-Australia); A.Prasad (Michigan-U.S.A.); M.Anke et al. (Jena-Germany); L.G. Marzilli (Atlanta-U.S.A.); C.F. Mills (Aberdeen-United Kingdom); J. Durlach et al. (Paris-France); H. Sigel (Basel-Switzerland); J.Mc. Howell (Perth-Australia); V. Ferm (New Hampshire); P.J. Sadler (London-United Kingdom); M. Gielen (Brussels-Belgium); R. Smetana (Vienna-Austria); T. Theophanides (Athens-Greece); Joan Silverstone Valentine (Los Angeles-U.S.A.); G. Chazot (Lyon-France); I. Pais (Budapest-Hungary); A.S. Kiss (Szeged-Hungary); M.J. Halpern (Lisbon-Portugal); P.F. Zatta (Padova-Italy); M. Abdulla (Stockholm-Sweden); S. Ermido-Pollet and S. Pollet (Athens-Greece); T. Itp (Tohoku-Japan); I. Haiduc (Cluj-Napoca-Roumania) a.o.

Special thanks are addressed to Msgr. Sebastian Kräuter – bishop of the Roman Catholic Diocese Timișoara, who made possible to organize in the Roman-Catholic Cathedral an Organ recital and Vocal-organ concert for the participants of the symposium. Also, we thank to Msgr. Martin Roos-director of the Bishopric Office of the Roman-Catholic Diocese of Timișoara, and to Pr. Lázló Böcskey-secretary of Bishopric Office of the Roman-Catholic Diocese of Timișoara for their help. The concert was performed by Lecturer Teodora Ciucur, soprano of the Opera Timișoara and assistant Marcela Costea from the Faculty of Music, West University Timișoara. Also, we mention also the contribution of the cathedral’s chapel master Prof. Walter Kindl PhD, from the Faculty of Music, University Timișoara.

At the opening of the symposium’s works brief speeches were presented by Prof. Gheorghe Ciuhandu PhD, Eng.-rector of the University “Politehnica” Timișoara, university wich hosted the symposium, Acad. Prof. Toma Dordea PhD, Eng. on behalf of the Roumanian Academy-Branch Timișoara and Acad. Prof. Ionel Haiduc PhD, vice-president of the Roumanian Academy, on behalf of Roumanian Academy.

Finally, we wish to thank all the students from University “Politehnica” Timișoara, University of Agricultural Sciences and Veterinary Medicine Timișoara, University of Medicine and Pharmacy Timișoara and West University of Timișoara who helped us in different moments for a successful organization of the Symposium.

Timișoara, December 10, 1998

Gârban Zeno
Drăgan Petru

BIOLOGICAL ESSENTIALITY AND TRANSFER OF CHROMIUM IN THE FOOD CHAIN – DOES CHROMIUM DEFICIENCY EXIST IN EUROPE?

Anke M., Glei M., Dorn W., Illing-Günther Heike, Holzinger Sylvia, Jaritz M.,
Anke Sabine, Arnhold W., Hartmann Esther, Lösch Edda,
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ABSTRACT

Chromium is taken up by plants according to its geologically or anthropogenically induced occurrence in the soil. According to the present level of knowledge, it is not essential for the flora, it accumulates in leaves and seed husks as well as in barks and twigs. The Cr content in starch- and sugar-rich cereals, pasta, bread, cakes and pastries was between 80 and 400 µg/kg dry matter. Vegetables are the main supplier of chromium (250 to 1300 µg/kg dry matter). The Cr content in plant foods increased between 1988 and 1996. On an average, animal foodstuffs contain between 250 and 600 µg Cr/kg dry matter. Water is Cr-poor with 1.5 µg/l. Other beverages contribute considerably to the Cr supply. The Cr intake of adults meets their requirements and exceeds the recommended Cr intake. Apart from breast-feeding women, the Cr balances of adults are positive. This finding explains the high Cr content in the skeleton of adults. The slightly negative balance of breast-feeding women requires further investigations.

Key words: Chromium: transfer in the food chain, foodstuffs, essentiality, requirement, intake, balance of humans, toxicity

INTRODUCTION

Chromium occurs abundantly in the earth's crust with about 100 mg/kg and occupies the 21st place in the frequency list. Thus, it occurs more frequently than Cu and Zn. The annual production of Cr ores amounts to 10 million tons. Cr is used for the production of special steels in the metal-processing industry, for chromium coating in the galvanic industry, as a pigment and catalyst in the chemical industry, as dye in the textile industry, for the leather production in tanneries and for the impregnation of products in timber industry. The use of Cr-rich products led to the Cr accumulation in the environment. Local Cr exposures of soils and the environment can occur via sludge, water and air.

But, three-valent Cr is less toxic than six-valent Cr. When nutritive Cr amounts are taken in, six-valent Cr is quickly reduced to three-valent Cr. Six-valent Cr can trigger cement eczema and asthmatic bronchitis. Cr exposures at the place of work are assumed to be the cause of the frequent occurrence of lung cancer.

According to our present knowledge, Cr is not essential for the flora. On the other hand, Cr belongs to the essential ultratrace elements which must be available to the fauna and humans in minimal amounts (Schwarz and Mertz, 1957, 1959; Mertz, 1969, 1981) although it has not yet been finally detected as a component or activator of

proteins, enzymes and/or hormones (Offenbacher and Pi-Sunyer, 1997). Due to its toxicity and essentiality, the laws of the Cr transport in the food chain from soil via flora and fauna to humans should be systematically investigated and conclusions should be drawn for the Cr supply of humans in Central Europe.

MATERIALS AND METHODS

The matrix release of the biological samples was carried out with dry ashing at 450°C. The ashes of 20 to 30g dry matter were put in 25% HCl, diluted with bidistilled water to 2.5%, heated in a water bath and poured into 100 ml volumetric flasks. Cr was determined with ICP OES (Spectroflame-D Spectro Analytical Instruments, Kleve, Germany) (Anke et al., 1997a). The repeatability of the measurements was tested with an apple and a cereal sample on 9 different measuring days. The accuracy of the Cr determination was checked with the help of the reference material "IAEA H-9 Mixed Human Diet" and "CTA-OTL-1 Oriental Tobacco Leaves and "GBW 07802 Bush Branches and Leaves", resp.

RESULTS AND DISCUSSIONS

1. Influence of the geological origin of the site and anthropogenic chromium emissions on the chromium content in the flora

On an average, the flora of the weathering soils (table 1) of the lower strata of new redsandstone had the highest Cr content. Therefore, its individual Cr content was

Table 1: Influence of the geological origin of the site on the chromium content in the flora

Geological origin of the site	Relative number
Lower strata of new red sandstone	100
Granite, porphyry, syenite	96
Moor, peat	87
Phyllite	80
Slate	81
Diluvial sand	79
Muschelkalk	79
New red sandstone	79
Gneiss	77
Boulder clay	77
Loess	74
Keuper	74
Alluvial riverside soils	74

equated with 100. The higher Cr content in the indicator plants on the weathering soils of the lower strata of new red sandstone may result from the higher phosphate content of these sediment weathering soils. The flora on loess, keuper weathering soils and alluvial riverside soils contained only 26% less Cr than that of the lower strata of new red sandstone. Compared with other trace elements, the geological origin only affected the Cr content in the vegetation to a moderate extent. The Mn, Zn, Cu, I and Se concentration of plants is much more varied by the geological origin of the site (Anke et al., 1994). In spite of that, the Cr content in the soil takes a significant effect on the Cr content in the flora. The more Cr was found in the soil of the plant site, the higher its content in its flora (Kabata-Pendias and Pendias, 1992).

Even after decades, the chromium emissions of a former cement plant induced a significant increase of the Cr content in wild and cultivated plants without triggering phytotoxic and nutritional damage in the flora, fauna and humans (table 2).

Table 2: Effects of the emissions of a former cement and incandescent phosphate plant on the chromium content in wild and cultivated plants ($\mu\text{g/kg}$ dry matter)

Species (n:n)	Control area		Saale valley		p	% ¹⁾
	s	$\bar{x}$	$\bar{x}$	s		
Cucumber (15,8)	293	685	1628	2016	>0,05	238
Tomatoes (13,5)	238	343	760	597	<0,05	222
Onion leek 86,7)	108	380	608	215	<0,05	160
Lettuce (16,8)	521	1035	1406	625	>0,05	136
Meadow red clover (5,6)	84	216	287	149	>0,05	133
Sweet clover (15,4)	67	214	277	129	>0,05	126
Tancy (14,4)	50	298	362	67	<0,05	124

¹⁾control area = 100 % middle Saale valley = x %

In contrast to other trace and ultratrace elements, the age of plants did not take effect on the Cr content of the flora.

The influence of the species of plants on the Cr content in the flora remained within moderate limits. Barks, tips of twigs and several perennial plant species which serve as winter grazing for hoof game proved to be particularly Cr-rich. Little Cr is accumulated in leaves. On an average, the Cr content in tubers, roots and stem bulges, fruit and seeds was smaller than that in leaves. The skin of fruit, vegetables and seeds accumulates more Cr than their content. Hence it follows that foods produced from them are Cr-poorer (Anke et al., 1997a).

2. Chromium content in plant foodstuffs

The investigated foodstuffs and beverages were bought in 1988 (n 9) before the reunification of Germany with local food production and in 1992 (n 5) with global trade and - to a certain extent - in 1996 in East Germany. A total number of 142 foodstuffs and beverages were investigated. When there were no significant differences between the Cr contents, they were summarized. Otherwise, the year shows when the foods were bought. Apart from oat flakes and several cocoa products which store more Cr, the Cr content in sugar- and starch-rich cereals, pasta, bread, cakes and pastries varies between 80 and about 400 μg Cr/kg dry matter (table 3).

Table 3: Chromium content in different kinds of bread, cakes and pastries in $\mu\text{g}/100\text{g}$ edible proportion (fresh matter) and in $\mu\text{g}/\text{g}$ dry matter

Kind, year	Dry matter %	Fresh matter $\mu\text{gCr}/100\text{g}$ μg	Dry matter $\mu\text{g Cr}/\text{kg}$	
			$\bar{x}$	S
Biscuit (1988)	97	16	80	24
Crumble cake (1988)	81	7,5	93	18
Cornflakes	87	9,9	102	68
Rolls	75	9,5	127	27
Crumble cake (1992)	81	11	138	23
Wheat and rye bread	61	9,8	160	38
Rusk	94	15	163	96
Toast	68	11	164	96
Biscuit (1992)	97	7,7	170	48
Cake with many eggs (1988)	38	7	183	36
Stirred cake	77	15	199	54
Crispbread	94	22	237	84
Cake with many eggs (1992)	38	13	335	169
Coarse rye bread	54	19	360	162
White bread	64	23	361	234

Herbs and spices have a mean Cr content of between 400 and 1800 $\mu\text{g}/\text{kg}$ dry matter (marjoram). Thus, their contribution to the Cr supply of humans remains unimportant because they are consumed in small amounts.

Table 4: Chromium content in different kinds of vegetables in $\mu\text{g}/100\text{ g}$ edible proportion (fresh matter) and in $\mu\text{g}/\text{kg}$ dry matter

Kind, year	Dry matter %	Fresh matter $\mu\text{gCr}/100\text{ g}$	Dry matter $\mu\text{g Cr}/\text{kg}$	
			$\bar{x}$	S
White cabbage (1988)	9,1	2,3	250	78
Potatoes	18,0	6,0	333	235
Radish	6,5	2,5	378	160
Carrots	7,0	2,7	383	271
Sauerkraut (glass)	9,7	3,7	386	161
Leek	11,0	4,6	419	191
Green beans	6,6	2,8	421	164
White cabbage	9,1	4,8	535	202
Kohlrabi	10,0	6,1	612	282
Cauliflower	8,0	5,7	714	123
Red cabbage	9,2	6,9	748	466
Spinach	8,4	6,7	799	139
Carrots (glass)	6,3	5,0	800	480
Sauerkraut (loose)	7,3	5,9	813	784
Asparagus	4,6	4,4	948	312
Cucumbers	5,2	3,4	981	866
Mixed mushrooms	6,0	6,3	1062	451
Lettuce	7,3	9,2	1260	694
Mushrooms	5,2	6,6	1276	402

Apart from tomatoes, fruits also accumulate low Cr amounts. Apart from potatoes, vegetables are the main chromium suppliers of humans. This is particularly true for kohlrabi, carrots, spinach, asparagus, cucumbers, lettuce and mushrooms (table 4).

The Cr content in plant foods increased between 1988 and 1996 in Central Europe. The differences were significant for rice, mustard seeds, white beans, oatflakes, oat pulp, biscuit, crumble cake, cake with many eggs, lemons and white cabbage. Only pepper and cocoa contained more Cr in 1988 than in 1992. There were no significant differences between the Cr contents for all other foodstuffs in 1988 and 1996 (Anke et al., 1997 b).

3. Chromium content in animal foods

In spite of the modest Cr incorporation of only 1%, the Cr transfer from the flora to the fauna takes place without problems 4 days after the intake by herbivores. The renal Cr excretion begins very quickly after the absorption. The incorporated Cr remains in the skeleton, brain, muscle and liver for so long a time (Anke et al., 1971) that a Cr content of > 4000 µg/kg ribs must be regarded as normal for several game species. Cerebrum, kidneys and liver accumulated 500 to 1000 µg Cr/kg dry matter. The Cr content in the organs of game is species-specific. Hares and moufflons stored more Cr in the tissues than red deer (table 5).

Table 5: Chromium content in different parts of the body of game (µg/kg dry matter)

Part of the body (n)		hare	roe deer	moufflon	wild boar	red deer	Fp	% ¹⁾
Liver (29,21,37,9,17)	$\bar{x}$ s	807 ^{xx} 185	628 167	728 580	581 109	495 ^x 139	<0,001	61
Ribs (25,8,13,21,0)	$\bar{x}$ s	7652 ^{xx} 1343	6120 1666	4532 ^x 1259	5400 1302	—	<0,05	59
Kidneys (0,14,2,10,8)	$\bar{x}$ s	—	858 318	1971 ^{xx} 684	734 ^x 184	799 663	<0,001	37
Cerebrum (0,8,10,2,4,)	$\bar{x}$ s	—	1022 194	1545 668	1098 10	1022 ^x 262	<0,001	66

1) ^{xx} = 100 %; ^x = x %

Sausage (250 µg/kg dry matter) contained less Cr than poultry meat, beef, mutton and pork (500 µg Cr/kg dry matter) whose Cr content varies very little. Liver (770 µg Cr/kg dry matter) and kidneys (990 µg Cr/kg dry matter) accumulated much Cr. Salt-water fish (300 - 500 µg Cr/kg dry matter) store less Cr than trout (650 µg Cr/kg). Fish fillet with few bones contained less Cr than sardines.

Butter (60 µg/kg dry matter) is Cr-poor whereas cow's milk and cheese (600 µg/kg dry matter) are Cr-rich like the same organs of game (table 6). Cheese spread (1413 µg/kg dry matter) contains twice as much Cr than all other investigated kinds of cheese. Its high Cr content is probably due to the sodium phosphate supplementation before melting.

Milk, curd cheese and the different kinds of cheese are excellent Cr suppliers for humans which can contribute essentially to the Cr supply of people with mixed diets and vegetarians (Anke et al., 1997c).

Table 6: Chromium content in different dairy products in $\mu\text{g}/100\text{ g}$ edible proportion (fresh matter) and in $\mu\text{g}/\text{kg}$ dry matter

Kind, year	Dry matter	Fresh matter $\mu\text{g Cr}/100\text{ g}$	Dry matter $\mu\text{g Cr}/\text{kg}$	
			$\bar{x}$	s
Margarine	77	3,1	40	36
Butter	85	5,4	63	51
Yoghurt	18	4,4	245	136
Tollenser	55	29	519	42
Edamer	55	32	575	80
Milk	12	7,1	591	239
Altenburg "goat's cheese"	48	29	597	111
Curd cheese	8	11	614	136
Condensed milk	21	13	618	55
Limburger	45	28	638	57
Emmentaler	63	42	659	47
Tilsiter	57	38	660	186
Camembert	46	30	664	292
Gouda	58	39	668	161
Cheese spread	41	58	1413	434

4. Chromium content in beverages

The German decree on drinking water as well as the WHO/FAO/IAEA (anonymous, 1996) allow a Cr content of $50\text{ }\mu\text{g}/\text{l}$ in the drinking water. $5\text{ }\mu\text{g Cr}/\text{l}$ drinking water are regarded as the normal value and $112\text{ }\mu\text{g Cr}/\text{l}$ as the maximum value in the literature (anonymous, 1977). On an average, 60 samples of drinking water contained $1.5\text{ }\mu\text{g Cr}/\text{l}$ in Germany (1996). All other beverages proved to be Cr-richer. They can contribute essentially to the Cr intake (table 7).

Table 7: Chromium content in different kinds of beverages ($\mu\text{g}/\text{l}$)

Kind	$\bar{x}$	S	Kind	$\bar{x}$	s
Drinking water	1,5	1,0	Beer	35	20
Lemonade	2,9	2,6	Vermouth	39	23
Corn schnapps	4,2	1,3	Champagne	44	6,0
Brandy	12,0	0,5	White wine	46	25
Coke	13,0	4,0	Red wine	81	58
Juice	22,0	10,0	Advocaat	87	13

5. Chromium intake in humans

Schwarz and Mertz (1957) observed an insufficient glucose tolerance in rats with Cr-poor rations. They postulated the lack of a glucose tolerance factor. 3-valent Cr was assumed to be its active form (Schwarz and Mertz, 1959; Mertz, 1969). The structure of this insulin-intensifying Cr complex has not yet been verified (Anderson, 1987). In spite of that, it was possible to trigger different deficiency symptoms in animals and humans with Cr-poor rations. Signs and symptoms of Cr-deficiency are: Insufficient glucose tolerance (man, rat, mouse, guinea pig), increased circulating

insulin amounts (man, rat), slower growth (rat, mouse, turkey), reduced life expectancy (rat, mouse), the more frequent occurrence of plaques in the blood vessels (rabbit, rat, mouse), increased cholesterol and triglyceride in the serum (man, rat, mouse), neuropathies (man), encephalopathies (man), damaged corneas (rat, squirrel) as well as reduced reproduction performances and numbers of spermatozoa (rat) – Anderson, 1987.

The basal Cr requirement of humans amounts to $< 20 \mu\text{g/day}$. The minimum chromium intake of a population should be $25 \mu\text{g/day}$. The WHO recommends the intake of $33 \mu\text{g/day}$ (anonymous, 1996).

Depending on sex, age, body weight, season (summer, winter), living area (Germany, Mexico), performance (breast-feeding) and kind of diet, the Cr intake of adults was investigated in 1988 (4 test populations), in 1992 (n 6) and in 1996 (n 8) with the help of the duplicate method. Apart from the people with mixed diets and vegetarians from Jena (n 10), the test populations consisted of 7 women and 7 men who collected the visually estimated duplicates of all consumed foods, beverages and sweets on 7 consecutive days. The dry matter intake of women and men was only varied by 2 to 4% due to the reunification and the wider offer (Anke et al., 1997d).

The mean Cr intake of people with mixed diets varied between 61 and 88 $\mu\text{g/day}$ in women and between 84 and 100 $\mu\text{g/day}$ in men during the three test years. Due to their 24% higher dry matter intake, men took in 24% more Cr than women.

Table 8: Chromium intake of adult people with mixed diets in Germany depending on time ($\mu\text{g/day}$)

Period (n;n)	women		men		Fp	%
	S	$\bar{x}$	$\bar{x}$	s		
1988 (196,196)	27	75	97	37	< 0,001	129
1992 (294,294)	41	88	100	36		114
1996 (217,217)	31	61	84	55		138
Fp	< 0,001				—	
%	81		87			

Thus, the Cr intake exceeded the recommended intake by the double and the assumed Cr requirement by the threefold. A world-wide Cr intake of 20 to 146 $\mu\text{g/day}$ was found according to the references summarised by Parr et al. (1992). The lowest Cr intake was registered in India (Soman et al., 1969) and the highest in Italy (Cocchioni et al., 1988). The abundant Cr offer may be due to the serpentine weathering soils, which deliver much Cr into the food chain. None of the people with mixed diets in Germany took in $< 20 \mu\text{g Cr}$ on the average of the week (Fig. 1). All of them took in $> 20 \mu\text{g Cr/day}$. Thus, their individual Cr requirement was met. The recommended Cr intake of $33 \mu\text{g/day}$ is also realised on the average of the week. A marginal Cr supply was only registered in 4% of women and 1% of men.

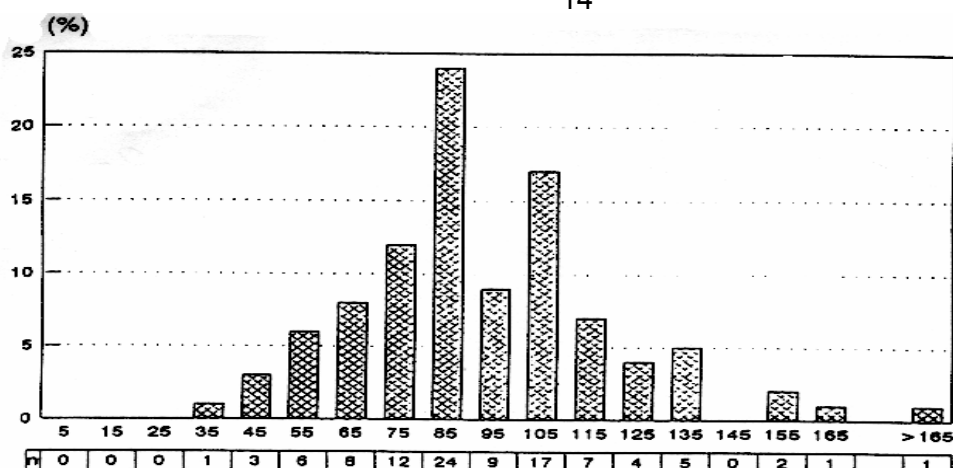


Fig. 1. Chromium intake of men on the average of the week ($\mu\text{g/d}$)

The Cr intake/kg body weight varies between 1.4 and 0.9 $\mu\text{g/kg}$ body weight on the average of the test years and sexes. A Cr intake of 1 $\mu\text{g/kg}$ body weight meets the Cr requirements of adults abundantly.

Season as well as the age of the test persons did not affect the Cr intake of sexes but the body weight did. Heavyweight women and men consumed a Cr-rich dry matter and also took in more Cr/day than lighter ones.

Due to their high dry matter consumption, breast-feeding women took in more Cr than other women.

The Cr content in the dry matter consumed by people with mixed diets and vegetarians only differed to an academic extent. Eating preferences due to sex did also not take any effect on it. Due to their higher dry matter intake, however, vegetarians took in significantly more Cr/day than people with mixed diets (table 9). This statement is even more valid for the Cr intake/kg body weight since, on an average, vegetarians were lighter than people with mixed diets. The latter consumed about 1 $\mu\text{g/kg}$ body weight.

Table 9: Chromium intake of adult people with mixed diets and vegetarians in Germany depending on the form of diet ($\mu\text{g/day}$)

Form of diet (n;n)	Women		Men		Fp	%
	S	$\bar{x}$	$\bar{x}$	S		
Mixed diet (217,217)	31	61	84	55	< 0,001	138
Vegetarians (70,70)	25	85	99	40		116
Fp	< 0,05				—	
%	139		118			

The influence of the living area is best reflected by the Cr content in the consumed food and beverage dry matter since the varying influence of the dry matter consumption does not apply (table 10).

The food dry matter consumed at Steudnitz where a former cement plant emitted much Cr and where the spices, vegetables and fruit produced in the house gardens still contain significantly more Cd than those from other living areas contain most Cr (Anke et al., 1997a). However, the Cr intake of adults does not point to a Cr exposure in this living area. The test population from the neighbouring Jena also had an abundant Cr supply whereas that from Ronneburg took in less Cr/kg dry matter.

Table 10: Chromium content in the food and beverage dry matter consumed by adult people with mixed diets in Germany depending on the living area ($\mu\text{g/kg}$ dry matter)

Living area/year	Women		Men		Fp	% ¹⁾
	S	$\bar{x}$	$\bar{x}$	S		
Steudnitz 1996	136	301	303	165	> 0,05	101
Jena 1988	73	297	281	86		95
Bad Liebenstein 1992	59	277	301	84		109
Wusterhausen 1992	112	314	254	78		81
Bad Langensalza 1992	111	296	267	75		90
Chemnitz 1992	148	300	254	66		85
Greifswald 1992	74	270	261	98		97
Mexiko (98;98)	110	261	253	105		97
Wusterhausen 1988	97	260	256	95		98
Freiberg 1992	85	259	253	57		98
Bad Langensalza 1988	91	240	240	72		100
Vetschau 1988	79	229	241	70		105
Jena 1996	36	208	220	73		106
Rositz 1996	59	183	183	43		100
Ronneburg 1996	35	157	195	36		124
Fp	< 0,001				—	
% ²⁾	52		64			

1) women n = 100 %, men r = x %; ²⁾ Steudnitz 1996 = 100 %, Ronneburg 1996 = x %

The Cr intake of women in Germany varied between 101 and 45 $\mu\text{g/day}$, that of men between 116 and 67 $\mu\text{g/day}$ on the average of the test populations. Cr deficiency need not be expected in adult Germans with mixed diets.

The two Mexican test populations which did not differ with regard to their Cr intake and which, therefore, are summarized in table 10 correspond with the range of the Cr intake of the German test populations. Cr deficiency need not be expected there as well.

6. Chromium excretion and chromium balance of adults and breast-feeding women

The test populations of people with mixed diets and vegetarians as well as 7 breast-feeding women from Jena (Thuringia) were available for these investigations in 1996. They also put the 15 milk samples at our disposal. It was not possible to quantify their milk amount. It was calculated at 750 ml/day (Rother, 1997). Adult people with mixed diets only excreted 2% of the Cr amount via urine and 98% via faeces (table 11).

Table 11: Chromium excretions of adult people with mixed diets via urine and faeces in $\mu\text{g/day}$ and in per cent of both excreta (n 147;147)

Excretum	Women		men		p	%
	S	$\bar{x}$	$\bar{x}$	S		
Urine, $\mu\text{g/day}$	0,5	0,6	0,7	0,4	> 0,05	117
Faeces, $\mu\text{g/day}$	31	35	43	32	< 0,05	123
Urine, %	1,7		1,6		—	
Faeces, %	98		98			

Anderson (1987) estimated the renal Cr excretion of humans via the 24-hour urine at 1 µg. In accordance with their higher dry matter intake, men also excreted 17 and 23% more Cr than women via urine and faeces.

The renal and faecal Cr excretion of vegetarian populations follows similar rules. They only excreted 1 percent Cr via urine and 99% via faeces. Sex did not affect the Cr excretion of vegetarians. The fibre-rich nutrition of vegetarians did not take a significant effect on the excretion ways of Cr. The renal Cr excretion of breast-feeding women was in accordance with that of people with mixed diets and vegetarians. It amounted to 1 µg/day. The faecal Cr excretion does also not differ much from that of people with mixed diets. In the case of an assumed milk production of 750 ml/day, women excreted 12 µg Cr/day (table 12).

Table 12: Chromium excretion of breast-feeding women with mixed diets via urine, faeces and milk in µg/day and in per cent of the excreta (n 49)

Excretum	$\bar{x}$	S	%
Urine, µg/day	1,0	2,1	1
Faeces, µg/day	79	53	86
Milk, µg/day	12	3,0	13

On the average of the available investigations, mother's milk only contained one quarter of the Cr amount found in cow's milk (Anke et al., 1997c). The data in the literature on the Cr content in mother's and cow's milk vary extremely. Cow's milk produced in America delivers the same Cr amounts as that produced in Germany. In the USA, the Cr content of mother's milk is in accordance with that of cow's milk and only amounted to 0.3 to 0.4 ng Cr/ml (Anderson, 1987; Casey and Hambidge 1984; Offenbacher et al. 1997). In the available investigations, breast-feeding women secreted 12 µg Cr/day into the milk.

After the oral intake, Cr is very quickly and dominantly absorbed in the jejunum. The extent of the absorption is influenced by the time of measuring, the amount of Cr taken in, the composition of the diet (interactions) and probably by the species as well. E.g. hens incorporated 15% of the Cr in the body 6 hours after a Cr offer into the crop. 24 hours after the application only 7% were left. The incorporated Cr amounts were reduced to <1% four days after the intake in other species. The mechanism of the Cr absorption is not completely understandable (Anke et al., 1971; Hennig et al., 1971). The passive diffusion of the Cr is probably completed by an active one with the help of transferrin. The Cr absorption increases when the Cr offer is low. The supplementation of Zn and other essential trace elements may take a negative effect on the Cr utilization.

Cr may be incorporated in 3 body compartments of humans with a half-time of 5 to 12 h, 1 to 14 days and 3 to 12 months. The Cr in the blood is excreted very quickly via urine. 0.2 to 0.8 µg Cr/day or 1 to 2% of the consumed Cr amount leave the body via urine. Most of the Cr taken in is excreted faecally.

The Cr balance of women with different forms of diets and performances varies between -11% and +35% (table 13). Breast-feeding women had a slightly negative balance, vegetarians a balanced one and non breast-feeding women with mixed diets had a clearly positive balance. They incorporated about one third of the Cr.

Table 13: Chromium balance of adult people with mixed diets and of vegetarians in 1996

Parameter	Women			Men	
	Vegetarian	mixed diet		vegetarian	mixed diet
		non breast feed	breast feed		
Intake, $\mu\text{g/day}$	85	55	83	99	75
Excretions, $\mu\text{g/day}$	87	36	92	76	44
Balance, $\mu\text{g/day}$	- 2,0	+ 19	- 9	+ 23	+ 31
Balance, %	- 2,0	+ 35	- 11	+ 23	+ 41

amount taken in. Independent of the form of diet, men also had a positive Cr balance. Considerable Cr amounts can apparently be incorporated. Goats accumulated 45% of the absorbed Cr in the skeleton (Anke et al., 1971). The high Cr concentrations in the ribs of different game species confirm this statement (Anke et al., 1997c). The Cr absorption of animals and humans is more comprehensive than demonstrated. Only limited Cr amounts leave the body via urine (milk, eggs). The main amount of the absorbed Cr is brought back to the intestines via bile and intestine secrets and excreted faecally. An intensive reabsorption of the Cr takes place in the kidneys, which is also shown by their high Cr content (Anke et al., 1971, 1997c; Hennig et al., 1971).

Breast-feeding women secrete about 13% of the excreted Cr amount into the milk. They showed a slightly negative balance. Men and non breast-feeding women had a positive Cr balance, vegetarians a balanced one.

The Cr absorption is much more comprehensive than that registered in urine and milk. Most of the absorbed Cr is excreted into the intestines and leaves the body faecally.

Cr deficiency need not be expected in adults in Germany. Attention must be paid to the Cr supply of breast-feeding women. Although they have a considerable Cr depot in the skeleton, it is unknown how long it can compensate for the slightly negative balance (Anke et al., 1997c).

7. Chromium intoxications

Less than three-valent Cr, six-valent Cr induces health injuries. Normal amounts of six-valent Cr taken in via food are quickly reduced to three-valent Cr.

Exposures to chromate dust correlates with the more frequent occurrence of lung cancer and dermatitis. Six-valent Cr may be gene toxic and mutagenic. Six-valent Cr is probably reduced to three-valent Cr, OH radicals come into being. Six-valent Cr causes DNA protein cross links in different cell lines. Three-valent Cr does not lead to DNA damage.

Six-valent Cr can trigger cement eczema and asthmatic bronchitis which often occur in construction workers, workers in the leather industry (tanners) and, years ago, in milk inspectors who used chromate for the preservation of milk (Offenbacher et al., 1997).

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ORGANOMETALLIC COMPOUNDS IN THE ENVIRONMENT, MEDICINE AND BIOLOGY

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ABSTRACT

Two types of organometallic compounds can be distinguished: a) environmentally stable organometallics, and b) environmentally sensitive organometallics. The first group can be environment hazards because of their toxicity (organomercury, organolead, organoarsenic and other derivatives) or may serve useful purposes, being used as medical drugs, pesticides etc. The sensitive organometallics are rapidly degraded to inorganic species.

The exposure of human beings to organometallic compounds, in laboratory, industry and everyday life, should be treated with caution and serious consideration must be given to potential hazards.

Key words: organometallic compounds

INTRODUCTION

Organometallic compounds are chemical species containing direct metal-carbon bonds, with a negative polarity at carbon and positive polarity at the metal, i.e. $M^{\delta+} - C^{\delta-}$. According to this definition, organic derivatives of semimetal or metalloid elements, such as boron, silicon, arsenic, antimony, with a similar bond polarity, are also described as organometallics. In common organic compounds, the bond of carbon to other elements, e.g. oxygen, nitrogen or halogens, displays opposite, i.e. $E^{\delta-} - C^{\delta+}$ polarity.

The chemistry of organometallic compounds represents a bridge between traditional organic and inorganic chemistry, sometimes called "the third chemistry" and became a broad field of intensive research. Since the chemistry operates with almost all chemical elements of the periodic Table (at least 90), which can be combined with any possible organic fragment, it is obvious that the synthetic possibilities are enormous and surpass the potential of both organic and inorganic chemistry taken separately. In recent years this field enjoyed an explosive development and became one of the major areas of interest in chemistry. Not only intensive academic research is currently going on in the area, but important industrial and everyday applications have emerged and as a consequence a large number of people are exposed to this type of compounds. This includes the numerous researches involved in the synthesis and investigation of these compounds, the industrial workers participating in their manufacture, the users and in some case even "innocent bystanders".

With a very few notable exceptions (e.g. methylcobalamin or B₁₂ vitamin containing a Co-C bond, and the organoarsenic betaine $Me_3As^+-CH_2COO^-$) the organometallics are man-made compounds, i.e. completely unnatural species, foreign to living organisms. Therefore, the exposure of humans and other living organisms to organometallic compounds can be a source of potential accidents

(provided that their "unnatural" character leads to toxicity) and an aggression of the nevironment may also result. On the other side, moderate or controlled toxicity may be used for destruction of harmful species, and thus, organometallic compounds can be of interest as bactericides or pesticides. Other beneficial effects upon living organisms may convert some organometallics into usefull medical drugs. In line with the theme of this Symposium, some pertinent aspects of organometallic chemistry will be briefly outlined.

It is important to mention that two types of organometallic compounds can be distinguished:

- a) Environmentally stable organometallics, which are not affected by oxygen, water (liquid or vapour), i.e. are stable under normal atmospheric conditions;
- b) Air and water-sensitive organometallics, which can be prepared and handled only under rigorous inert atmosphere and anhydrous conditions. These cannot be encountered in the normal environment and their synthesis and uses are confined only to laboratory and industrial conditions.

The stability of organometallics towards air (oxygen) and water is determined by the properties of the metal-carbon bond. Compounds with low polarity of the M-C bonds are less reactive. The bonds to carbon of elements such as boron, silicon, germanium, tin, lead, arsenic, antimony and bismuth, are basically inert towards oxygen and water and therefore the corresponding organometallic compounds can be handled without special precautions. They survive the contact with the open atmosphere and can travel within living organisms, interacting with other chemical species. Thus, environmentally stable organometallic compounds can be either harmful or beneficial in their contact with the living organism.

Air and water sensitive compounds are those containing very polar metal-carbon bonds, e.g. organic derivatives of alkali metals, alkaline earths, aluminium, zinc and others. In contact with water they eliminate the organic groups as hydrocarbons and form inorganic oxo and hydroxo derivatives, as products of chemical degradation.

The transition metals may also form water and air stable organometallic compounds, but many are sensitive, mainly in solution, and as a general rule they are usually prepared under inert atmosphere and only after their stability in the open air is established they are handled without special precautions and can be considered for potential uses. Examples are the ferrocene and titanocene derivatives.

THE COMPOSITION OF ORGANOMETALLIC COMPOUNDS

Another important aspect of organometallic compounds is related to their composition. In addition to homoleptic compounds, MR_n which contain only organic groups attached to the metal atom, there are numerous functional derivatives, R_nMX , where R is the organic group and X is an anionic functional group, e.g. halogen, hydroxo or alkoxo, thiolato, amino, carboxylato, phosphato etc. The functional groups are labile, in general can be readily replaced, and much chemistry is based upon their reactions. Chelating or other ligands, can also replace the functional groups X. In the living organisms, the interactions of organometallic compounds with the biomolecules is usually based upon replacement of the initial functional groups and fixation of the organometallic moiety at reactive sites of the biomolecules.

Many environmentally stable organometallic compounds are very toxic, and thus are potential pollutants. Some famous accidents are known, for which the toxicity of organometallic compounds was responsible. Thus, the so-called Minamata case (1953-1960) in which many Japanese fishermen were killed by methylmercury compounds formed in the marine environment is a famous example. The investigations prompted by this case lead to the discovery of biomethylation of mercury and other metals, notably lead, the natural methyl group transfer reagent being methylcobalamin. These cases prompted a careful investigation of the biological methylation of mercury in aquatic media and of the methylmercury toxicology. Methylmercury compounds are among the most toxic chemicals and a recent tragical poisoning of a chemist using dimethylmercury as standard for NMR spectroscopy added a new case to the known list of accidents. A mass poisoning with wheat seeds treated with an ethylmercury pesticide in Irak (1971) is also known. Another known accident caused by organometallics was that produced by Stalnon, a cosmetic preparation containing accidental traces of triethyltin species, Et_3SnX .

Akyl derivatives of other metals are also very toxic, mostly methyl- and ethyl-substituted monocationic species, e.g. R_3Pb^+ , R_3Sn^+ , R_2As^+ etc. Such species, e.g. Et_3Pb^+ are known to inhibit the oxidephosphorylation and the action of glutathione transferase. Diorgano-metal species, e.g. R_2Pb^{2+} block the enzymes containing neighbouring thiol groups. A similar mechanism explains the high toxicity of other thiophilic organometallic moieties. Degradation of some homoleptic organometallic compounds, e.g. tetraethyllead, PbEt_4 , to triorgano-metal species, explains their toxicity. For this reason tetramethyllead and tetraethyllead, used for many years as antiknock gasoline additives, were prohibited in many countries, as they are a source of serious atmospheric pollution, especially in congested urban areas.

THE TOXICITY OF ORGANOMETALLIC COMPOUNDS

In some cases the toxicity of organometallic compounds was put to good use. Thus, organotin compounds are used as fungicides or antifouling paint additives. Organoantimony compounds served to destroy larvae which are at the origin of some tropical diseases, such as leishmaniasis and schistosomiasis.

Direct medical use of some organometallic compounds were also notable. It is worth mentioning that the birth of chemotherapy was due to an organometallic compound. Thus, in 1909 Paul Ehrlich introduced Salvarsan, an organoarsenic derivative, for the very successful treatment of syphilis.

Organoborn compounds are useful in neutron capture radiotherapy, some are bacteriostatic and antimicrobial agents and exhibit antiinflammatory, antihyperlipidemic, diuretic and anticoagulant action.

Among the numerous biologically active organosilicon compounds the most interesting are the silatranes (triethanolamine monoorganosilicon derivatives). Some are highly toxic (phenylsilatrane is more toxic than strychnine), other exhibit strong wound healing properties and stimulate hair growth (used in the treatment of baldness).

Numerous organometallic compounds were found to display strong antitumor properties and some are promising anticancer agents. These include a germanium-containing heterocyclic compound known as Spirogermanium and 2-carboxyethyl-germanium sesquioxide ($\text{Ge } 132$), numerous organotin compounds (some more active in vitro than the clinically used Cisplatin), metallocene dihalides such as Cp_2TiCl_2 , Cp_2VCl_2 and others.

Organoiron compounds, some including ferrocene derivatives, also exhibit antitumor properties and were recommended as iron source in the treatment of anaemia.

The study of organometallic compounds in relation with the living organisms and the environment is now emerging as a new discipline, bio-organometallic chemistry, which can contribute to the discovery of new medical drugs and to the better understanding of the mechanisms of biological processes. The fate of organometallic compounds in the environment and in living organisms is an important subject of study, of both academic and applied interest, which merits further attention. It is obvious in the current literature and promises to be a continuous source of interesting results.

CONCLUSIONS

It can be concluded from this presentation that the contact with organometallic compounds in laboratory and industry should be treated with a certain amount of acution, since toxicity or other adverse effects can be encountered. On the other hand, the environmentally stable organometallic compounds, should be regarded as a potential source of useful solutions in medicine, agriculture and other areas related with the use of biologically active compounds.

This brief introduction serves only as a signal intended to attract wider attention towards organometallic compounds in the environment, medicine and biology. Much additional, more detailed information, as well as references to the original literature, can be found by the interested reader in the general references cited at the end.

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THE DETERMINATION OF PLATINUM AND GOLD IN THE AIR OF COPENHAGEN CITY USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS (INAA)

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ABSTRACT

Since the 1980's, the number of automobiles, using unleaded petrol and equipped with catalytic converters, has increased rapidly. Catalytic converters, containing platinum, are beneficial in removing nitrogen oxides and organic compounds, like polyaromatic hydrocarbons. However, despite this useful conversion, there has been some concern about the dispersion of platinum in the urban environment. Platinum and other precious metals in the catalytic dust are deposited along roads, on adjacent vegetation, and on soil. Concern has been expressed that platinum could effect the health of people by direct contact with platinum in the dust, by inhalation of dust and indirectly through the food chain. The knowledge about the influence of platinum on human health is – unfortunately – yet very limited. The detection of small amounts of platinum, present in environmental samples, necessitates the use of sensitive and reliable analytical methods. Therefore, instrumental neutron activation analysis has been used for the determination of Pt and Au in samples of dust, collected at streets with heavy traffic in the City of Copenhagen.

Key words: Platinum, dust, instrumental neutron activation analysis.

INTRODUCTION

The interest in the determination of low amounts of platinum in urban air has increased rapidly with the introduction of platinum containing automobile exhaust catalysts. The catalysts contain a catalytic active coating, mainly consisting of metallic platinum on different carrier materials. Although there are requirements in durability, the emission of catalyst material caused by abrasion cannot be avoided. Platinum is emitted during the traffic in the form of particles, which are deposited on the streets of heavy traffic, on all vegetation and the ground near these streets.

The knowledge concerning the effects of small amounts of platinum on man and plants are rather limited. On the other hand, it is the chemical species which primary induces allergene potential and toxicity of this element. The exposure and uptake of platinum by human beings was investigated in a study by Schaller et al., 1992. Employees exposed during the production and recycling of platinum based catalytic converters revealed clearly (up to 100 times) higher platinum levels in the urine and blood compared to non-exposed control individuals. This indicates an uptake of platinum via the respiratory tract.

Measurements of airborne particles containing platinum were conducted by several authors. Hodge and Stallard (1986) determined platinum concentrations in a range of 0.037 to 0.68 mg/kg in roadside dust collected on vegetation. Platinum

concentrations ranging from 0.073 to 0.184 mg/kg were determined in samples of airborne particles in Japan. (Mukai et al., 1990)

Using a sensitive method like instrumental neutron activation analysis it became possible to determine low concentrations of platinum in dust, collected at places with heavy traffic in the City of Copenhagen.

MATERIALS AND METHODS

Preparation of standards: Because the ^{47}Sc daughter of ^{47}Ca interferes with the detection of Pt as ^{199}Au , some combined Ca/Pt-standards had to be prepared. They were prepared as follows: Different amounts of CaCO_3 were weighed and placed into polyethylene containers ($\varnothing 17$ mm, height 57 mm). CaCO_3 was obtained from Merck, Germany, as calcium carbonate precipitate for the chromatography. Thereafter, cellulose powder, which acts as carrier material for the solution of Pt-standard, was added. The CaCO_3 -powder was thoroughly mixed with cellulose powder (Genuine Whatman cellulose powder, coarse grade, Great Britain).

Different amounts of Pt were added to the container onto the cellulose powder, using a Finn pipette. The standards were dried over night at 50°C . Next day the containers were closed by heat sealing, the powder in the containers was mixed again thoroughly by shaking and rolling each container. To obtain uniform irradiation conditions, the content of each container was kept at the same volume by adding different amounts of cellulose powder. For the addition of the solid materials, spoons of teflon were used.

Pt and Au-standards used for the determination of Pt and Au in filter samples, were prepared by diluting the corresponding primary standards (Pt: $960\text{ }\mu\text{g/ml}$ in 2 M HNO_3 ; Au: 20 mg/ml in 1 M HNO_3) with 1 M HNO_3 to the concentrations requested. The standard solutions were added onto 200 mg Whatman powder, placed in HNO_3 precleaned polyethylene vials. Thereafter, the vials were dried over night at 50°C . These vials were then heat-sealed before irradiation. The primary standards were prepared by dissolving a Pt wire (99.99 % Pt, Advent, Great Britain) resp. an Au wire (99.99 % Au, Advent, Great Britain) in 10 cm^3 of boiling aqua regia.

Sampling: Aerosol samples on mixed cellulose ester filters (Millipore Corp., Ireland) were collected during 24 hours using a membrane pump (total volume of air: ca. 60 m^3) at Jagtvej, a street with very heavy traffic (22000 cars/day) in Copenhagen City. The filters were washed with 10 ml of acetone (p.a. Merck, Germany) and the residue was transferred to polycarbonate or teflon filters (Costar, Cambridge, MA, USA; Millipore Corp., Bedford, MA, USA). Blank filters were treated in the same way.

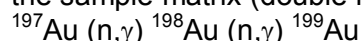
Irradiation: For irradiation the polyethylene vials ($\varnothing 17$ mm, height 57 mm), containing the combined standards, were placed into separate aluminium containers. For the determination of Pt in collected ground samples, the same type of irradiation containers was used. Samples and standards were irradiated together for 2 hours, using the CO_2 -cooled rotating facility of the D_2O moderated Danish Reactor DR3, operating with 20 % enriched ^{235}U at 10 MW (neutron flux approx. $4 \times 10^{17}\text{ nm}^{-2}\text{s}^{-1}$).

The dust on the filter samples was transferred to Teflon - or polycarbonate filters by using acetone (p.a. Merck), these filters were placed for irradiation into polyethylene vials ($\varnothing 12$ mm, length 24 mm) and thereafter heat-sealed. Each filter sampling was irradiated together with the corresponding Pt and Au standards for 6 hours, using the irradiation facility described above. The filter sample and the standard were placed in the same aluminium container.

Counting: All filters were counted at a distance of 4 cm (counting position 31) from a 178 cm³ γ -X Ge detector (Ortec, Oak Ridge, TN, USA) using 8192 channels at a gain of 0.2 keV/channel. The detector has a resolution of 1.77 keV at 1333 keV and a relative efficiency of 35 %. The decay times of the filter samples were in the range of 120 to 273 hours and the counting times were in the range from 9 to 23 hours. The decay times of the standards were in the range of 120 to 140 h, and the counting reel time was 1 h.

RESULTS AND DISCUSSIONS

The results have been obtained by a method, developed and described by Alfassi et al., 1998. The method allows the determination of platinum by the ¹⁹⁹Au daughter of ¹⁹⁹Pt in the presence of the spectral interference from the ⁴⁷Sc daughter of ⁴⁷Ca. The contribution of the Pt and Ca signals to the integral 157-161 keV peak were separated by calculating the number of disintegration's due to the ⁴⁷Sc from the signal of her parent ⁴⁷Ca at 1297.1 keV γ -ray. The method was used to calculate the trace concentrations of Pt in air samples, collected on filters. This method is especially suited for Pt-determinations of samples, which also contain Ca. This is very often the case when air samples are collected in Copenhagen. Altogether, 23 samples have been taken in the period January 1995 to April 1997. A significant interference's comes from the second order reaction of the naturally occurring gold in the sample matrix (double neutron capture):



Correction for this interference is based on the counting of a gold comparator for 24 hours, so that the precision of the Pt determination for the filter sample is not significantly affected. Therefore, all Pt measurements are corrected for this interference. The following tables show the results obtained.

Table 1: Platinum and Gold in the air of Copenhagen City

Sample no.	Collecting date	Pt concentrations, corrected ng/m ³ $\pm$ SD	Pt concentrations, uncorrected ng/m ³	Au concentrations ng/m ³ $\pm$ SD
179156a	1995-01-23	2.740 $\pm$ 0.720	2.740	not detectable
179099	1995-02-22	1.450 $\pm$ 0.080	2.000	0.3654 $\pm$ 0.0012
179100	1995-02-23	1.800 $\pm$ 0.100	2.430	0.4177 $\pm$ 0.0013
179101	1995-02-24	2.520 $\pm$ 0.100	3.140	0.4355 $\pm$ 0.0014
179112	1995-02-25	2.740 $\pm$ 0.270	4.700	1.0360 $\pm$ 0.0020
179123	1995-02-26	0.289 $\pm$ 0.122	0.496	0.1380 $\pm$ 0.0006
179122	1995-02-27	0.502 $\pm$ 0.022	0.640	0.1100 $\pm$ 0.0009
179121	1995-02-28	not detectable	0.268	0.1580 $\pm$ 0.0010
179258	1995-04-21	not detectable	< 0.100	0.0382 $\pm$ 0.0002
179259	1995-04-25	0.133 $\pm$ 0.085	0.346	0.0617 $\pm$ 0.0002
179260	1995-04-26	0.373 $\pm$ 0.088	0.476	0.0282 $\pm$ 0.0002
179484a	1995-05-22	1.090 $\pm$ 0.040	1.090	0.0620 $\pm$ 0.0002

a: no Ca interference

Table 2: Platinum and Gold in the air of Copenhagen City

Sample no.	Collecting date	Pt concentrations, corrected ng/m ³ ± SD	Pt concentrations, uncorrected ng/m ³	Au concentrations ng/m ³ ± SD
179232a	1995-12-23	1.600 ± 0.100	1.800	0.2013 ± 0.0006
179244	1995-12-25	0.543 ± 0.044	1.480	0.2241 ± 0.0004
179243a	1995-12-26	0.300 ± 0.060	0.320	0.0606 ± 0.0002
179485	1996-05-22	0.649 ± 0.076	not determined	0.0527 ± 0.0001
179195	1996-10-31	0.713 ± 0.034	0.841	0.0925 ± 0.0002
179492	1997-04-03	not detectable	not detectable	0.0521 ± 0.0001
179493	1997-04-03	< 0.100	not detectable	0.0496 ± 0.0002
179355	1997-04-07	0.713 ± 0.038	0.713	0.0965 ± 0.0002
179357	1997-04-07	not detectable	not detectable	0.5221 ± 0.0008
179358	1997-04-08	0.691 ± 0.050	0.691	0.1490 ± 0.0003
179486	1997-04-08	0.253 ± 0.067	not determined	0.1623 ± 0.0003

a: no Ca interference

As it can be seen from the tables, the highest Pt concentrations have been measured in the winter season. Their size may also very well depend on the measuring conditions, e.g. the weather (dry, rain, wind) and on the intensity of the traffic (average: 22000 cars/day at Jagtvej, Copenhagen City).

The Au-contents of the samples measured are remarkably high. Industrial activities, the emission from power stations and incineration facilities, may explain the Au content in the dust collected.

CONCLUSIONS

The question may then arise whether an increase in Pt concentrations in urban dust – cause by an increased number of cars equipped with catalysts poses a threat to human health through the inhalation of fine dusts. There is at present no evidence for any adverse health effects from platinum in the environment, particularly allergic reactions. Metallic platinum is considered non allergenic and since the emitted platinum is probably in the metallic or oxide form, the sensitising potential is probably very low. It is important to get more information concerning exposure of the human population to platinum in air and the health effects of such exposure. The use of instrumental neutron activation analysis (INAA) made it possible to detect the low Pt concentrations in the urban dust samples mentioned with adequate precision and accuracy.

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CLINICAL ASPECTS AND BIOCHEMICAL PATHOLOGY IN CYSTINIC UROLITHIASIS - PECULIARITIES OF THE METALLIC COMPOSITION

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ABSTRACT

Clinical, pathophysiological and pathobiochemical aspects specific in the case of urolithiasis reveal a multifactorial etiology. In this context the genetically induced pathology of urolithiasis may be discussed.

Cystinic urolithiasis is the consequence of cystinuria – an autosomal recessive pattern of inheritance – characterized by increased cystine excretion due to a defective renal tubular reabsorption of some amino acids. The transport mechanism for the reabsorption of cystine is not yet elucidated.

In the case of cystinic urolithiasis the metabolism of the amino acid cystine is perturbed: its concentration in blood and urine is augmented. Cystinuria, at patients, can be 0,5-2,0 g/24h. Elevated urinary concentration of other amino acids, e.g: lysine, ornithine, arginine, homocysteine, cysteinylsulfide, homoarginine, was also observed.

Researches performed on cystinic uroconcrements (surgically removed or spontaneously eliminated) by means of atomic absorption spectroscopy revealed their metallic composition. The concentration of metals in cystinic urolithiasis decreased in the series: $Na < K$ for alkaline metals; $Ca > Mg$ for alkaline-earth metals; $Zn > Fe > Cu > Mn$ for trace metal elements.

The essential bioinorganic mechanism in cystinic urolithiasis formation requires the presence of cystine (main amino acid involved) in high concentration in urine and the existence of metallic ions.

Key words: cystinic lithiasis – metallogram

INTRODUCTION

Urolithiasis is a multifactorial disease which belongs to one of the oldest symptoms in medicine and can appear after a period of excessive supersaturation of the urine with one of stone-forming salts.

Cystinic urolithiasis is the consequence of cystinuria resulting from the perturbation of the biochemical pathway of the basic amino acid called cystine. Classical cystinuria is due to an inherited metabolic lesion (aminoacidopathy) in which there is a transport defect of cystine, lysine, ornithine, arginine, homocysteine-cysteine and homoarginine (Watts, 1976; Galka et al., 1990). Cystine predominates in the extracellular fluids.

Initially it was believed cystinuria originated from an autosomal recessive pattern of inheritance. Amino acid analysis showed that cystinuria phenotype in homozygous persons comprises increased excretion of cystine but not necessarily for stone formation. The risk of stone formation depends on the urinary cystine content which has to exceed the solubility of amino acid in urine.

Usually cystine (the least soluble amino acid) is excreted in small amounts in the urine. When the rate of urinary cystine elimination exceeds 300-800 mg/L one can speak about cystinuria. This metabolic disease leads, in some conditions, to cystinic concretions formation. It is known that uroconcretions formation is based on a bioinorganic mechanism which constitute the crystalline nuclei, called "primers" or "starters". In our study the qualitative composition of calculi and than the quantitative metal composition of cystinic urolithiasis were investigated.

MATERIALS AND METHODS

Researches on cystinic urolithiasis became possible after the qualitative analysis of 234 calculi obtained from the Urological Clinic Timisoara. Some of them were surgically removed, while others were spontaneously eliminated.

In the first step we determined by infrared spectroscopy (IRS) the qualitative composition of the uroconcretions. For this purpose we obtained the "standard spectra" of the chemically pure compounds, such as: purine derivatives, oxalic acid, oxalates, cystine, cholesterol, phosphates, carbonates (compounds frequently found in urolithiasis). Then we recorded by a Specord 75 type apparatus the spectra of the studied urolithiasis. Comparing the spectra of urolithiasis with the standards, the types of urolithiasis were establishing. Details on the methods were presented in previous papers (Drăgan et al., 1981; Drăgan et al., 1985).

In the second step, by atomic absorption spectroscopy (AAS), the metal composition of the cystinic urolithiasis was found out. We used a PYE UNICAM type apparatus (Series SP 1900) whose spectral domain ranged between 189-855 nm. The concentration of the main alkaline (Na, K), alkaline-earth (Ca, Mg) and some trace elements (Zn, Mn, Fe, Cu,) were determined. For methodological details see previous paper (Gârban et al., 1981; Garban et al., 1996).

Definition of urolithiasis types was made by comparison with IR etalon - spectra for qualitative evaluation and also by a computerized system, i.e. method Pattern Recognition System (Holban et al., 1996)

The analytical determinations by IRS, in order to found out the type of urolithiasis and especially by AAS, for the metallic concentration, were made selectively for cystinic urolithiasis. Due to this fact there is not possible to make a percentage of casuistics total urolithiasis/ cystinic urolithiasis. Resulted data were processed statistically by a computerized method obtaining the mean value ($\bar{X}$) and the standard deviation (SD).

RESULTS AND DISCUSSIONS

It is known that the early formation of microcrystals presents an increased risk in the case of a supersaturated urine with various metallic ions. Though, related to the saturation, there are observed zones of supersaturation (instability), metastability and undersaturation (Robertson and Peacock, 1984). A general presentation of these zones is given in Figure 1.

Literature data showed that the frequency of cystinic urolithiasis is about 1-6% of among all the urolithiasis types, it appears in childhood (until 15 years) and may have a regional disposal. The results of a comparative study revealed that in East Germany the frequency of cystinic urolithiasis was 0,3%, in Bonn (West Germany) was 0,98%, in Paris 2,5%, in Budapest 3,1% and in Argentine 3,2% (Matouschek and Huber, 1981).

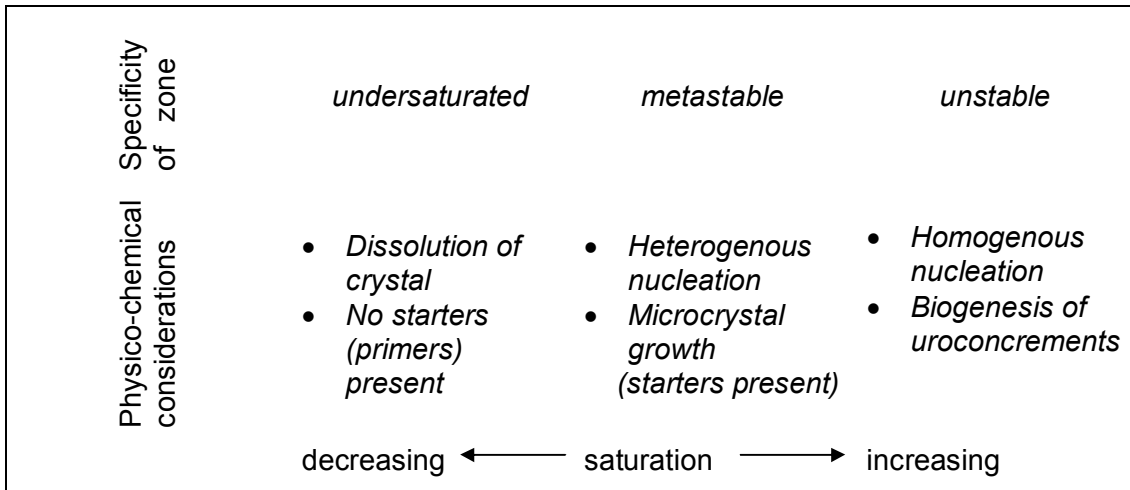


Fig. 1. Diagrammatic presentation of the relationship between the increase of saturation and uroconcrements biogenesis

Generally, the diagnostic of cystinuria in patients with urolithiasis is made at two years from the first renal colic caused by renal stone (Sakhaee, 1994). Pure cystine stones are yellow with a glistening surface and radio-opaque.

Normally, cystine is excreted in urine in low concentrations in 24 hrs, between 10-100 $\mu\text{mol/L}$. In case of cystinuria, in heterozygous patients it is increased to 20-600 $\mu\text{mol/L}$ and in heterozygous patients to 1400-4200 $\mu\text{mol/L}$. There are excreted similar amounts of lysine, ornithine and arginine and smaller amounts of homocysteine-cysteine and homoarginine (Heininger et al., 1986; Saravanan, 1997).

As fig. 2 shows, the urine of homozygous cystinurics is excessively supersaturated with cystine.

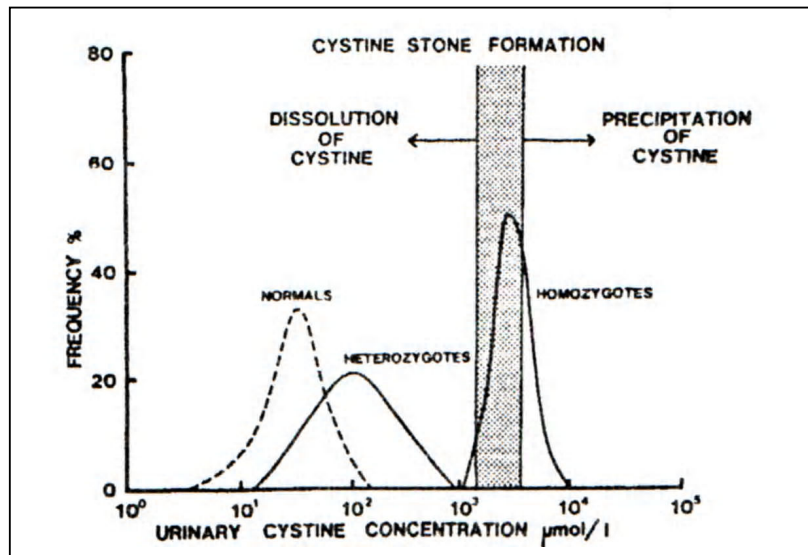


Fig. 2. Distribution of urinary cystine concentration and frequency in normal subjects, heterozygous and homozygous patients (after Robertson and Peacock, 1984)

The risk of stone formation in cystinuric patients depends on the extent to which the urinary cystine content exceeds the solubility of the amino acid in urine.

Urinary stones, or the passage of cystinic gravel or sand, are the only manifestations of cystinuria. The stones are usually multiple, they tend to recur after surgical removal, and bacterial infections often complicate the clinical signs. There is appreciable morbidity in childhood, although the third decade is the peak of symptoms. Cystinuria affects both the sexes equally, but the prognosis is worse in men than in women. In the absence of treatment the average ages of death from renal failure are 37:3 years for men and 53:8 years for women.

Pure cystine stones are yellow with a glistening surface, they are radioopaque although sometimes less so than calcium containing stones. Cystinuric patients had mixed stones or purely phosphatic stones.

Cystinuria is considered as a genetically disorder of amino acid metabolism which usually is inherited in an autosomal recessive fashion. This aminoacidopathy in which not only cystine but also the amino acids lysine, arginine and ornithine are handled inappropriately may have as a complication the appearance of cystinic urolithiasis.

For a better understanding of the aspects related to cystinuria the structural formula of the amino acids excreted in high amounts are presented in fig. 3.

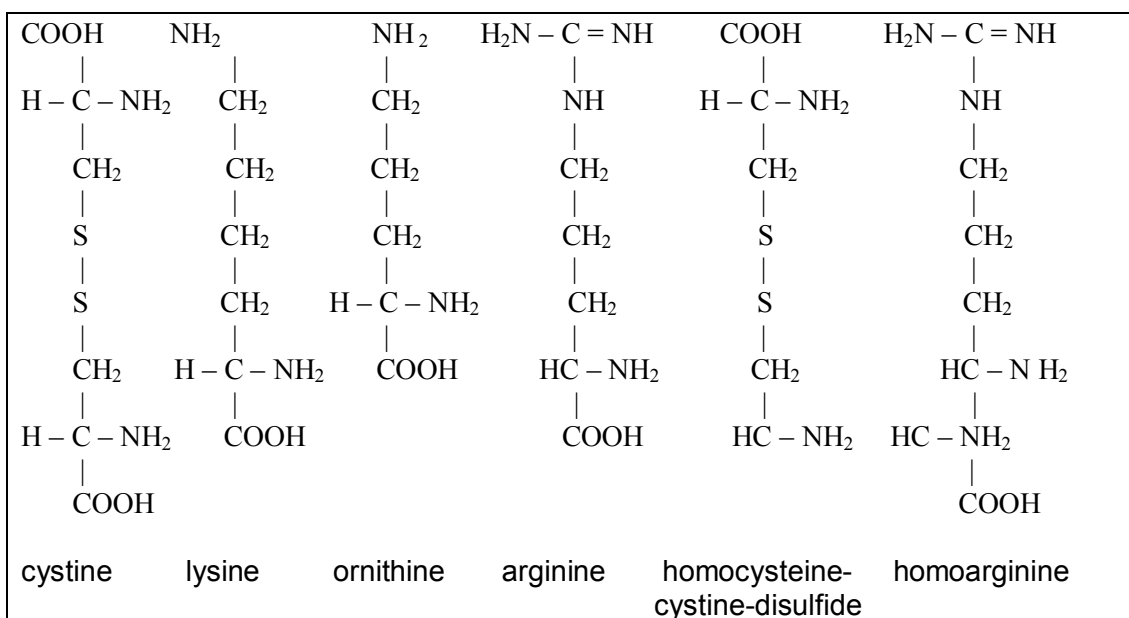


Fig. 3. Amino acids in excess decelated in cystinuria

In the urine of patients with cystinuria also an elevated quantity of homoarginine was found, considering that it is formed from lysine via homocitruline, this metabolic pathway being analogous to the formation of arginine form ornithine via citruline.

Cystine is the only one of the amino acids, increased amounts of which are excreted in cystinuria, which is sufficiently insoluble to precipitate and form stones (Lindell et al., 1995). The solubility of this amino acid in urine is between pH 5 and 7 is only about 300-400 mg/L.

Cadaverine and putresceine (diamines) intermitently are present in the urine of cystinuric patients. Some strains of *E. coli* metabolize arginine and ornithine to

putresceine, and lysine to cadavrine. These diamines are further metabolized and appear in the urine as pyrrolidine and piperidine respectively.

Numerous studies dealing with the tubular reabsorption of the amino acids: cystine, lysine, ornithine, arginine as well as the transport of this group of amino acids across the mucosa of small intestine, lead to the concept of a shared transport mechanism for the reabsorption of the mentioned amino acids from the lumen of renal tubule and to the proposition that failure of this energy mediated carrier system was the fundamental biochemical lesion in cystinuria.

In rare cases cystinuria was not associated with increased excretion of lysine, ornithine and arginine: cases in which the transport of cystine was dissociated from that of the other amino acids. Other investigations provided further evidence for the dissociation of cystine transport from the transport of other three amino acids in kidney.

Metal ions present in the blood and urine play an important role in the organism. Some of these metals can prevent the concrements formation at the level of urinary tract, gall bladder or salivary gland while the others inhibit the crystallization.

Urine is considered as a metastable medium, where organic and inorganic compounds may precipitate. Among these compound the alkaline, alkaline-earth and transitional metal ions play a major role. So, the presence of these ions in the uroconcrements reveal, indirectly, their contribution in the lithogenetic processes, at the basis of crystals nucleation and the appearance of the so-called "starters" or "primers". The role of metals in coprecipitative processes and implicitly in urolithogenesis, was noticed for the first time in 1961 by Boshamer (cited by Williams and Chisholm, 1976). Metals intervene in the crystallization process and uroconcrements formation.

Increased urinary excretion of calcium (hypercalciuria) is the most common noninfectious cause of urolithiasis in children and adults. Also, it is known that a magnesium supplementation leads to hypermagnesiuria which may prevent stone formation.

The appearance of metal elements in urolithiasis is a consequence of their presence in urine (considered as a metastable solution) where the coprecipitative processes start.

Nowadays the role of metal trace elements in the human organism and in uroconcrements is more and more studied. Some authors found that Co, Ni, Pb, Sn, V and Zn have an inhibitory effect on calcium oxalate crystallization rate while others reported that Al, Cu, Fe, Pb, Sn and Zn have no effect on calcium oxalate crystal growth. (Joost, 1987).

The first crystallization nuclei appear as a consequence of the interaction between cystine and metal ions (M^{n+}), i.e. monovalent alkaline metal ions – Na^+ , K^+ or divalent alkaline-earth metal ions – Ca^{2+} , Mg^{2+} and transitional metal ions – Zn^{2+} , Fe^{2+} , Cu^{2+} , Mn^{2+} . In this context the biochemical pathway of calcium involvement in cystinic urolithiasis, for example, is presented in fig. 4.

It is well known that the concentration of metals in the organism is due to homeostasis mechanism and may be influenced by environmental factors, by age, by accumulative processes. The excess of metals can lead to competitive interactions in the biological system modifying the normal metabolical status and sometimes inducing diseases (lithiasis, endocrinopathies, enzymopathies, homeopathies a.o.).

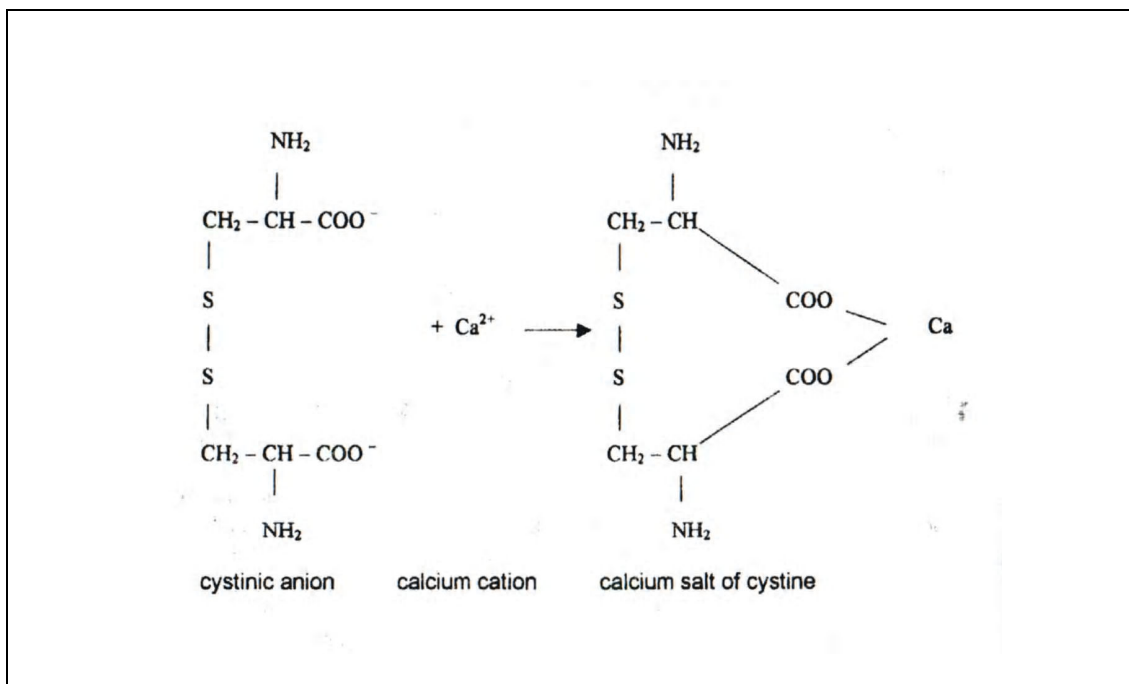


Fig. 4. Formation of the calcium salt of cystine

Metals are important components of the human body; are present in the food chain and environment (i.e. water, air, soil). The classification of the metals present in living matter takes into account their concentration in : macro- micro- and trace metallic elements. They have important role as bioconstituents of the human organism and are essential for many metabolic processes.

From chemical point of view the molecule of cystine is undissociated as Hcys in an acid medium. While the concentration is increasing a dissociation of the cystine molecule takes place, that can be expressed as an equilibrium reaction:



The H^+ ion can be substituted by alkaline metal ions (e.g. Na, K), alkaline earth metal ions (e.g. Ca, Mg), transitional metal ions (e.g. Zn, Cu etc.) In such conditions a nucleation process starts which leads to the formation of starters (primers) of cystinic nature, which ones can evolve to uroconcrements.

The metallic elements could enter in the humans organism by absorption from intestine, from respiratory tract and skin. They are distributed by blood in the liver, kindey and other organs, a part of them is accumulated and the remainder is excreted in the urine. So, by urinary tract are eliminated all the metals in excess and not necessary for organism (Williams and Chisholm, 1976; Drăgan et al., 1981; Leusman et al., 1990).

The existing metals in organism can act indirectly as effectors (activators-inhibitors) of metabolic processes or directly as bioconstituents in the formation and development of uroconcrements. In the lithogenetic processes. In the etiopathogeny of urolithiasis the metals play an important role. Their concentration is different dependent on the type of urolithiasis. The variation are due to the solubility differences of the compounds, the pH and the osmolality of the medium as well as to the morpho-functional state of the kidney and urinary tract.

Ions are present in the urine due to chronobiochemical processes specific for hydroelectrolytic metabolism. A hydroelectrolytic imbalance which implies changes in the concentration of urinary ions may be caused by an excessive food intake and by the existence of other favourising factors: infections, hormonal disturbances or anatomic malformations of the urinary tractus.

Role of the metals in urolithogenesis can be investigated indirectly by analytical methods applied to uroconcrements and finding out their metal concentration. The determination of concentration of the main alkaline, alkaline-earth is presented in table 1.

Table 1. Metallogram of cystinic uroconcrements (alkaline and alkaline-earth metals)

Metals		UM	No. of cases	Concentration of metals $\bar{X} \pm SD$
Alkaline	Na	$\mu\text{g/g calculus}$	23	239.84 ± 128.17
	K		23	610.37 ± 214.01
Alkaline-earth	Ca		23	3205.97 ± 1987.14
	Mg		23	315.83 ± 172.81

Determinations regarding transitional metals, that exist in more reduced quantities are presented in table 2.

Table 2. Metallogram of cystinic uroconcrements (transitional metals)

Metals	UM	No. of cases	Concentration of metals $\bar{X} \pm SD$
Zn	$\mu\text{g/g calculus}$	20	38.96 ± 12.09
Fe		20	17.02 ± 2.03
Cu		19	12.89 ± 2.98
Mn		20	5.01 ± 1.14

The presence of cystine in elevated concentration, of metallic ions as well as a protein and glycoprotein "matrix" in the urine allow the "straters" or "primers" formation. The terms "starter" or "primer" define the initial nucleus of crystallization. Generally these nuclei evolve to uroconcrements by the bioinorganic mechanism of lithogenesis.

CONCLUSIONS

1. Investigations on the qualitative composition of uroconcrements made by means of infrared spectroscopy permitted the establishment of the types of urolithiasis and the isolation of the cystinic urolithiasis for further investigations.
2. Atomic absorption spectroscopy used in the determination of the quantitative metallic composition of cystinic urolithiasis revealed a specific metallogram.

3. The metallogram of cystinic urolithiasis showed the followings:

- a) in the case of alkaline metals $\text{Na} < \text{K}$;
- b) in the case of alkaline-earth metals $\text{Ca} > \text{Mg}$;
- c) in the case of trace metals $\text{Zn} > \text{Fe} > \text{Cu} > \text{Mn}$

The series of decrease in the studied metals concentration could be characteristic for this type of urolithiasis.

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FORTEEN YEARS OF RESEARCH OF SELENIUM DEFICIENCY IN SERBIA

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ABSTRACT

Data on selenium (Se) deficiency in Serbia are presented after 14 years of research. The results include Se content in the ores, rocks, stream sediments, soils, cereal crops grown on these soils, food and in human population from 55 communities. Most of the results indicate a serious Se deficiency. In some communities the Se content of grain and human serum and scalp hair approach those of the low-Se belt in China. It is assumed that an extremely low Se status in the human population could be a risk factor for development of Balkan Nephropathy (BN), and very high incidence of Urinary Tract Tumors (UTT) in endemic areas, as well as the high mortality rates of malignant diseases. Some regions, on the other hand, with relatively higher serum Se status, have significantly lower mortality rates from cancer.

Key words: Se deficiency, soils, crops, human tissues.

INTRODUCTION

Deficiency or imbalance of bioessential trace elements, weather caused by geochemical processes or by human activities, has been shown to present a significant problem for the health of man and animals. In this respect Se stands as an outstanding example of the relation between geochemistry and human health.

In Serbia veterinarians were first to record areas with Se deficiency for domestic animals in the early sixties. They applied Se preparation for prevention and therapy of livestock in certain regions, especially in the Pester Plateau (Vujic, 1965). This work was followed by determination of Se in some forage crops, which showed a very low Se content of corn in some areas of the former Yugoslavia. These results, however, were practically unknown to the geochemists, nutritionists and medical doctors in this country, until it was established in the early eighties that Se is also essential for humans (Yang et al., 1984).

After 14 years of study of the Se status in geological and biological materials, and in the human population, Se deficiency has been revealed in most parts of Serbia. In this article, some of these results are presented and discussed.

MATERIALS AND METHODS

During the long period of study a large number of samples was collected, of stream sediments, rocks, surface soils, wheat, corn, and garlic grown on these soils, as well as human tissues from 55 communities in Serbia. Selenium was determined by

AAS and description of samples and methods was given elsewhere (Maksimovic et al., 1985; Maksimovic et al., 1992b; Radosevic et al., 1995)

RESULTS AND DISCUSSION

Geological materials

Sulfide ores

Selenium is a chalcophile element which, under endogenic conditions, enters the structure of sulfides as a substitute for sulfur. It is concentrated, therefore, in sulfide minerals and ores in numerous deposits and mineralizations in Pb–Zn–Sb– and Cu–geochemical provinces in Serbia, including the largest deposits of Pb–Zn, Sb and Cu in Europe. The highest concentrations of Se were found in sulfide minerals of the copper mines Bor and Majdanpek in the east Serbia. The commercial source of this element is the anodic slime from electrolytic copper refining. The production of refined Se was between 50 and 70 tons per year, and has been maintained for about fifty years. From 1990 on began the production of Se–salts, including sodium selenite (Na_2SeO_3) “food grade“, and sodium selenate (Na_2SeO_4) “for microfertilizers“.

Stream sediments

The first large scale geochemical investigation of Se in Serbia started using stream sediments (Maksimovic et al., 1985). Due to numerous sulfide deposits and mineralizations all over the country, a relatively high Se content in stream sediments, fraction $<50\ \mu\text{m}$, was expected. However, the Se content was low (average $229\ \mu\text{g/kg}$), and more or less uniformly distributed in active river sediments. It was lower than the mean Se content of soils in many countries. The low Se content of the stream sediments, in spite of the contribution of Se by numerous sulfide deposits and mineralizations all over the country, was the first indicator of Se deficiency in the large part of Serbia.

Igneous, metamorphic and sedimentary rocks

Selenium was determined in 285 igneous rock samples, including intrusive and volcanic rocks (Maksimovic et al., 1992a). The most widespread igneous rocks are those of intermediate composition. Most of the sulfide deposits and mineralizations were genetically connected with these rocks. After deposition of sulfides they have remained poor in Se, especially their volcanic equivalents, which were extremely depleted in this element ($\bar{x} = 22\ \mu\text{g/kg}$, $n = 90$). Such a low Se content is a cause of low Se status in soil derived from these rocks. It is a paradoxical situation that in the east Serbia, where the copper mines are located, the only producer of Se, there is a serious Se deficiency in rocks and soils.

The average Se content in 89 samples of metamorphic rocks in Serbia is $28\ \mu\text{g/kg}$ (Jovic et al., 1995). Such a low Se content may cause a low Se status of soil derived from them.

Sedimentary rocks were not the subject of a large scale research on Se. Still some important information came from the study of loess, the most widespread surficial geological formation in the Yugoslav part of the Pannonian Basin (Dangic et al., 1995a). The average Se content in loess of $26\ \mu\text{g/kg}$ is very low and soils derived from it are also poor in this element. As these regions are the most important agricultural and

food producing areas, Se deficiency and related health problems could be expected. In China, the Loessial Plateau is one of the major regions with Keshan and Kaschin–Beck diseases (Tan et al., 1996).

Soils

Selenium content in soils derived from volcanic rocks is low, $\bar{x} = 185 \mu\text{g/kg}$, $n = 11$. Other investigated soils were derived from sedimentary rocks of different age and composition. They are all poor in Se. The average Se content of 230 soil samples from 42 communities is $203 \mu\text{g/kg}$, a value about half those found in soils from other regions of the world. The range of Se in Serbian soils, $39\text{--}440 \mu\text{g/kg}$, shows that the highest value obtained is less than $500 \mu\text{g/kg}$, which is considered the upper limit for Se deficient soils (Mayland et al., 1989). pH of soils varies from 4.45 to 8.33, the acidic to neutral soils occurring most frequently. These conditions promote a low availability of Se in plants. The highest pH of soil was found in the southern Banat, in the north Serbia, where the amount of extractable Se was the highest, 27.6 % of the total (Maksimovic et al., 1992b).

Waters

River waters are poor in Se. In 11 samples of different rivers the range was $0.09\text{--}0.3 \mu\text{g Se/L}$, with the average $0.2 \mu\text{g/L}$ (Dangic et al., 1995b). Ground waters from 8 samples had an average of $0.26 \mu\text{g Se/L}$. Waters, therefore, could not be the source of this element in Serbia.

Selenium in crops

Samples of wheat, corn and garlic, grown on investigated soils, were collected from several agricultural regions in 30 communities.

Selenium content in wheat is low and ranges from 3.6 to $65.5 \mu\text{g/kg}$ with a mean value $20.0 \mu\text{g/kg}$ from 67 samples. There are wide variations of Se content in wheat in various regions: for example, in Banat area the average Se content is $25.2 \mu\text{g/kg}$, while the lowest value originates from hilly areas in western Serbia ($\bar{x} = 9.4 \mu\text{g/kg}$), where the pH of soil is acidic.

Corn is very poor in Se, in the range $2.0\text{--}82.0 \mu\text{g/kg}$, with an average of $12.6 \mu\text{g/kg}$ from 88 samples. Most of the communities had extremely low Se levels in corn ($< 10 \mu\text{g/kg}$). They correspond to values found in corn in the low–Se belt in China (Tan and Huang, 1991).

Garlic is known to accumulate more Se than most other crops. Garlic and soil were collected from 75 sites in 22 communities. The analyses revealed wide variations of Se in garlic, with a mean content of $12.6 \mu\text{g/kg}$. The highest content was found in three communities in Banat, with an average of $35.4 \mu\text{g/kg}$ from 13 sites. In these cases soil was alkaline and extractable Se was high. However, most of the communities had extremely low Se levels in garlic ($< 10 \mu\text{g/kg}$) not reported in the literature so far.

An example of a Se deficient community is Barajevo, situated 25 km south of Belgrade. It comprises 12 villages in a picturesque countryside, where many weekend cottages were built. However, soil and crops are very poor in Se, with comparable or even lower values than those in a Se deficient area in China (Table 1). Se deficiency was found in the human population, as well as related health problems (Maksimovic et al., 1995a).

Table 1. Se content in soil, grain and garlic in the rural community Barajevo (Belgrade), compared with a Se-deficient area in China, Buteha Qi, Inner Mongolia (Tan and Huang, 1991).

Specification	n	Se, µg/Kg Range	Mean ± SD
Barajevo			
Soil	16	39.0 – 331.0	157.0 ± 77.0
Wheat	9	12.4 – 27.0	16.9 ± 4.6
Corn	9	2.0 – 4.2	3.3 ± 0.8
Garlic	9	1.5 – 10.5	4.9 ± 2.9
Se – deficient area, China			
Soil	14		143.0
Wheat	11		12.8 ± 8.0
Corn	12		25.0 ± 16.0

Food

Confirmation of the results on Se content in crops came from another study. During spring and winter 1991, samples of wheat, wheat flour, potatoes, milk and pork were collected in Serbia according to the sampling protocol designed by the FAO European Research Network on Trace Elements. These were pooled to obtain nationally representative sample. The results were lower than in most of the European countries (Djujic, 1996).

Analyses of food items collected in 4 different regions of Serbia during the 1996–1997 period showed that concentrations of Se in analyzed animal products were significantly lower than in samples collected in 1991 (Table 2). The reason is in the reduction of Se supplements for the livestock in the period 1996–1997 because of economical problems. Results obtained for the both period, however, indicate a very low Se intake for the Serbian population compared with Recommended Daily

Allowances (US RDA), and similar to those in the Finish diet before soil

Table 2. Concentration of Se in food from Serbia and dietary intake

S a m p l e	Se, µg/kg	
	1991–1992	1996–1997
Wheat	23	23
Corn	13.5	12
Potatoes	8 ¹	7 ¹
Milk	98 ²	52 ²
Pork	357 ²	232 ²
Beef	310 ²	176 ²
Average daily intake	26.7 ³	25.6 ⁴

¹ air dried; ² freeze dried powder; ³ µg/day Se calculated on the basis of data for Se in foods, household budget survey and cooking loss; ⁴ µg/day Se on the basis of 7 days duplicate diet samples.

Allowances (US RDA), and similar to those in the Finish diet before soil fertilization with selenium (Kumpulainen et al., 1987).

Investigation of Se intake with 7–days duplicate diet carried out in 15 healthy adult persons (the average age 22) in three different annual periods has shown the following average daily intake of Se: in 4 volunteers were below 21 µg/day, in 8 volunteers between 21 and 41 µg/day and only in 3 of them over 41 µg/day.

Selenium in human population

Interchange of foodstuffs occurs in Serbia from one district to another. However, low Se content in soil, grain in the main agricultural regions, and very low daily intake is reflected in the low Se status of the human population. Here, also, large variations exist.

The results obtained during the last 10 years for Se in blood, serum/plasma, erythrocytes, scalp hair and daily urine samples of adult healthy population in Serbia are presented in Table 3. The mean values are lower than those reported in other countries.

Table 3. Se concentration in healthy population in Serbia

S a m p l e	n	Sex	Se, µg/L	Se, µg/kg
Whole blood	462	M+F	58.2 ± 18.3	98.0 ± 15.7
Serum/plasma	1681	M+F	52.9 ± 16.5	
Erythrocytes	1288	M+F	61.8 ± 19.6	
Scalp hair	472	M+F		
Urine	678	M+F	14.8 ± 4.5 ¹	

¹ µg/day

A high correlation was found between Se levels in blood and scalp hair ($r = 0.87$, $p < 0.001$), serum/plasma and scalp hair ($r = 0.82$, $p < 0.001$), and erythrocytes and scalp hair ($r = 0.77$, $p < 0.001$). As a result of obligatory addition of Se to animal feed since 1989 and the popularity of self-medication with commercial Se preparations, there was a slight increase of serum Se in the Serbian population between 1990 and 1996. However, the mean serum Se value, obtained in 55 communities, including the Belgrade area ($\bar{x} = 52.9 \pm 16.5$ µg Se/L, $n = 1681$) is still lower than that in serum of the Finnish population before fertilization of the soil with Se (55.3 µg/L ± 18.2 , $n = 964$; Virtamo et al., 1987). It is almost 50 % lower than data from the literature for the world blood Se concentration (Iyengar and Woittiez, 1988).

The lowest serum Se values in Europe were found in Serbia, lower than in the neighbouring countries: Bulgaria 54.8 µg/L, Hungary 55.8 µg/L and Croatia 64.2 ± 11.5 (Alfthan and Neve, 1996).

Se deficiency and possible effects on health

Epidemiological studies of population in the Western countries have shown that a suboptimal Se intake, as determined by serum or toenail selenium, is inversely associated with an increased risk of death from cardiovascular diseases and cancer (Aaseth, 1996). According to data obtained, in 78 % of investigated communities in

Serbia the mean serum Se is below 50 $\mu\text{g/L}$, and in 26 % is less than 40 $\mu\text{g/L}$. Thus a significant number of inhabitants in this country may have an extremely low Se level.

There is no record in Serbia of a specific disease in humans caused by Se deficiency. However, a serious Se deficiency in rural inhabitants could be a major risk factor in the development of Balkan Nephropathy (BN) and high incidence of Urinary Tract Tumors (UTT) in endemic areas (Maksimovic, 1991; Maksimovic et al., 1955b). Geographic correlation between BN and UTT was established both in the former Yugoslavia and Bulgaria (Petkovic et al., 1971; Chernozemsky et al., 1977).

A high incidence of different human cancers, as well as BN, occurs in some communities and areas with a serious Se deficiency in crops and human population. One of these cases is the rural community Barajevo, near Belgrade. It is one of the areas with the lowest Se content in soils, crops (Table 1) and serum Se ($\bar{x} = 38.1 \pm 10.9 \mu\text{g Se/L}$, $n = 18$; Maksimovic et al., 1995a). In this rural population the average cancer incidence rate, for the period 1985–1991, was 664/100.000 – higher than in the old urban community Stari Grad, in Belgrade, with much older population (569/100.000). It is also important to note that the rural community Barajevo is part of the region with a high incidence of BN. Another region with a serious Se deficiency is in the eastern Serbia, in the districts of Zajecar and Bor. In this area the total mortality rates in 1988 was the highest in Serbia (1263/100.000), with a high mortality from malignant diseases, and villages with a high incidence of BN. Serum Se level in human population is very low (Table 4).

Table 4. Mortality rates (1: 100.000) of death from malignant diseases in some communities of Serbia (1978–1988) (Djordjevic et al., 1992) and serum Se concentration of healthy adults

Communities	Mortality rates	Mean Serum Se $\pm$ SD	n
Belgrade area	144	54 ± 15	662
Barajevo ¹	181	38 ± 11	18
Zajecar ¹ and Bor district (East Serbia)	150	38 ± 18	59
Zlatibor area (West Serbia)	108	63 ± 15	158

¹ High incidence of cancer and Balkan Nephropathy

In these two areas a large number of inhabitants have extremely low Se levels, approaching those found in the low-Se belt in China. In both areas a high percentage of UTT in patients and villages with BN, and the high incidence of other human cancers, are indicative of a common co-factor, promoting both cancerogenic and nephropathogenic agents. So far, Se deficiency is the only observed common denominator in both areas.

Data on the mortality rates from malignant diseases (Table 4) are also indicative: the higher the serum Se the lower mortality rates. The lower mortality rates of cancer and cardiovascular diseases were found in the Zlatibor district, in western Serbia, where the serum Se level was the highest (Maksimovic et al., 1998).

CONCLUSIONS

From this long period study of Se in Serbia the following conclusions could be made:

1. Serbia is the biggest producer of Se in Europe from the sulfide copper deposits in the eastern part of the country. On the other hand, the other geological materials all over the country, including: stream sediments, igneous, sedimentary and metamorphic rocks, soils on these rocks, and waters, are poor in Se.
2. Crops are also poor in Se, in some regions approaching those of the Se-deficient belt in China.
3. Low Se status in crops is the cause of the low Se concentration in food, and in very low average daily Se intake of Serbian population (25.6 µg/day, in 1996–1997).
4. Low Se content in food is reflected in low serum Se status of the human population, the lowest in Europe (52.9 ± 16.5 µg Se/L). Very low serum Se status in some regions could be a risk factor for development of Balkan Nephropathy and very high incidence of Urinary Tract Tumors in endemic areas, as well as high mortality rates of malignant diseases.

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TRACE ELEMENT INDUCED CLINICAL CONDITIONS IN ANIMALS AS MODELS FOR THE HUMAN MEDICINE

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ABSTRACT

A review is made of the main trace elements induced clinical conditions in animals with some of their corresponding diseases in human. The iron deficiency of baby pigs is a peculiar example for the iron deficiency of the human infants, despite the fact that the physiopathological patterns of the two conditions are different. The copper deficiency is related in bovine with the myocardial sclerosis and with severe atheromatous like alterations in chicken; it's significance in the pathogenesis of human atherosclerosis was recently noted. In calves a genetical disturbance of the zinc absorption is known in the so-called Itai-itai disease (chronic gastroenteritis and osteomalacia) and for osteomalacia in chicken and maybe in other species. The selenium deficiency induced the well known nutritional myodystrophies in mammals and birds, corresponding to some muscular dystrophy in human as it was signaled in China. The primary and / or secondary iodine deficiency is the main cause of the goiter in human as well as in animals, with an endemic pattern in some definite areas. Excessive fluorine is responsible of the early dental fluorosis in bovine animals, but also in human. And finally the excess of lead acts as a chronic toxicant with encephalopathy and amaurotic epilepsy in human as well as in dogs.

Key words: trace elements, deficiency, excess.

INTRODUCTION

The early recognition of many clinical entities in different animal species offered the possibility to extrapolate some of their pathogenic peculiarities in human medicine, with suggestions to preventative and therapeutic protocols.

IRON DEFICIENCY ANAEMIA

One of the best studied iron deficiency syndromes is that which affects the baby pigs. In fact, piglets denied access to other iron sources than sows milk develop anaemia within 2-4 weeks postnatum. Their hemoglobin levels drop from about 10g/dl to 3-4 g/dl. The breathing becomes labored, the skin has parchment-like colour and some of the sick piglets die. The surviving animals recover only slowly and incompletely but only as they begin to eat solid food. The piglet anaemia is often complicated by *E. coli* infection and toxico-sis. The disease is due to: a. low iron content of the body, including the liver stores; b. the relatively low iron content of the sows colostrum and milk; c. the high growing rate.

The iron deficiency in human infants is the cause of the anaemia at 4-24 months of age. It is due to a true iron depletion by a high rate of iron excretion through the faeces.

Both conditions respond to iron therapy, either in injectable form or by oral administration of iron preparations.

COPPER DEFICIENCY

A congenital copper dysmetabolism in children is known as Menkes disease (trycho-polydystrophy) which is characterized by a progressive degenerative encephalopathy

encephalopathy which is lethal under 3 years of age. The main symptoms are hypotonia, "inky" hairs ("pili torti"), hypothermia, progressive disfunction of the medulla oblongata, a scorbut-like osteopathy, and a generalised arteriopathy consisting in arterial occlusions and fragmentations of the internal elastic lamina. The blood copper level is 25% lower than normally, as well as the liver copper; the urinary copper excretion is high. Hypothetically, there is a disturbed intestinal absorption of copper.

Although not identical with the above mentioned condition, we found in chicken experimentally deprived of copper fragmentation of the elastine in the aortae as well as an scorbut-like osteoporosis which responded well to a copper supplementation (Ghergiu, 1972).

In fact, the copper deficiency is responsible for severe cardiovascular disturbances in animals. The deficiency is responsible for the myocardial sclerosis in adult bovine with sudden death (falling disease) which is copper responsive. The experimental copper deficiency in pigs is associated with vascular disturbances due to a defective elastinogenesis very similar with the alteration in chicken.

In human patients there are data attesting the involvement of copper (and zinc) in atherosclerosis either acting directly on the arterial wall or by rising the cholesterolemia (Howari, 1993).

COPPER EXCESS

A true natural copper intoxication is rarely encountered in human and in monogastric species. However, in human a genetic copper dysmetabolism is known, namely the Wilson's disease (the hepatolenticular degeneration). The disease is transmitted as an autosomal recessive gene, which is associated with an excessive urinary depletion of copper, low serum copper and ceruloplasmin levels and a high copper accumulation rate in the liver, the brain and the kidneys.

The blood copper is as low as 40-60 $\mu\text{g/dl}$, the ceruloplasmin between 4 and 20 mg/dl and the urinary copper excretion may be as high as 17-60 $\mu\text{g/day}$ together with aminoaciduria.

A very similar disease in dogs is known as the "hereditary progressive hepatic dystrophy" or as the "copper intoxication of Bedlington terriers". Although the disturbance was initially described in this breed, later it was noted in other breeds too. In the Bedlington terriers the disease is transmitted as an autosomal recessive gene, but this way of transmission was not demonstrated in other breeds.

The main clinical symptoms are of hepatic insufficiency. There is an excessive copper accumulation in the liver, above 2,000 $\mu\text{g/g}$ DM instead of about 350 $\mu\text{g/g}$ but the blood copper level is normal as well as the alimentary copper intake. It seems that the pathogenesis consists in a defective copper lysosomal excretion.

ZINC DEFICIENCY

In both human and bovine there are two genetic zinc deficiencies namely the acrodermatitis enteropathica in human infants and the Adema disease in calves. Both of them are the consequence of an autosomal recessive gene whose effect is poor zinc transfer from the mucosal surface of the gut to the serosa.

Acrodermatitis enteropathica was described in maternal milk-deprived human infants but also at weaning. There are eczema-like symmetrical skin lesions from bulae to verrucous plaques especially at the extremities of the legs. Frequently total alopecia, stomatitis, conjunctivitis with photophobia and mental retardation were also noted. The sick children are particularly sensitive to various infections due to the thymic hypoplasia.

Adema disease (congenital thymic hypoplasia, congenital parakeratosis) was described in 1-2 months old Friesian calves. The main lesions are prekeratotic with a preferential but not exclusive localization on the hind legs, but also of the oral mucosa. The sick calves have a low cellular-mediated humoral immunity and if not treated they die within 3-4 months after birth, by intercurrent infections.

In both acrodermatitis and parakeratosis there is an accumulation of zinc inclusions in the Paneth intestinal cells attesting the low transfer of zinc to the serosa. In both diseases the blood zinc levels are low, as well as the alkaline phosphatase activity.

Interestingly the feeding of the sick children with human milk may alleviate partially the symptoms due to a ligand which may improve the zinc transfer through the intestinal wall. Such an effect is not known in the case of the cow's milk. The oxyquinoline drugs may exert beneficial effects but the healing seems to be conditioned only by a high zinc intake, at least in calves.

An acquired natural zinc deficiency syndrome is hardly known in human. Earlier reports of Prasad et al. from the sixtieth described a syndrome in young adults and youngsters subsequent to a high intake of phytates, consisting in a retarded growth and sexual maturity, hepato- and splenomegaly, slow ossification of the skeleton, anaemia, low serum and hair zinc and iron levels. But low serum zinc levels may be associated with many other conditions in human like kwashiorkor, burns, intestinal malabsorption, total parenteral feeding and so on, which hardly may be recognized as a true zinc deficiency (Ghergariu, 1980).

The natural zinc deficiency is known in calves and pigs as an acquired parakeratosis of the skin. It is due mainly to high levels of Zn-antagonists in feed, like Ca, phosphates, phytates, Cd, Cu, Mo, Fe, various chelators, MTU, vitamin D and so on. Raising the Ca level to 1.3% in the feed, the parakeratosis may be induced in 90% of the pigs.

CADMIUM EXCESS

The acute cadmium toxicity syndrome is known in human especially as a result of its inhalation in a contaminated environment. It is illustrated by bronchial irritation, cough, dyspnea and pulmonary emphysema. In the subacute and chronic exposure the main symptoms are a yellowish pigmentation of teeth, loss of smell and gustative capacity, chronic bronchitis and pulmonary emphysema and bone painful fragility.

On the other hand the eating of cadmium-contaminated rice was responsible in Japan for the so-called "Itai-itai" disease characterized by chronic gastritis and osteomalacia.

Such syndromes are known in animals only in experimental conditions and only following the oral intake of cadmium. In fact, cadmium supplemented diets induced osteomalacia-like bone alterations in chicken and pigs.

SELENIUM DEFICIENCY

The selenium deficiency, alone or in combination with a vitamin E deficiency is related with many clinical conditions in animals. Among them the nutritional myodystrophy (NMD) which was diagnosed under natural conditions in ruminants, pigs, horses and hens. NMD is characterized by a cerous (hyaline) muscular degeneration of Zenker type which may be fatal especially if the cardiac and respiratory muscles are involved.

The most prominent Se-related condition in human is the so-called Keshan disease which is endemic in certain regions of China. It is a cardiomyopathy characterized by heart failure, cardiac enlargement, ECG abnormalities, gallop rhythm and even fatal cardiac shock. The Se in blood of the Keshan patients was in the range of 0.020-0.026 µg/ml and in the hair of 0.07 to 0.12 µg/g instead of 0.027 µg/ml in the blood and 0.16 µg/g in the hair of normal patients in the same area. The levels for Se-adequate population is 0.095 µg/ml blood and 0.36 µg/g hair. Treatment of Keshan disease was successful with a weakly dosage of 0.5-1.0 mg sodium selenite (Koller and Exon, 1986).

SELENIUM TOXICITY

May be the most ancient description of selenotoxicosis in animals was made by Marco Polo seven centuries ago, during his historical trip to China. In fact he described a disease in horses characterized by deformed and sloughed horn which was recognized later to be a chronic selenotoxicosis or the so-called "alkali disease". It consists mainly in lack of vitality, anaemia, stiffness of joints, roughened hair coat, lameness and deformed hoofs in herbivorous animals.

There is an acute type of Se-toxicosis namely the "blind staggers". The animals exhibit impaired vision, abdominal pain, anorexia, ataxia, paralysis and death. Both are due to the consumption of some vegetables which accumulate Se like *Astragalus*.

Yatrogenic Se-toxicity is not unusual in animals overdosed with Se-preparations.

In human the Se toxicosis is associated with a garlic odour of the breath, thickened and brittle fingernails with white spots and longitudinal streaks, the hair is dry, brittle and easily broken, red and swollen hands and feet (they may blister and ulcerate), excessive tooth decay, convulsions and paralysis.

IODINE DEFICIENCY

The primary iodine deficiency is well known both in human and animals throughout the world in some geographical areas. The main clinical expression is the simple nontoxic goiter as it was demonstrated in humans as well as in animals.

It is interesting that as we found it in piglets and in calves, the goiter is pronounced only in newborn and in young animals where the enormously enlarged thyroid may induce the death by asphyxy (Ghergariu et al. 1993; Ghergariu and Roca, 1995). In adult animals the iodine deficiency is infraclinic and their thyroids are moderately enlarged only. But even in such dramatic cases the iodine supplementation may alleviate the symptoms.

However it must be underlined that in animals an important role is played by the goitrogenic substances contained by some feed like many cruciferous plants and the incompletely heat-treated soya beans. They may precipitate the appearance of the goiter and may accentuate its severity.

FLUOROSIS

The plants are rarely responsible for fluorosis both in man and animals unless they are contaminated either by the soil high fluorine content or by industrial dusts, fumes or waters (Underwood, 1971).

In animals especially in ruminants the dental fluorosis is a very early symptom and in this respect the bovines are considered as a "sentinel species". The teeth become modified in shape, size, colour, orientation and structure. The incisors are pitted and the molars irregularly abraded.

Later, the osseous lesions consist of exostoses, particularly of the jaw and long bones, together with a general thickening and change in shape of the bones. The animals are reluctant to walk, the joints are stiff and painful.

In human the dental fluorosis is also very well known and consists in mottled enamel of the teeth which become mottled with chalky-white patches and secondarily with a brown infiltration. As in animals only the permanent teeth are affected and appear only during their formation. In the case of a severe fluorine excess the skeleton may be involved too: osteosclerosis, calcification of ligaments and tendons, exostoses. The involvement of the fluorine deficiency in the pathogenesis of the dental caries is still questionable in animals like in human.

LEAD EXCESS

The most sensitive species to even a moderate lead excess in the environment is the horse which is considered the "sentinel" of the industrial contamination. Horses are affected by an extremely dramatic syndrome consisting mainly in pharyngeal and laryngeal paralysis which may be fatal either by asphyxia or by aspiration pneumonia. The lead poisoning in bovines is peracute or acute with dramatic symptoms of a cortical syndrome: teeth grinding, salivation, dromomania, muscle tremor and fasciculations, sometimes even rabiform behaviour and death.

The lead poisoning in dogs and primates is very similarly manifested with that of the human: cyclic anorexia, abdominal pain, loss of weight, vomiting, diarrhea or constipation and a variety of nervous symptoms, like amaurotic epilepsies, convulsions, ataxia, paralysis of the hind leg and so on.

The chicken are extremely resistant to the lead excess. Indeed doses as high as 1000 ppm are not harmful with the exception of a drop of the weight gain.

Table 1: Trace element induced syndromes in man and animals

Involved trace element	Clinical syndrome in man	Nature of the disturbance	Corresponding clinical syndrome in animals/species	Nature of the disturbance
Copper	Menkes' disease: progressive encaphalopathy, scorbut like	Autosomal recessive sexlinked	Enyootic ataxia (swayback) in lambs, kystik-gelatinous degeneration of the white matter of the brain dysmyelination	Natural, primary or secondary Cu-deficiency
	Atherosclerosis	Natural, Cu + Zn (?) deficiency	Fragmentation of the internal elastic lamina of the arterial wall; dissecting aneurysma chicken, pigs	Experimantal Cu-deficiency
			Myocardial sclerosis (<i>falling disease</i>) in bovine	Natural Cu-deficiency
	Wilson's disease (<i>hepatolenticular degeneration</i>) High Cu levels in liver, kidneys, brain Blood Cu level normal or even subnormal	Autosomal recessive	"Cooper intoxication of the Bedlington Trriers" Hereditary progressive hepatic dystrophy (others breeds) High Cu levels in liver (above 2000 µg/g DM) Normal blood Cu level	Autosomal recessive in Bedlingtons Genetic (?)
Zinc	<i>Acrodermatitis enteropathic</i> Eczema – like simmetrical skin lissions High sensitivity to intercurrent infections (thymic athrophia) Low blood Zn and alkaline phosphatase	Genetic	Adema disease (congenital parakeratosis), calves Parakeratotic skin lessions especially of the hind legs Thymic atrophia. Low cell-mediated immune response High sensitivity to intercurrent infections Low blood Zn and alkaline phosphatase	Genetic (autosomal recessive)
	Natural zinc deficiency syndrome in youngster due to excessive phytate intake. Retarded growth and sexual maturity, deffec-tive ossifications, hepato + spleno-megaly, low blood and hair levels	Phytate excess in the food (?)	Acquired parakeratosis in calves and pigs Parakeratotic lessions of the skin, thymic involution, low immune response Oesophagogastric ulcera in pigs (?) Perosis in chicken and turkeys	Natural Zn deficiency due to high Zn-antagonists intake (Phytates, especially Ca) The syndrome may be experimentally induced in pigs by rising the Ca intake to 1,3% in the diet. Experimental in many species (goats, rats, horses, dogs)
Cadmium	Acute toxicity – bronchial irritation, pulmonary emphysema Suacute chronic – same respiratory symptoms+bone painful fragility,	Inhalation of dusts or fumes with high Cd content Consumption of Cd contaminated	Unknown Osteomalacic-like lessions, chicken and pigs	Experimental high oral Cd intake

	infertility "Itai-itai": chronic gastritis osteomalacia	rice		
Selenium	Keshan disease, endemic in some parts of China: heart failure, cardiac enlargement, may be fatal cardiac shock Se in blood: 0,02-0,26 µg/ml (N=0,027- 0,095); hair: 0,07- 0,12 µg/g (N=0,36) Se-toxicosis: thickened and brittle fingernails and hair, swollen extremities, excessive tooth decay, convulsions, paralysis	Primary se- deficiency in the food Excess Se- content of the food (accidental)	Nutritional myodystrophy in many mammals and birds Acute: blind staggers, cyaloreea, amaurosis, respiratory failure (fatal) in horses, cattle and pigs Chronic: alkali disease, depression, emaciation, rough hair, alopecia, brittle and deformed hoofs, all in herbivorous species in pigs	Natural Se deficiency, frequently combined with an excess of polyunsaturated fatty acids (PUFA) Frequently iatrogenic- overdosing of Se- preparations Consumption of vegetables with high Se-content; accumulating plants like Astragalus
Iodine	Simple non-toxic goiter	Low iodine intake through food and water	Simple non-toxic goiter	Primary iodine deficiency Goitrogenic substances in cruciferous plants Other goitrogens
Fluorine	Dental fluorosis, skeletal fluorosis	High F intake due to industrial contamination	Dental fluorosis, skeletal fluorosis Cattle particularly sensitive ("sentinel species")	High F intake due to soil or industrial contamination
Lead	Cyclic anorexia, abdominal pain, loss of weight, vomiting, diarrhea or constipation, "amaurotic epilepsy", paralysis of the inferior legs, dental abnormalities	Industrial contamination, cosmetics, water pipes, cigarette smoking (inhalation, oral intake)	Horses very sensitive ("sentinel species"): pharyngeal and laryngeal paralysis, asphyxia, aspiration bronchopneumonia Cattle Acute: a cortical syndrome primates, dogs: like in human	Industrial contamination, lead containing paints, lead from feed containers, motor vehicle exhaust (oral, intake, inhalation?)

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EFFECTS OF TI-ASCORBATE ON HEAVY METAL INDUCED CARBOHYDRATE METABOLISM IN WHEAT SEEDLINGS

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ABSTRACT

Hydroponically-grown seedlings of wheat (Triticum aestivum L.) were exposed to Cd²⁺ (10⁻⁵, 10⁻⁴, 10⁻³ M) and Cd-Ti ascorbate stresses. Total water-soluble carbohydrate (WSC), glucose and fructose content were determined from samples of leaves and glucose and fructose were from roots. Cadmium treatment caused significantly higher, concentration-dependent total water-soluble carbohydrate content in wheat seedlings with the highest increment at 10⁻³ M concentration. Increase in soluble sugar of 10⁻⁵ M Cd treated plants mostly based on the increase in all main sugar components and under 10⁻³ M Cd²⁺ treatment glucose, fructose and oligo-glucan content showed the most characteristic change. Cadmium treatment resulted increase of glucose and fructose content in roots too. In the presence of Ti-ascorbate the Ti-ascorbate moderated the toxic effects of Cd²⁺, it lowered the soluble carbohydrate content and accumulated fructan and glucan instead of reducing sugars.

Key words: cadmium, titan-ascorbate, wheat

INTRODUCTION

Heavy metals from various sources of pollution accumulate in soil and they are taken up by plants (Leap, 1981). Some of the heavy metals, e.g. cadmium, are toxic even at low levels while others, e.g. copper, are essential at low concentration and toxic only at elevated concentration.

Heavy metal stress affects many physiological and biochemical processes in plants resulting in the alteration of some metabolic pathways (Assche and Clijsters, 1990). Changes in carbohydrate content are of particular importance on account of their direct relationship with such physiological processes as photosynthesis, translocation and respiration (Foy et al. 1978; Ernst 1980).

Several studies, mostly in the laboratory, indicate an altered carbohydrate metabolism in plants affected by heavy metal pollution (Darshan et al. 1992; Greger and Bertel 1991, 1992).

We started to search the physiological effects of titanium at the Department of Chemistry and Biochemistry of the University of Horticulture and Food Industry in 1968. We found that titanium-ascorbate has very broad positive physiological effects on plant life (Pais, 1983). Later we also registered that Ti-ascorbate has a protective effect against some herbicides (Farkas et al. 1981), some stress-effects can be corrected by rapid application of Ti-ascorbate solutions (Tóth et al., 1985) and Ti-ascorbate increase activities of some enzymes (Fehér et al., 1984).

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In animal experiments we found antistress effect of Ti-ascorbate against toxic heavy metals (Pais et al., 1994).

In this study we attempt to assess and characterize the involvement of major sugar components in the adaptive processes of wheat seedlings under cadmium stress. Furthermore, the effect of Ti-ascorbate on Cd-induced changes in carbohydrate content was also presented.

MATERIALS AND METHODS

The seeds, winter wheat (*Triticum aestivum* L. cv. Martonvásári 21) were grown hydroponically in a growth chamber containing complete nutrient solution in a 16 h daylight period at 23 °C, and in a 8 h dark period at 18 °C and during 3-11 day period were exposed to Cd²⁺ (10⁻⁵, 10⁻⁴, 10⁻³ M) and Cd+Ti-ascorbate stresses. Ti-ascorbate was used in 5 ppm concentration. The plants were removed on the 3th, 7th and 11th day. In this study data of the 3 day old leaves and roots were detailed.

Samples of 2,5 g fresh weight were extracted twice in 40 mL boiling water, and twice in 40 mL boiling aqueous (80% v/v) ethanol for 15 minutes for each extraction. Fractions were collected and cleared by filtering through Whatman No.42 (pore size: 2.5 µm) paper. Filtrates were dried under reduced pressure (40 °C), using vacuum evaporator (Büchi model SB, Sweden) and were dissolved in distilled water.

Oligosaccharides were hydrolysed by boiling in 0,5 % HCl for 60 minutes. The amount of free (analyzed before hydrolyses) and bound (oligosaccharides, analyzed after hydrolyses) glucose, fructose and sucrose were measured using Boehringer Mannheim GmbH glucose/fructose/sucrose, No. 716 260 Kits (Wagner et al., 1983).

Total water soluble carbohydrate (WSC) determination was based on the phenol-sulphuric-acid method (Dubois et al., 1956), involving adding 1 mL of 5% phenol solution and 5 mL of concentrated sulphuric acid to 200 µL of samples and reading the absorbance at 510 nm after 20 min. Sucrose was used as standard.

Glucose and fructose content of roots were analysed by OPLC. The fresh parts of plants were frozen with liquid N₂, powdered and extracted with methanol (300 mg plant powder/ 300 µL of methanol and H₂O mixture, 80:20 V/V). This suspension was centrifuged at 15000 g for 10 minutes at 4 °C. The clear supernatants were used to overpressured layer chromatographic (OPLC, developed by OPLC-NIT Engineering Ltd., Budapest, Hungary) separations. OPLC separations were carried out on TLC and HPTLC silica gel 60 F₂₅₄ (Merck Co.) precoated chromatoplate. Staining by aniline - diphenyl amine - phosphoric acid reagent. For densitometric measurement TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ = 540 nm was used.

Three replication of each experiment were performed.

RESULTS AND DISCUSSIONS

The cadmium-induced changes in carbohydrate content were evident during the whole experiment, but in this study the early response data of the 3 day treatment was detailed. Results are given in Table 1. and Fig 1.

Considering the components of WSC considerable differences were measured in the presence and in the absence of Ti-ascorbate, results are given in Table 2. and Fig 1.

Table 1. Effect of Cd ²⁺ treatment on carbohydrate content of leaves

treatment mg/g fresh weight	controll	Cd ²⁺ 10 ⁻⁵ M	Cd ²⁺ 10 ⁻⁴ M	Cd ²⁺ 10 ⁻³ M
WSC	11.45	13.56	17.47	28.34
glucose	3.77	4.60	3.75	7.42
fructose	0.39	0.78	0.51	1.95
glucan	3.33	4.59	4.15	8.56
fructan	0.51	0.56	0.01	0.01

Table 2. Effect of simultaneous Cd ²⁺ and Ti-ascorbate treatment on carbohydrate content of leaves

treatment mg/g fresh weight	controll Ti- ascorbate	Cd ²⁺ 10 ⁻⁵ M +Ti- ascorbate	Cd ²⁺ 10 ⁻⁴ M +Ti- ascorbate	Cd ²⁺ 10 ⁻³ M +Ti- ascorbate
WSC	13.78	11.32	14.02	20.47
glucose	5.72	3.43	4.33	6.28
fructose	0.39	0.47	0.46	0.66
glucan	6.10	3.84	4.59	7.28
fructan	0.31	0.51	0.61	0.66

Cadmium treatment in all applied concentrations caused significant changes in total water-soluble carbohydrate level of wheat seedlings (Fig.1. A). The highest increment was caused by 10⁻³ M Cd (247 %), followed by 10⁻⁴ M (152 %) and 10⁻⁵ M Cd (118 %).

In the presence of Ti-ascorbate this increment rate was lower but Ti-ascorbate controll showed higher values than the absolute controll (Fig.1. A).

Differences were observed among the effects of various cadmium concentration too (Fig.1). Increase in WSC of 10⁻⁵ M Cd treated plants mostly based on the increase in all main sugar components. The highest increase was detected in fructose (200 %) (Fig.1.C) and oligo-glucan (137 %) concentration (Fig.1.D) and the following was glucose (122 %) (Fig.1.B)

There were glucose (196%), fructose (500 %) and oligo-glucan content (259%) which mostly caused the high WSC concentration under 10⁻³ M Cd treatment

The WSC level did not change in 10⁻⁵ M Cd+Ti-ascorbate treatment, but it means a considerable increase in fructan (164 %)(Fig.1.E) but decrease in glucose (59 %) and oligo-glucan (62 %) titer.

The effect of 10⁻⁴ M Cd+Ti-ascorbate resulted in a significantly higher fructan (196 %), while 10⁻³ M Cd+Ti-ascorbate in fructan (212 %) and glucan (119 %) content (Fig.1.E.D).

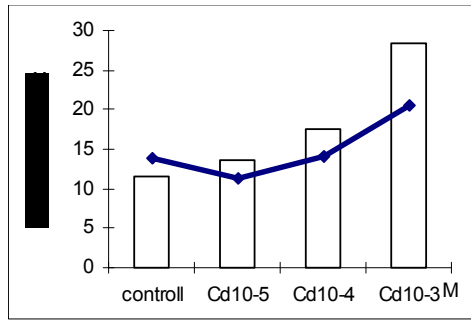
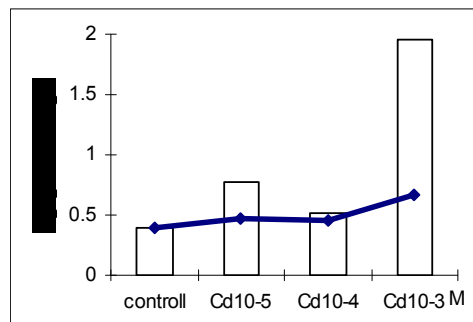
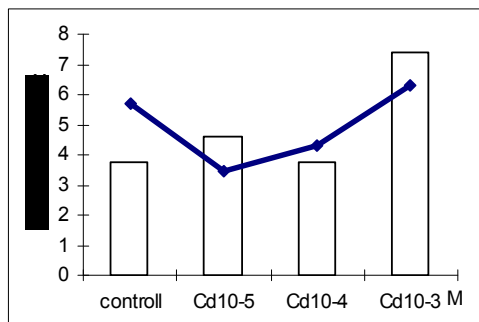
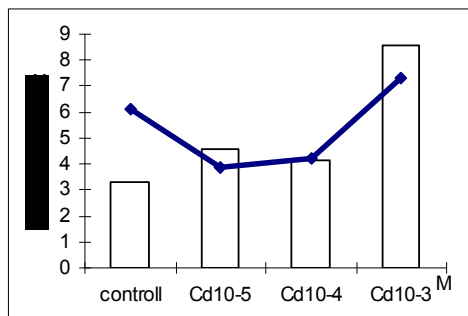
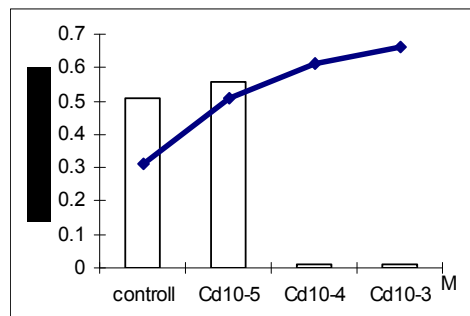
**A****B****C****D****E**

Figure 1. Effect of Cd^{2+} ($\square$) and simultaneous Cd^{2+} and Ti-ascorbate ($\blacklozenge$) treatment on carbohydrate content, (A)-water-soluble carbohydrates, (B)-glucose, (C)-fructose, (D)-glucan, (E)-fructan in leaves

Cadmium treatment resulted characteristic increase of glucose and fructose measured by OPLC in roots. Degree of increment of glucose and fructose was stronger in roots than in leaves. The effect of Ti-ascorbate treatment was more

expressed in roots than in leaves. Results of treatments are shown in Table 3, 4 and Figure 2.

Table 3. Effect of Cd^{2+} treatment on carbohydrate content of roots

treatment mg/g fresh weight	controll	$\text{Cd}^{2+} 10^{-5} \text{ M}$	$\text{Cd}^{2+} 10^{-4} \text{ M}$	$\text{Cd}^{2+} 10^{-3} \text{ M}$
glucose	1.37	3.61	3.95	7.39
fructose	0.63	1.32	1.32	4.14

Table 4. Effect of simultaneous Cd^{2+} and Ti-ascorbate treatment carbohydrate content of roots

treatment mg/g fresh weight	controll Ti- ascorbate	$\text{Cd}^{2+} 10^{-5} \text{ M}$ +Ti- ascorbate	$\text{Cd}^{2+} 10^{-4} \text{ M}$ +Ti- ascorbate	$\text{Cd}^{2+} 10^{-3} \text{ M}$ +Ti- ascorbate
glucose	4.83	1.14	1.06	5.52
fructose	1.63	0.24	0.32	2.68

It is well documented, that Cd^{2+} inhibits plants growth (Foy et al.1978; Malone et al.1978) but the degree of inhibition depends on the metal level. Cadmium probably acts on the active transport of sucrose at the tonoplast or stimulates the passive transport at protoplasma (Greger and Bertel, 1992).

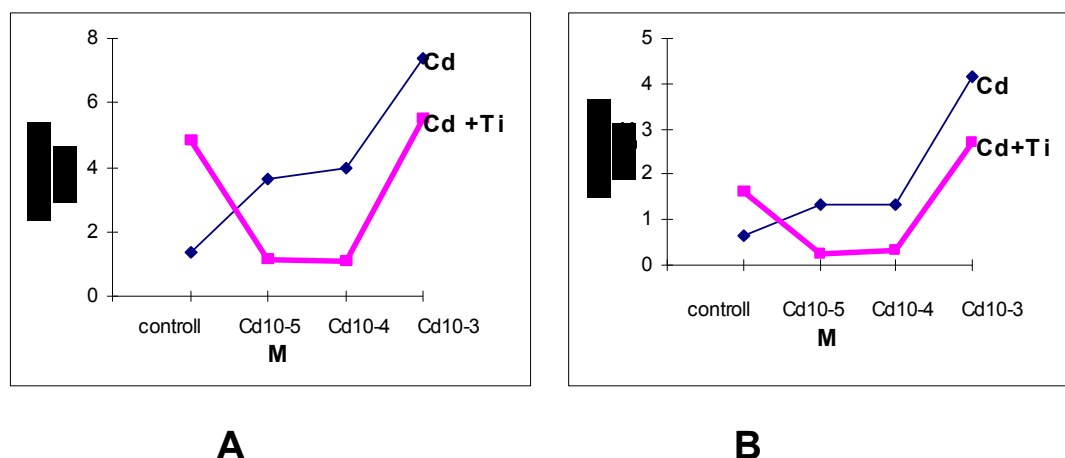


Figure 2 Effect of Cd^{2+} (□) and simultaneous Cd^{2+} and Ti-ascorbate (I) treatment on carbohydrate content, (A)-glucose, (B)-fructose in roots

Increased accumulation of Cd^{2+} resulted in reduced photosynthesis (Malic et al.) Heavy metal could, in this way, indirectly affect carbohydrate metabolism. There are some contradictory results in literature related to the effects of Cd^{2+} on the changes in water-soluble carbohydrate content. These data depend on the type of plant, metal concentration or duration of exposure.

Malic et al. found that the concentration of various metabolites, including glucose, fructose and sucrose decreased in 10 and 20 mM cadmium treated 30 day old wheat seedlings. Greger et al. referred to an increasing sugar level in 14 day old sugar beet exposure to cadmium (0.6-20 μM) in daily increments.

Our results in agreement with Greger's results clearly show that 10^{-3} , 10^{-4} and 10^{-5} M concentration of cadmium during a short term (3 day) interval increased the water-soluble sugar content in the leaf of wheat seeds. An explanation is that the decrease in utilization of carbohydrate for growth is more pronounced than the decrease in CO_2 fixation, thus resulting in an increased accumulation of carbohydrates.

This observation is in agreement with previous results considering the role of these carbohydrates in stress adaptation processes. Considering the WSC component, it was evident, that higher Cd^{2+} concentration induced the monosaccharide accumulation which is very unfavourable for the plant because high glucose concentration causes membrane damage.

Our results clearly show, that Ti-ascorbate moderated the toxic effects of Cd^{2+} , it lowered the soluble carbohydrate content and accumulated fructan and glucan instead of reducing sugars.

Fructan is not only a reserve carbohydrate, but it is considered to play a key role in stress-induced metabolic processes (Albrecht et al., 1993; Bancal and Triboi, 1993). Perhaps fructans - similar to sucrose - can protect membranes or other cellular component against the adverse effects and may influence the process of cell-wall hardening (Pilon-Smits et al., 1995). In this respect high fructan content indicate adaptation process while high glucose and fructose content is a marker of toxicity.

CONCLUSIONS

Our data, while providing further evidences on causal relationship between carbohydrate accumulation and heavy metal effect, indicate that the changes in main sugar component might be a useful trait to indicate heavy metal pollution. Furthermore, Ti-ascorbate seems to be a very useful trait to reduce the toxic effects of heavy metal pollution.

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A SURVEY ON THE EFFECTS OF Hg ON THE WATER PERMEABILITY OF RED BLOOD FROM VARIOUS SPECIES

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ABSTRACT

Mercury – containing compounds such as p-chloromercuribenzenesulphonate (RBC), are powerful inhibitors of the water permeability of red blood cell (RBC) membranes. They act as rather specific blockers of sulphhydryl (RBC) groups in the membrane proteins accommodating the water channels, the so-called aquaporins. Although the aquaporin 1 from human RBC was isolated and the details of inhibitions with Hg were previously reported studies on RBCs from other animal species are scarce. Detailed studies of the maximal inhibitions of water diffusions in RBCs from various species with variety of mercurials have been performed. Differences in the behaviour of RBCs from various species in regard with the conditions of incubation with Hg (concentration of reagent, duration and temperature incubation) required to inhibit the water permeability, as well as the degree of inhibition were noted. These differences might suggest peculiarities in the number and/or the turnover of aquaporins in RBCs from various species. In addition, the lowest values of permeability (i.e. after maximal inhibition) correspond to the basal permeability to water of the RBC membranes; such values were calculated for over 20 species. Comparative studies of water permeability in human and animal RBC might help in a better understanding of the molecular basis and significance of water diffusions across the RBC membrane.

Key words: mercury compounds, water permeability, red blood cell membrane.

INTRODUCTION

A prominent feature of the red blood cell (RBC) membrane is its very high permeability to water (for reviews see Benga 1988; Benga 1989). Investigations of RBCs from various species could shed new light on the physiological significance of the high permeability of the RBC membrane, since correlations between this parameter and other characteristics of the whole organism might be established. As part of a programme of comparative measurement in various species the characteristics of water permeability of RBCs from some wild, laboratory and domestic animal species have been reported. (Benga et. Al. 1993 a-e; 1994 a; 1995; 1996; 1999).

An important characteristic of water permeability of RBCs is its inhibition by sulphhydryl-binding mercurial reagents. Macey and Farmer (1970) found that p-chloromercuribenzenesulphonate (RBC) inhibited osmotic permeability by about 50% elevated the activation energy and reduced the ratio of osmotic to diffusional permeability to unity. Water transport properties of RBCs under these conditions are hardly distinguishable from lipid bilayers. A straightforward interpretation was that mercurials react with the sulphhydryl groups or proteins associated with water channels proteins, resulting in the closure of channels.

The first water channel protein (aquaporin 1) was discovered in human RBCs (Preston et al. 1992) and in recent years a whole family of aquaporins have been identified in a variety of cell, from bacteria and plants to animal and humans.

We have studied in detail the effect of a mercurial (RBC) on the diffusional water permeability of human RBCs and also on the RBC from a variety of mammalian species. A short overview of the most significant findings is presented below.

MATERIALS AND METHODS

Samples of blood were collected from domestic and wild animals in Cluj-Napoca, Romania, or in Sydney, Australia. The samples were collected into heparin (15 I.U./ml) refrigerated within 30 min and used routinely within 72 hr. The RBC was isolated by centrifugation, washed three times in medium S: 150 mM NaCl, 5.5-mM glucose, 5 mM Hepes 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4, and suspended at a hematocrit of 30-40% in the same solution supplement with 0.5% (w/v) bovine serum albumin. Samples for nuclear magnetic resonance (NMR) measurements were prepared by carefully mixing 0.2 ml cell suspension and 0.1 ml doping solution (40 mM MgCl₂, 100 mM NaCl).

The diffusional water permeability (P_d) of RBCs was measured by a NMR technique as previously described (Benga et al. 1990 a). The inhibition of water diffusion across the RBC membranes was calculated from the formula:

$$\%INHIBITION = \frac{P_{dcontrol} - P_{dsamples}}{P_{dcontrol}} \times 100$$

The incubation with RBC was performed at 20°C or at 37°C for different durations and various concentrations. After the incubations three washes were performed to remove the mercurial and then cells were used for measurements.

RESULTS AND DISCUSSION

It was previously shown that the conditions for exposure of human RBCs to mercurial (concentration, temperature and duration) are critical for the degree of inhibition (Benga 1989). Consequently, we have performed systematic studies of the effects of RBC on RBCs from various species to find the conditions for maximal inhibition of water diffusion. The degree of inhibition appears to be dependent upon the RBC concentration and incubation time.

The values of P_d of the maximal inhibition and the condition of the incubation with RBC for RBCs from various species are listed in Table 1. On the basis of P_d and inhibition by RBC these species could be divided into three groups. First, the chicken RBCs has a low value of P_d and this is not influenced by the incubation with RBC. The RBCs from sheep, cow, camel and man have higher values of P_d and the maximal inhibition is reached in 30-60 min with 1-2 mM RBC.

The third group is formed by RBCs from mouse, rat, guinea pig and rabbit having twice-higher values of P_d compared to the first groups above and also having higher values of inhibition (57-71%) reached in 30-45 min with 2 mM RBC. An exception appear to be the RBCs of rabbit with the highest value of inhibition (71%) reached in 15 min with 2 mM RBC. The significance of our findings may be discussed taking into consideration the present knowledge of the molecular basis of water permeation across the RBC membrane.

In chicken RBCs the water permeability (which is much lower than that of mammalian RBCs and is not effected by RBC) is mediated via the lipid bilayer and no functional water channel proteins are present in the membrane. For mammalian RBCs the water permeability is mediated by aquaporin 1. It appears that amount and/or turnover of aquaporin 1 in the

Table 1. Parameters characterizing the water diffusional permeability of red blood cells from various species*.

Source of red blood cells	Conditions of incubation for maximal P_d (cm/sec x 10^3)					Inhibition (%)
	PCMBs (mM)	Time (min)	Temp. (°C)	Control	Inhibited	
Man	2.0	30	37	6.1	3.3	46.0
Chicken	2.0	60	37	4.4	4.4	0
Sheep	1.0	60	37	5.0	2.2	56.0
Cow	1.0	60	37	5.0	2.7	46.0
Camel	2.0	45	37	6.6	2.9	56.0
Mouse	2.0	30	20	11.0	4.7	57.3
Rat	2.0	45	37	11.2	4.6	59.0
Guinea pig	0.5	10	37	9.2	3.1	62.9
Rabbit	0.5	15	37	10.7	3.1	71.0

*The permeation of washed red blood cells, measurements and calculation of P_d and %inhibition were performed as described in Materials and Methods. The results represent the mean of 4-30 measurements.

membrane of aquaporin 1 in the membrane of RBCs from various species is different. This makes further comparative studies of the membrane water permeability in RBCs from different species of relevance to a better understanding of the molecular basis of water diffusion across their membranes.

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DEUTERIUM CONCENTRATION OF THE CULTIVATION MEDIUM AFFECTS THE REDOX SYSTEM OF BRYOPHYLLUM DAIGREMONTIANUM

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ABSTRACT

A change in deuterium concentration of the cultivation medium from the average natural value (ca. 150 ppm atomic ratio) affects the redox system of plants, which was modeled by that of Bryophyllum Daigremontianum in this experiment. It was observed, that when a cultivation medium containing deuterium in either higher or lower than natural concentration was used, both the glutathion activity and concentration of $\cdot\text{OH}$ radicals decreased with respect to the control. The plant was found to adapt relatively quickly (in 10-15 days) to the change in the deuterium concentration. This was evidenced by that a smaller difference was seen in the operation of the redox system of older plants than in the case of younger ones. Both the found adaptation ability of the plant to the change in the deuterium concentration and the biochemical effect of this concentration change are new evidences to that Deuterium is an essential and toxic microelement.

Key words: Deuterium, $\cdot\text{OH}$ radicals, glutathion, microelement, lipid peroxidation

INTRODUCTION

Deuterium is an essential (Somlyai et al., 1993) and toxic (Galbács et al., 1996) microelement. The biological effects of aqueous solutions containing Deuterium in a higher than natural (150 ppm D/H atomic ratio) concentration have been extensively studied (Kotyk et al., 1990; Cope et al., 1965; Sacchi et al., 1992) for a long time. In opposition to this, only a relatively small number of research groups and publications have dealt (Somlyai et al., 1993; Kiss et al., 1996a; Kiss et al., 1996b; Kiss et al., 1996c; Kiss et al., 1996d; Kiss et al., 1998) with the effects Deuterium depleted (20-150 ppmD) solutions. It has been observed, that - among others - a lowered deuterium concentration hinders cell division, growth of coleoptyls and tumours (Kiss et al., 1996b), similarly to the effect of advanced deuterium concentration (Cope et al., 1965). Beside plant studies, experiments on animals have also been carried out, in which the impact of deuterium depleted solutions was observed to affect the operation of the redox system of *Drosophylla Melanogaster* (Kiss et al., 1998).

The generation of free radicals (e.g.: $\cdot\text{OH}$) can be imagined through either photosynthetic or mitochondrial electrotransport processes, taking place in both the cell and body tissue (Mehdy, 1994). Even under normal conditions of cell respiration, only 90% of the oxygen is used to build up water and the oxygen containing radicals also formed destruct some parts of the cells through oxidation. The harms caused by these processes are characterised by Szántay et al. (1993) as the following: lipid

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peroxidation (membrane destruction), excretion of NAD and thiols, decrease in ATP concentration due to the hindering of ATP-ase.

In *Drosophila*, the lowered Deuterium level (20 ppm D) induced a significant change in the operation of the redox system with respect to the control (Kiss et al., 1998). Upon these precedents we performed experiments on *Bryophyllum Daigremontianum* in this study to examine the effect of Deuterium depleted water on plants. The current paper reports on our results obtained in this experiment.

MATERIALS AND METHODS

We performed experiments to investigate the free radicals eliminating glutathion (GSH) activity, lipid peroxidation (LPO) and hydroxide free radical ($\cdot\text{OH}$) concentration in exposition to cultivation media of different deuterium concentration. The plant used in the experiments, *Bryophyllum Daigremontianum*, has a CAM (Crassulaceae Acid Metabolism) type metabolism.

Cultivation of *Bryophyllum Daigremontianum*. 20-20 shoots of *Bryophyllum* from the same leaf were weighed on an analytical balance and then cultivated on wet filterpaper. The solutions were prepared according to Prjanyisnyikov (Hortobágyi, 1963) and only differed in their Deuterium content. During the preparation of solutions we started off from 20 ppm D distilled water and set the D content to (20), 150 and 300 ppm using a microliter syringe. The wet filterpaper with the plants were put in a tightly closed (ground-edge) Petri dish. This precaution was taken to ensure that no isotope exchange could take place between the solution and the air humidity of the laboratory. The dishes with the plants were kept under scattered sunlight. No thermostating was used, but the conditions with respect to the temperature and light exposure were identical for the parallel measurements. After the cultivation period, the exterior water to the plants were absorbed by dry filter paper and then the plants were weighed again. Following this were the analytical measurements performed.

Sample preparation. A known amount of plants were homogenised with physiological NaCl solution using a cooled Potter homogenizator to 1:10 ratio. The resulting liquid was then centrifuged at 2000 g and the samples were taken from the upper phase.

Reduced glutathion (GSH) measurement. The determination was carried out following the description of Tietze (1969), in which the GSH is brought together with Elman reagent [5,5-dithio-bis-(2-nitro-benzoic acid, DTNB)] and the resulting yellow solution is measured spectrophotometrically at 412 nm.

Lipid peroxidation (LPO). LPO means the indirect quantity of the oxygen radicals playing a role in the metabolism of the manifold unsaturated fatty acids. We used the method of Plancer et al. (1966) for the determination of LPO, which is based on the measurement of the reddish-yellowish substance at 532 nm formed in the reaction of malon-dialdehyde (MDA) and thiobarbituric acid (TBA).

$\cdot\text{OH}$ radical measurement. We employed the procedure developed by Halliwell-Gutteridge (1990). In this procedure, $\cdot\text{OH}$ radicals attack pentose (2-deoxy-D-ribose) and this product, when heated in a 1% sulphuric acid medium with thiobarbituric acid, gives a reddish colour. Absorbance is to be measured at 532 nm wavelength.

Protein concentration. The determination was performed by the Folin method described by Lowry et al. (1951).

RESULTS AND DISCUSSIONS

Our results appear in Table 1. as averages of six replicate measuring series. For the sake of clarity, the given percentage values refer to the results of the control.

Table 1. Effect of Deuterium content of cultivation solution on the redox system of *Bryophyllum Daigremontianum*.

Cultivation period (days)	D content (ppm)	GSH	[•] OH	LPO
4	20	47%	72%	64%
4	150	100% *	100% **	100% ***
4	300	62%	37%	45%
10	20	75%	73%	90%
10	150	100%	100%	100%
10	300	83%	80%	77%

* GSH $\equiv$ 0.50 nM/mg protein

** [•]OH $\equiv$ 11.0 nm MDA/mg protein

*** LPO $\equiv$ 175 nm MDA/mg protein

It can be clearly seen, that the deviation is smaller for older (10 days) plants than for younger ones. This, together our former similar findings with sprouts (Kiss et al., 1996b; Kiss et al., 1996c), supports that plants can adapt to a change in D concentration of the medium. In the case of sprouts, it was found that the initial big deviation ceases after 14 -16 days.

CONCLUSIONS

1. The Deuterium content of the cultivation medium affects the redox system of the plant. One of the reasons to explain this can be that a larger activation energy is needed to bring of deuterated compounds into reactions, than non-deuterated ones. For instance, the decomposition of deuterated formic acid with formic dehydrogenase requires 29% more (activation) energy than the non-deuterated formic acid (Simon and Palm, 1966). The deuteration of proteins and the change of built-in D content of proteins can occur at normal temperatures, therefore the D concentration of medium can largely affect many biochemical reactions (Tamura and Akasaka, 1992).

2. Plants adapt to the change in D level during „ageing”. The biochemical processes will shift to provide the redox conditions typical to the natural (cca. 150 ppm) D concentration. In the initial period, the D concentration of the medium has a significant impact on the growth of plants. In other words, Deuterium can be essential or „toxic”, depending on its concentration (Galbács et al., 1996).

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APPLICATIONS OF CIRCULAR DICHROISM IN THE STUDY OF THE INTERACTION BETWEEN DNA AND METAL IONS OF SOME TRACE ELEMENTS

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ABSTRACT

Investigations concerning the relationship between the chemical structure and biological activity of the deoxyribonucleic acid (DNA) macromolecule revealed conformational changes in the secondary structure. The phenomenon of circular dichroism (CD) permits to reveal modifications in the secondary structure of DNA and can yield valuable information about the biological macromolecule. The CD method is particularly powerful in monitoring conformational changes. Our researches by means of CD permitted to evaluate the modifications that appeared in the systems of deoxyribonucleic acid / divalent metallic ions (M^{2+}), usually noted as DNA / M^{2+} . In such systems adducts of type DNA - M^{2+} are formed and evident modifications appear in the dichroic spectra, depending on the wavelength and also on the concentration of metallic ions. Among the divalent metallic ions there were used Zn^{2+} , Cu^{2+} and Mn^{2+} from chloride salts. The studies on the interaction of DNA with Cu^{2+} and Mn^{2+} ions were made by circular dichroism. The modifications in the CD spectra showing the variations of Cotton effect were evaluated. The obtained data on DNA and on Zn^{2+} / DNA-P, Cu^{2+} / DNA-P, respectively Mn^{2+} / DNA-P systems revealed the correlation between Cotton effect variation and conformational modifications of the secondary structure type B-DNA (native form of the macromolecule) evolving to structures of type A-DNA or C-DNA defined by linear and angular parameters. The adducts DNA - Cu^{2+} and DNA - Mn^{2+} produced more marked modifications as compared with DNA - Zn^{2+} adduct.

Key words: circular dichroism – DNA, trace metal ions

INTRODUCTION

Polarization of light - defined as the orientation of the electric vector that composes the electromagnetic wave can be described in terms of circular polarization states. In such states, the electric vector of light traces out a helical path, rotating once around the direction of propagation in one wavelength. Circular polarization states of light are therefore chiral and the interaction of photons with chiral molecules leads to circular dichroism.

Nucleic acids are optical active substances. The circular dichroism (CD) phenomenon - differential absorption of circularly polarized light - is evidenced by the Cotton effect. Circular dichroic effects occur when the wavelength of the incident light approaches that of the absorption band. Cotton effects in DNA are determined by the optically active deoxyribose, by the purine and pyrimidine bases whose chromophores become optical active due to their binding to the deoxyribose, as well as by the double helical structure of the macromolecular edifice of DNA (Yang and Samejima, 1971).

Circular dichroism, characterised by accuracy and sensibility, offers an efficient method for the study of conformational transitions of DNA during the interaction with transitional metals (Balasubramanian and Kumar, 1976; Eichhorn et al., 1977; Gârban et al., 1980). The investigation

of conformational transitions of DNA is important in order to elucidate the disturbing mechanisms in molecular genetics and pathology. Various metal ions can bind differently depending on the conditions of reaction. At low metal ion concentration a relatively unstable interaction appears between the polynucleotide strands and at high metal concentration hydrogen bonds appear between the less stable base pairs. Studying the interaction of DNA with metals, which in some conditions can induce mutagenic and oncogenic processes, interesting data are obtained regarding the destabilization of the macromolecule, followed by destructive processes and even cytological modifications. In this way, circular dichroism becomes a valuable method that helps the investigations in pharmacology, molecular biology, toxicology with applications in human and veterinary medicine.

MATERIALS AND METHODS

The interaction between DNA with some trace metal elements was studied on DNA from thymus at a concentration of 10^{-4} M in a solution of sodium perchlorate ($\text{NaClO}_4 \cdot 10^{-2}$ M). The metals studied were Zn, Cu, Mn (with the electronic configuration of the last stratum $3d^{10}$, $3d^9$ and $3d^5$) used as chlorides: $\text{ZnCl}_2 \cdot 2\text{H}_2\text{O}$; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, at the same concentrations 10^{-4} M in the same medium.

Out of the initial solutions of DNA and chlorides, there were further on prepared solutions with two increasing molar ratios, defined by the fraction $M^{2+} / \text{DNA-P}$ (related to the number of phosphate groups from the DNA macromolecule). The ratios were: 0.5/1; 1.0/1; 2.5/1; 5.0/1; 10.0/1 (for details see Gârban et al., 1980). The solution of sodium perchlorate does not affect neither the conformational state of DNA nor the interaction with divalent metals.

Dichroic spectra were obtained with a Roussel-Jouan dichrograph composed of: a monochromator, a polarizer and a crystal of monoammonia phosphate under an electric field. For a correct orientation, the plane polarized light can be divided into right and left components which are unequal absorbed in an optical active medium and recombine in the region of electronic absorption, resulting the elliptic polarized light.

In order to express the Cotton effect detected by means of CD spectra there can be established the following parameters: molecular ellipticity (θ), specific ellipticity $[\Psi]$, dichroic absorption $\Delta\epsilon$. The wavelength (λ) domain used was between 220-320 nm. The calculations and the transformations regarding the above mentioned parameters were presented by Crabbé (1965), Balasubramanian and Kumar (1976), helping to interpretate the notions of circular dichroism.

In our investigations the specific ellipticity (evaluated in $\text{grd} \cdot \text{ml} \cdot \text{g}^{-1} \cdot \text{dm}^{-1}$) was expressed in relationship with the wavelength $[\Psi] = f(\lambda)$. Due to the different absorption of the circular polarized components a positive Cotton effect $[\Psi] > 0$ and a negative Cotton effect $[\Psi] < 0$ were observed, related to the wavelengths.

RESULTS AND DISCUSSIONS

Circular dichroism spectra for distinct types of secondary structure present in peptides, proteins and nucleic acids are different. The analysis of CD spectra can therefore yield valuable information about secondary structure of biological macromolecules. Our investigations of DNA in solutions and the $M^{2+}/\text{DNA-P}$ systems at various molar ratios were followed by the calculation and the interpretation of CD spectra and the establishment of maximum and minimum Cotton effects.

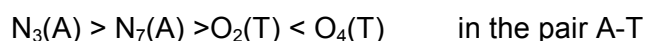
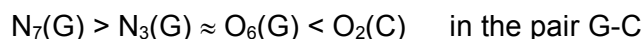
The CD spectra show a geometrical minimum - negative Cotton effect - and a geometrical maximum - positive Cotton effect. The curve has the form of the letter S. The point where the specific ellipticity anneals and the curve changes the sign corresponds to the wavelength characteristic for the absorption in UV (Crabbé, 1965).

Correlating the CD spectra of DNA with the conformation of the macromolecule it is attested the presence of B-DNA type which corresponds to the Watson-Crick model. The polarity of the medium, the used solvents, the nature of

the ions in solution, the pH, the DNA content of guanine and cytosine can determine the appearance of another DNA types: A-DNA, C-DNA.

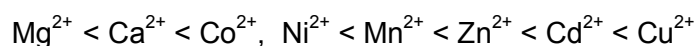
The different conformational types are characterised by the variation of the sterical parameters (Gârban, 1997).

Transitional metals bind predilectly at the nucleobases forming electronic bonds between the N, O atoms which are donors with the d orbitals of the transitional metals which are acceptors and only in some cases at the phosphodiester groups (Sisoeff et al., 1976; Marzilli, 1977). According to Pullman and Yagi (1980) the series of binding capacities at the nucleobases is:



with N_7 at guanine the most attractive position.

Experimental considerations led to the existence of a characteristic affinity of M^{2+} related to the phosphodiesteric centers and the DNA bases. According to Zimmer (1971) the binding tendency to the nitrogenous nucleobases increases in the series:



and the binding tendency to phosphodiesteric centers increases in opposite sense.

The N, O, C atoms from the nitrogenous bases contribute to the formation of the π orbital system. These have the lowest energy and are occupied by the pairs of available electrons from the system. The π orbitals have the highest energy. Electronic interactions π - π^* have an important contribution at the circular dichroism of DNA.

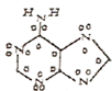
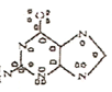
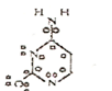
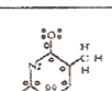
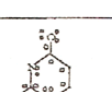
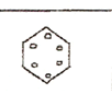
In the π system there can be distinguished orbitals with low energy occupied by the pairs of available electrons – type π - and high energy orbitals - type π^* , unoccupied. In the case of heterocyclic nitrogenous nucleobases from nucleic acids, numerous π - π^* transitions are possible (Sprecher and Johnson, 1977). Electronic transitions in nucleobases offer a new evaluation modality of the chemical structure of macromolecules in nucleic acids with implications on the perturbation of biological activity.

In table 1 there are presented the electronic structures of the purinic and pyrimidinic nucleobases. As to the number of occupied / unoccupied orbitals in these nucleobases it is compared with the electronic structure of benzene.

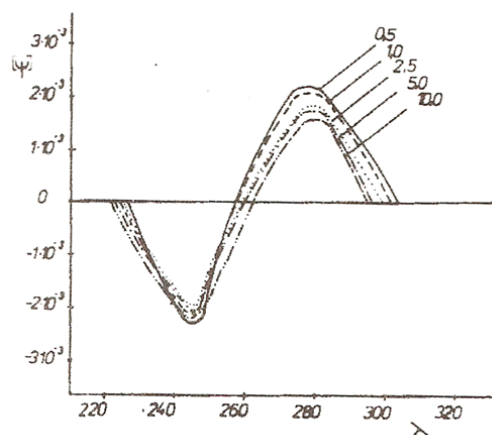
Such transitions can accompany the building of bioincompatible structures characteristic for mutagene and/or oncogene processes. In that way the concept of molecular pathology can be substituted with that of electronical pathology.

Studies on circular dichroic spectra in the M^{2+} / DNA-P spectra reveled modifications which express the disturbance of the secondary structure of DNA. At the origin of these modifications is the interchangeability of conformational types of the DNA double helix.

Table 1. Electronic structures of purinic and pyrimidinic nucleobases

No	Compound	Structure	Molecular π orbitals	Electrons	Orbitals	
					Occupied π	Unoccupied π^*
1	Adenine		10	12	6	4
2	Guanine		11	14	7	4
3	Cytosine		7	10	5	2
4	Thymine		8	10	5	3
5	Uracil		8	10	5	3
6	Benzen		6	6	3	3

In the case of the reaction of Zn^{2+} with DNA the effects are characterised by modifications of the DNA conformation (fig. 1). Dichroic modifications reveal the depression of negative and positive Cotton effect.



The increasing concentration of Zn^{2+} ions occurred in the system Zn^{2+} / DNA-P leads to the decrease of the absorption maximum, for example:

Zn^{2+} / DNA-P (ratio)	Specific ellipticity [ψ]
0.5/1	$2.22 \cdot 10^{-3}$
1.0/1	$2.00 \cdot 10^{-3}$
5.0/1	$1.71 \cdot 10^{-3}$

According to Zimmer (1971) the modifications indicate the perturbation of the electronic system of the bases by coordination of Zn^{2+} ion at the bases.

Fig.1: CD spectra of Zn^{2+} /DNA-P at various molar ratios

Chelatisation between complementary nucleobases presents an intrahelical disposal of transitional metal ions: Cu^{2+} binds to N_1 of guanine and N_3 of cytosine. Another way for the internal chelatisation of DNA - Me^{2+} type consists in the binding of the metal ion to a purine base in the positions N_7 and N from C_6 of adenine or N_7 and O at C_6 of guanine. As a consequence it appears a local denaturation of the

macromolecular structure. The N₇ position is electron-donor and from the quantum chemistry point of view represents the center with the highest electron density at the group of complementary nucleobases of DNA (Gârban et al., 1980; Gao et al., 1993; Gârban et al., 1996; Gray, 1996).

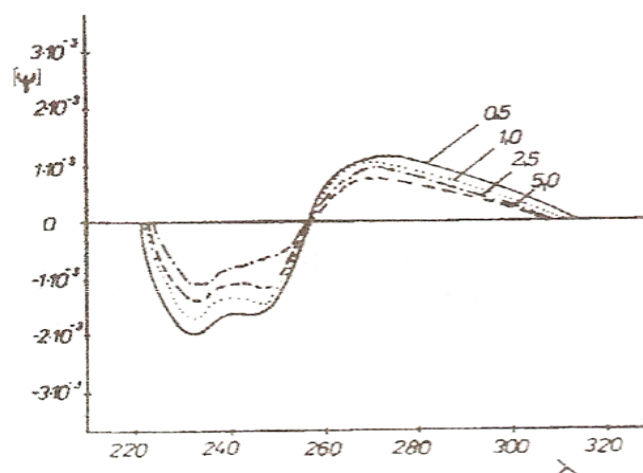


Fig. 2: CD spectra of Cu²⁺ / DNA-P at various molar ratios

The effect of Cu²⁺ on DNA reveals also dichroic modifications (fig. 2). The dichroic curve decreases with the increase of the molar ratio.

A peculiarity at these spectra consists in the existence of a discontinuity in the negative domain. It may correspond to a transitional state knowing that the system develops to a complex and the DNA moves off from the initial B-DNA type.

Important modifications of the Cotton effect are

induced by the Mn²⁺ cation on the positive domain (fig.3).

A special characteristic for Mn²⁺ is that in the positive domain the curves present two modulations. It was considered by Zimmer that conformational

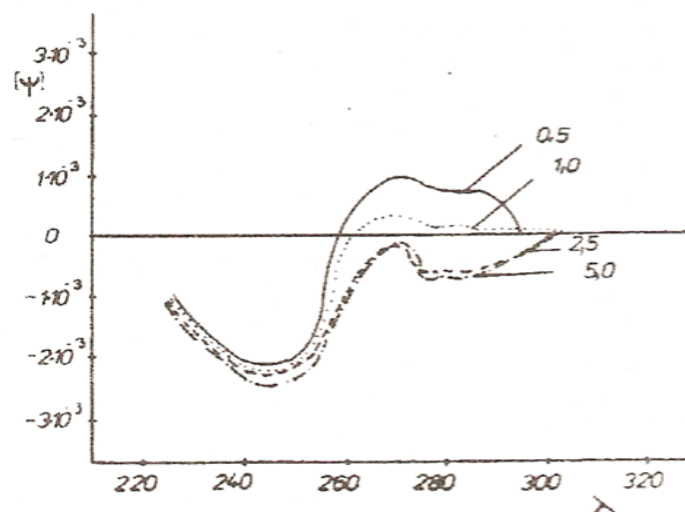


Fig.3: CD spectra of Mn²⁺ / DNA-P at various molar ratios

modifications at the pairs of bases G-C take place.

Comparing the Cotton effects induced by the studied transitional metals one can see that the structural modifications of DNA are also influenced by the nature of the divalent cations. Thus, for Cu²⁺ and Mn²⁺ there are differences of the electronic structure which governs the geometry of the complex that is formed.

Theoretical studies presented in literature confirm the discussed aspects showing that there

is a linear and monotone dependence on the different geometrical parameters which define the double helix of the DNA.

CONCLUSIONS

1. The double helical DNA conformation presents a greater sensibility to the action of transitional metals due to the affinity of the electron-donor parts of the nucleobases in comparison with *d* electronic configuration of the ions.
2. Metallic cations Mn^{2+} , Zn^{2+} which bind to the phosphodiesteric groups enhance the dichroic curve in the negative domain of Cotton effect.
3. Divalent transitional metals chelate at the level of intrahelical nucleobases and, in some cases (Mn^{2+} , Zn^{2+}), they can simultaneously chelate at the phosphodiesteric groups and at the bases.
4. In case of Cu^{2+} ion the CD spectra showed a peculiarity - the existence of a discontinuity in the negative domain, i.e. a double inflexion.
5. Transitions of the macromolecule depend on the ionic interactions in solution, on the modifications of solubility given by the decrease of water activity when the ions concentration increase. Once decelated, these transitions can be considered as structural modifications of DNA which determine, later on, functional modifications.

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POLLUTION OF NATURAL WATER BASINS WITH HEAVY METALS IN A ZONE WITH NONFERROUS METALLURGICAL INDUSTRY

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ABSTRACT

In our doup are of the most difficult problem of water utilization has become the protection of its quality, the prevention and control of its pollution. The general characteristic of water is the great and rapid variability of their physicochemical and biological traits due to the permanent and direct contact with the environment. That is why an investigation of the pollution with heavy metals has been carryall out for four years in a zone with nonferrous metallurgical industry.

Samples have been collected in two series each year from water worst, fountains and surface water in three places: one in the industrial zone, the other two 20 kilometers upstream and downstream of it. After sample processing lead, cadmium; and zinc were determined by means of atomic absorption spectrophotometry (AAS).

In the water collected in the industrial zone lead and cadmium were absent or below the accepted limits in fountains, but above there in the central water supply. In this case the mean values were larger, for lead by 2.4 times in the raw water, 1.7 times in the pure water, 1.6 times in the tap water, while for cadmium it was 1.2 larger in the raw water only. In surface waterslead reached a maximum (1.05 mg/dm^3), zinc was higher both upstream (1.898 mg/dm^3) and downstream (4.0 mg/dm^3), while cadmium had its highest value downstream (4.1 mg/dm^3). As a result of the presence of heavy metals in water sources it is necessary to check strictly and permanently the water quality in order to prevent the appearance of irreversible ecological phenomena.

Key words: water, lead, cadmium, zinc

INTRODUCTION

Water represents one of the most important factors of natural environment being indispensable for any kind of life; it encounters in atmospheric phenomena, in most physical, chemical and biological transformation in soil and air, influencing both directly and indirectly the development of industry, agriculture and transport (Cotarau, 1988; Dudea, 1986; Popescu, 1985).

For living bodies, water has a special importance representing the real cause of all biologic reactions. It is the environment where most organic and inorganic substances from cells and intercell spaces are solved which it transports from one tissue to another. It has a nutritive and antitoxic part because penetrating the composition of organic liquids carries a part of nutritive substances at the level of all organs on one hand and on the other hand it loads itself with some products resulted from metabolism which it transports up to excretory organs (Hyde, 1977; Popescu, 1985).

Surface water has a very variable composition because it chains surfaces of land which they come into contact and the same time receive a part of meteoric waters from the waste one, as well.

Chemical pollution of waters is the most frequent and important due to the exaggerated increase of industry, which generates polluting substances and elements. The pollution of these waters is due to the penetration of foreign substances into their normal composition, which leads to an increased modification of organoleptic, physical, chemical and biological characters (Dudea, 1986; Hyde, 1977; Rutkovici, 1992).

MATERIALS AND METHODS

The research carried out for a period of 4 years following the level of lead, cadmium, zinc from water factories, wells and surface water from three places: in the industrial zone, the other two 20 kilometers upstream and downstream from the industrial area, by water samples harvesting and processing. The samples were harvested two times a year in 3 l plastic cans prepared in advance. The processing of samples was done considering the contents of heavy metals both in suspension and solved. This quantity from the samples was filtered and prepared for determination by spectrophotometry techniques with atomic absorption (AAS).

The data obtained were compared with the values admitted by STAS 1342/91 (drinkable water) and 4706/88 (surface waters).

RESULTS AND DISCUSSION

The results obtained from the analysis of water in wells pointed out concentrations under the level of STAS in those three places where samples had been harvested both for lead ($0.02\text{--}0.05\text{ mg/dm}^3$) and for cadmium ($0\text{--}0.005\text{ mg/dm}^3$) (table 1).

Table1: Results of water samples from fountains

Place of collection	Pb (mg/dm^3)	Cd (mg/dm^3)
B	0.100	0.010
M ₁	0.030	0.002
M2	0.040	0.003
M3	0.020	0.000
M4	0.030	0.005
M5	0.020	0.002
M6	0.020	0.003
T	0.050	0.003
CM1	0.000	0.000
CM2	0.040	0.000
SM	0.000	0.000

Although these concentrations are not too high still their accumulation with other quantities in the animal body from air and feeds can be harmful.

As a consequence of water treatment as plants, the concentration of lead and cadmium decreases very much from the source of raw water to the running one almy pipes.

The running water on pipes from the locality situated at 20 km upstream from the industrial area presented values much under the STAS for lead and cadmium.

These quantities were found in a suspension, which remained at the level of filtration in water plants. Following the quality of waters used for processing in the water plants the other two localities, we find out an exceeding of lead concentration for about 2.4 times in raw water and 1.7 times for finished water, 1.6 times for running water. The exceeding percentage of cadmium was for 1.2 times only for raw water (table 2)

Table 2: Results of sample analysis at water plants

PLANT	Water type	Pb mg/dm ³	$\bar{X}$	Cd mg/dm ³	$\bar{X}$
M	Raw water	0.230		0.004	
		0.194		0.003	
		0.030	0.151	0.002	0.003
	Pure water	0.050		0.001	
		0.190		0.001	
		0.025	0.088	0.004	0.020
	Tap water	0.030		0.008	
		0.025		0.001	
		0.019	0.025	0.001	0.003
CM	Tap water	0.002		0.0005	
		0.200		0.001	
		0.035	0.079	0.002	0.003
D	Raw water	0.201		0.003	
		0.090	0.145	0.002	0.016
	Pure water	0.070		0.008	
		0.024	0.047	0.001	0.004
	Tap water	0.036		0.003	
		0.021	0.029	0.001	0.002

In case of surface water, higher levels were recorded in winter months as well as in July and August due to large quantities of rain. The medium annual values for heavy metals searched in those three places for samples harvesting varied as follow: for the place situated upstream in the industrial area these values were under the maximum admitted limit for surface waters.

In the industrial area, lead reaches values of 0.091 mg/dm³, zinc 1.898 mg/dm³, cadmium 0.10 mg/dm³ upstream in the industrial area the values of metals are: 1.05 mg/dm³ for lead, 4.00 mg/dm³ for zinc and 4.1 mg/dm³ for cadmium, overpassing the maximum admitted limits (table 3).

Table 3: Evolution of mean annual concentrations of the main pollution indices of surface waters

Check section		20 km upstream				Industrial area				20km upstream			
indices	CM mg/dm ³	1	2	3	4	1	2	3	4	1	2	3	4
Pb	0.050	0	0	0	0	0.0025	0.046	0.044	0,099	1.05	0.50	0.017	0.320
Zn	0.030	0	0	0	0	0.0700	0.130	0.980	1,898	0.50	4.00	3.200	1.990
Cd	0.003	0	0	0	0	0.0000	0.006	0.100	0,033	4.10	0.10	1.020	0.036

CONCLUSIONS

After the determination for the main levels of heavy metals which pollute surface waters in an area with nonferrous metallurgic industry we reached the following conclusions:

1. Waters from wells in the industrial area remain under the admitted limit or reach values admitted by STAS for drinking waters.
2. The concentrations of lead and cadmium pass over the admitted standards in water plants 1.2 times to 2.4 times.
3. in the locality situated upstream from the industrial area, there are no exceeding of heavy metals concentration.
4. The highest pollution is in the industrial area and upstream reaches the maximum value 1.05 mg/dm³, zinc 4 mg/dm³ and cadmium 4.1 mg/dm³.

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TI-6AL- 4 V ELI AND Co-Cr-Mo BIODUR ALLOYS BIOCOMPATIBILITY: RESULTS OF AN EXPERIMENTAL STUDY

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ABSTRACT

According to regulations in force, alloys bioavailability has to be tested on animals. The study was performed on 12 white, New Zealander rabbits - age between 1.5 and 2 years, male and female, weight between 4.5 and 5.2 kg, equally divided in 4 groups, and 8 metis dogs - male and female, weight between 11-17.5 kg, equally divided in 2 groups. All animals came from the Bio-base of the Laboratory for Surgical and Micro-surgical Experimental Research of the University of Medicine and Pharmacy Timișoara.

Previously to the femoral implantation, both the test and the control prothese were introduced in distilled water and sterilized.

After 2, 4 and 6 month we have performed the second surgical intervention for the extraction of the bony fragments with implants.

From the hystological point of view, the presence of the test implants did not involve significant hystological changes in the bone or in the neighboring tissues, in comparison with the control group, involving currently used orthopedic materials.

Key words: alloys biocompatibility, orthopedic materials, experimental

INTRODUCTION

Nowadays orthopedic surgery uses metallic, ceramic, silicon and biological prothese. The metallic prothese are most frequently used. The metal implant can be manufactured using a pure metal or an alloy. The additional metals increase bioavailability and improve the medical characteristics. Best protheses are made of steel, Cobalt, Titanium, Platinum.

Some protheses undergo "in vivo" testes – corrosion after contact bodily fluids or plastic deformation due to mechanic pressure on the joint.

Total hip prothesis represent the best solution in femoral neck fracture but because of the above-mentioned changes (corrosion and/or deformations) animal testing is necessary.

Institute for Research Development for Warm Sectors (INTEC S.A.) - has initiated the manufacturing of Ti-6Al-4 V ELI and Co-Cr-Mo BIODUR alloys for the production of autochthonous total hip protheses.

According to regulations in force, alloys biocompatibility has to be tested on animals. The two above mentioned alloys have been obtained from TISTO and CARPENTER - American firms that produce biocompatible materials. INTEC SA produced mini-protheses for animal implants, according to ASTM 136 and ISO 5832/3.

MATERIALS AND METHODS

Based on contract no. 4574&20.07.1998 between the University of Medicine and Pharmacy Timisoara (UMPT) and the Institute for Research Development for warm sectors, the Laboratory for Surgical Experimental Research of the UMPT designed an experimental study regarding the biocompatibility of Ti-6Al-4 V ELI and Co-Cr-Mo BIODUR alloys. The project was approved by the Committee for Animal Research Ethics and was performed under the coordination of Assist. Prof. Avram Jecu, M.D. and the Project Coordinator Florentina Cadariu, M.D. The experiment has been conducted under the standards of "Standard Practice for Assessment of Compatibility of Metallic Materials for Surgical Implants, with Respect to Effect of Materials on Tissue" F361 of the American National Standard Committee.

The study was performed on 12 white New Zealander rabbits - age between 1.5 and 2 years, male and female, weight between 4.5 and 5.2 kg, equally divided in 4 groups and 8 metis dogs - male and female, weight between 11-17.5 kg, equally divided in 2 groups. All animals came from the Bio-base of the Laboratory for Surgical and Micro-surgical Experimental Research of the UMPT.

The mini-implants were cylindrical, with hemispheric (rounded) heads, with a 2 mm diameter head and 6 mm length - for rabbits and with a 4 mm diameter head and 12 mm length for dogs. The materials used for the implants were those currently used in Orthopedics (SUTUPAK, STAHLDRÄHT monofilament, strahlensterilisiert, ETHICON).

Previously to the femoral implantation, both the test and the control prostheses were introduced in distilled water and sterilized.

The surgical implantation technique consisted of:

- general anesthesia with Ketasten I.V. - 0.1 mg/body kg;
- fur removal of the surgical area;
- washing the surgical area with iodine tincture;
- longitudinal hip incision of the skin, subcutaneous tissue, aponeurosis; muscular tissue dissociation, denudation of the cortical portion of the femoral lateral side;
- 1.6 mm (in rabbits) and 3.2 mm (in dogs) deep orifices were made, 2 cm away from each other, with the electrical screw tap (variable rotation speed), on the lateral side of the femur;
- the implants were then introduced in these orifices (manually) - 2 in each rabbit femur and 6 in each dog femur;
- rebuilding the muscular, aponeurotic, subcutaneous and cutaneous plans with isolated sutures.

The post surgical care of animals has been performed in accordance with the standards of the "Guide for the Care Use of Laboratory Animals" (NIH) 78-23 (1978). There were no general or local complications; the post surgical evolution was good.

Table 1. The situation of implants and the surveillance periods:

Experimental animal	Group number	Implant type		Surveillance period
		Right femur	Left femur	
RABBITS (12 animals)	I	Co-Cr-Mo BIODUR	SUTUPAK stainless steel	2 months
	II	Ti - 6Al - 4 V ELI	SUTUPAK stainless steel	2 months
	III	Co-Cr-Mo BIODUR	SUTUPAK stainless steel	4 months
	IV	Ti - 6Al - 4 V ELI	SUTUPAK stainless steel	4 months
DOGS (8 animals)	I	SUTUPAK stainless steel	Co-Cr-Mo BIODUR	6 months
	II	SUTUPAK stainless steel	Ti - 6Al - 4 V ELI	6 months

The hystological preparation of the bony fragments has been performed according to the following scheme:

- fixation in 10% formalin solution;
- decalcification in HNO₃ - 1N solution;
- extraction of implant from bone (no visible changes on the implant surface were noted);
- paraffin embedding;
- paraffin blocks sectioning;
- coloring with haematoxylin-eosin.

RESULTS AND DISCUSSIONS

During the second surgical intervention, for the extraction of the bony fragments with implants, we observed:

- the surgical scar;
- normal muscular tissue with sutures;
- the presence of a periostal reaction at the implantation site, secondary to the implantation traumatism.

The results of the hystological analysis were the following:

a) **In rabbits group I and II the hystological changes were similar:**

- necrosis and necrobiosis areas in the muscular tissue and, more limited, in the bony tissue; they are due to the high speed technique used in making the bone orifices;
- absence of reactive inflammatory changes with the exception of one case of implant with Ti - 6Al - 4 V ELI, in which moderate infiltrates with macrophages, lymphocytes, granulocytes and giant multinuclear cells were observed;
- absence of fibrosis.

b) **In rabbits group III:**

- compact bony tissue, with no significant hystological changes;

- normal haematopoietic marrow;
- sharply delimited implant sites;
- sharply delimited plasmatic overflow, in the shape of an omogenous eosinophils strip;
- absence of cellular inflammatory reaction;
- absence of fibrosis.

c) **In rabbits group IV:**

- sharply delimited implant sites;
- normal, compact bony tissue;
- normal haematopoietic marrow;
- the presence of a continuous, thin body matrix, with no Haversian systems, placed between the medullary area and the implant site;
- absence of inflammatory reaction elements;
- absence of fibrosis.

d) **Dogs - groups I and II:**

Control implants:

- area of bony necrosis and necrobiosis in the proximity of the implantation site;
- absence of inflammatory reaction;

Test implants:

- linear, sharp delimitation of the implant site;
- compact bony tissue, within normal histologic limits;
- histologically normal haematopoietic marrow;
- presence of an uniform, normally calcified bony matrix, with no Haversian systems, situated in the near proximity of the implant site, towards the medullary canal;
- absence of inflammatory reaction;
- presence of a layer of conjunctive-fibrous tissue along the the periosteum, at the implant site, on the bone surface.

CONCLUSIONS

Generally speaking, we can conclude that, from the histological point of view, the presence of the test implants did not involve significant histological changes in the bone or in the neighboring tissues, in comparison with the control group, involving currently used orthopedic materials.

The post surgical evolution of the experimental animals and also the absence of significant histological changes, support the biocompatibility of Ti - 6Al - 4 V ELI and Co-Cr-Mo BIODUR alloys and recommends them for the manufacturing of total hip prostheses.

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THE ROLE OF “KONDI” TABLETS IN DIMINISHING PLUMBEMIA

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ABSTRACT

Based on the physiological antagonism between microelements, the authors studied the effect of zinc (Zn) and magnesium (Mg) containing “Kondi” tablets on lead (Pb) elimination in a voluntary group exposed to chronic lead intoxication. A “Kondi” tablet contains: 100 mg soia lecithine, 200 – 400 mg yeast powder enriched with 1.8–2.0 mg Zn in organic compounds, vitamins B₁₋₆, and E, and 10 mg Mg. Personal and anamnestic data, clinical signs and somatometry were recorded together with laboratory parameters, as well as the treatment (one “Kondi” tablet / 10 kg bw, 3 months long).

Results: 54 persons (35 children and 19 adults). Laboratory data: hypocalcemia 19 (35.1%), hypomagnesemia 18 (33.3%), decreased alkaline phosphatase activity 29 (53.7%). Blood lead level was checked in 50 subjects before and in 37 after the treatment (74.0%), between the lasts in 19 (51.3%) the mean plumbemia decreased from 3.23 $\mu\text{mol/l}$ to 2.78 $\mu\text{mol/l}$, in another 15 cases (40.5%) increase was noted from 2.32 to 2.81 $\mu\text{mol/l}$, and in 3 cases (8.01%) were no changes. According to this results the authors consider that the “Kondi” tablets could have a prophylactic role in chronic lead poisoning

Key words: chronic lead intoxication, zinc and magnesium therapy

INTRODUCTION

History of mankind has been close linked to lead industry (Poór and Mituszova, 1989) and the lead poisoning appeared under various aspects (Manuwald, 1989). Preclassic Greeks suffered on account of mining, the Romans had been intoxicated with the vine drank from lead cups (even lead syrup was used to improve taste and colour of red vines !) Epidemics of lead poisoning were seen two centuries BC., during celebration of the harvest and large amounts of vines used. This gave the name “ saturnism “ to lead poisoning (saturnalia = celebration of the harvest between 17 – 23 December). In the year 1845 the Franklin Expedition suffered from lead poisoning because of conserves (Kowal et al., 1991), but it happens nowadays also with paprika falsified with lead oxide - PbO (Hungary, 1992). Other ways of alimentary poisoning are: the contaminated drink water (Shannon and Graef, 1989), use of ceramic vessels with enamel - mostly when used for lemon tea or vine (Vázsonyi et al., 1965).

Development of industry brought in front the professional poisoning in mining, metallurgy, paint industry, typography, ceramics and porcelain industry, battery manufacture etc.

The third form of lead poisoning is environment pollution, mainly of atmosphere with emission gases of vehicles which affects also vegetation and culture plants (grain, grapes, etc).

Penetration into human organism is possible on different ways: inhaled lead particles or vapour (30 – 50% is absorbed), ingestion with 10 – 15% rate of absorption and transdermic - only for organic compounds (Balla and Kiss, 1996).

The study was made in Corund, a locality where for centuries the greatest part of inhabitants are dealing with ceramics and in the last century they are using enamel based on lead. The technology used produces chronic intoxication during the burning process, pollution of air, soil and water. Great part of the potters are suffering from chronic saturnism.

MATERIALS AND METHODS

Based on the antagonism between microelements as are zinc (Zn) and lead (Pb), we tried a treatment with Zn and Mg on potters and their family suffering from chronic lead poisoning. For this purpose we used “Kondi” tablets, with: 100 mg soia lecithine, 200 - 400 mg yeast powder, enriched with 1.8 – 2.0 mg Zn in organic compound, natural vitamins B₁, B₆, E and 10 mg Mg. The study was made using a standard protocol, on data sheets were recorded: personal data, professional circumstances (toxicological), clinical signs (lead border on gums, Chwostek's sign, etc.), somatometry and laboratory results. Before and after the treatment we checked the hematology: hemoglobin (Hgb), hematocrit (Htc), plumbemia (with atomic absorption spectrophotometry), kalemia, calcemia, magnesemia and phosphatemia, alkaline phosphatase activity, SGOT and SGPT, serum urea, creatinine and 24 hour urine delta levulinic acid (D – ALA). Treatment used was also registered: daily one “Kondi” tablet for 10 kg bw, for three months.

RESULTS AND DISCUSSIONS

The study group had 54 persons coming from lead polluted environment: 35 were children (64.8%), between 3 – 16 years of age and 19 adults (35.1%). Sex distribution was: 30 (55.5%) male, 24 (44.4%) female.

The main clinical signs found were:

- 44 (81.45%) lead border on gums
- 19 (35.1%) Chwostek's sign of latent tetany
- 6 (11.1%) somatic retardation

Main values of the laboratory data recorded during the study:

- Hgb $\bar{x} = 10.25 \text{ g/dl.} \pm 0.84$, significantly decreased ($p < 0.002$)
- Htc $\bar{x} = 34.72\% \pm 1.74$ decreased ($p < 0.002$)
- lead in the serum (determined with atomic absorption spectrophotometry), plumbemia in 54 subjects before treatment: $\bar{x} = 3.32 \mu\text{mol/l}$. After the treatment this parameter could be checked only in 37 patients, were plumbemia decreased in 19 cases (51.3%) to $2.78 \mu\text{mol/l}$. Other 15 patients (51.3%) had increased serum lead level after our treatment with $\bar{x} = 0.49 \mu\text{mol/l}$ (because of insufficient cooperation) and 3 patients (8.0%) had no changes.
- kalemia $\bar{x} = 4.22 \text{ mEq/l}$ (normal)
- 19 cases with mild hypocalcemia $\bar{x} = 2.10 \text{ mmol/l}$ ($p < 0.05$)

- 18 cases with significant hypomagnesemia $\bar{x} = 0.68 \text{ mmol/l}$ ($p < 0.001$)
- phosphatemia $\bar{x} = 1.29 \text{ mmol/l}$ (normal)
- alkaline phosphatase activity was significantly diminished in 30 cases (55.5%) $\bar{x} = 45.23 \text{ } \mu\text{u/l}$ ($p < 0.001$)
- SGPT $\bar{x} = 9 \text{ } \mu\text{u/l}$ (normal)
- Gamma GT was increased in 4 cases (7.47%) $\bar{x} = 26.2 \text{ } \mu\text{u/l}$
- Blood urea was slightly increased in 5 cases (9.25%) $\bar{x} = 46 \text{ mg/dl}$, but after treatment diminished: $\bar{x} = 32 \text{ mg/dl}$.
- Serum creatinine slightly elevated in 5 cases (9.25%) $\bar{x} = 1.2 \text{ mg/dl}$, came back to normal levels after treatment: $\bar{x} = 0.9 \text{ mg/dl}$.
- 24 hour urine delta levulinic acid was increased in 39 cases (72.2%) $\bar{x} = 14.2 \text{ mg/dl}$

From the results and parameters presented the following conclusions could be taken:

- chronic lead poisoning generally causes anemia
- non significant hypocalcemia (31.4%), but significant hypomagnesemia (33.3%) was seen because of antagonism between magnesium and lead, with decreasing absorption and increasing elimination of magnesium (Balla and Kiss, 1996).
- the decrease in serum alkaline phosphatase activity (81.0%) in persons with high plumbemia was a surprise, as we did not find similar data in literature studied. The reason may be the inhibition of enzyme activity by lead (as the activity of hem - synthetase is also inhibited) or the magnesium deficiency (Balla and Kiss, 1996) or both.

The decrease of serum lead level after the treatment with "Kondi" tablets proved the antagonistic effect of zinc which decreased the absorption and increased the elimination of lead from the organism. This effect was stimulated by the presence of magnesium in the "Kondi" tablets.

CONCLUSIONS

1. We conclude that for therapeutic studies is better to choose a homogenous group, adults and children separated, and to use monotherapy containing only zinc.
2. In Corund the best period for such studies is the winter when the potters and their families are staying at home.
3. To follow therapeutic results we consider as best parameters the Hgb, Htc, serum Pb, Ca, Mg and serum alkaline phosphatase activity.
4. We are intending to repeat this study in better circumstances, using only zinc, to have much more reliable results.

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RICKETS AND HYPOMAGNESEMIA

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ABSTRACT

Rickets associates with many cases less sensitive to usual treatment and prevention. There are partial or temporary resistant forms, due to the multiple organic or cellular dysfunction.

New research allowed knowledge of the metabolic stages of vitamin D. These findings intensified because of the importance of magnesium in many biological processes inside the human body and in the phospho-calcium metabolism too.

The authors studied a group of 83 children aged between 4 months and 3 years, hospitalized with rickets-resistant forms to usual treatment. In those cases the paraclinical investigations showed: hypomagnesiemia between 0.66-0.7 mmol/l (38 cases) and between 0.72-0.8 mmol/l (45 cases).

We applied a hygienic diet regime ensuring a balanced food supply corresponding to the age, and we administered a treatment using magnesium in a dosage of 6-8 mg Mg/kg/day, during 4-6 weeks, and vitamin B₆ 50 mg/day associated with vitamin D therapy.

The study reveals the beneficial effect of the magnesium therapy standing out the correction of the hypomagnesiemia, hypocalciemia and clinical manifestations of rickets.

Key words: rickets, magnesium therapy

INTRODUCTION

Rickets, important variety of defective pediatric pathology is still a matter of public health.

Along with the defining clinical and paraclinical data, the biochemical tests also evidence the existence of a form of defective rickets caused by

hypomagnesemia. The observations are also confirmed by the increasing role of the magnesium ion in the human body in general and pediatric pathology in particular.

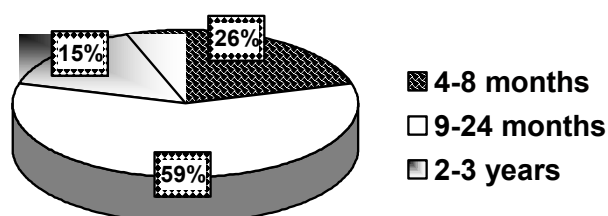
MATERIALS AND METHODS

A group of 83 children aged between 4 months and 3 years hospitalized with rickets-resistant forms to usual treatment, was studied.

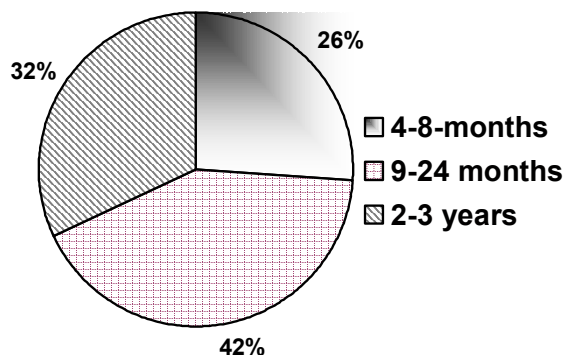
The clinical examination and paraclinical investigations of the mentioned children group permitted us to make the following classification of rickets forms:

- A. Precocious rickets: in babies from 4 to 8 months (26% of the cases)
- B. Rickets caused by prolonged absence of vitamin D in children aged 9 months up to 24 months (59% of the cases)
- C. Sequel rickets in children older than 2 years (15% of the cases)

Data referring to age groups repartition are presented in the next figures.



Age-groups repartition in rickets cases



Age-groups repartition in rickets and hypomagnesemia cases

Calcemia, magnesemia and phosphatemia were determined in all cases. According to the results there were established the therapy.

RESULTS AND DISCUSSIONS

Out of the total amount of magnesium in the human body (0,045% of the body weight), 60 to 65% is in the bony system in the form of labile carbonate. The remaining 34 to 40% is shared by the intra- and extracellular systems. Intracellular magnesium concentrates especially in the nucleus thus ensuring the cellular morphofunctional integrity. Extracellular magnesium is predominantly found in the serum in a concentration ranging from 1,8 to 2,4 mg%. Serum magnesium takes three forms: ionized, in combination and protein bound, mostly bound to albumin and the globule fractions α_1 , α_2 as principal means of transport.

Regarding the distribution of magnesium in the human organism a general scheme is presented in fig.1.

The physiological needs of magnesium in the body are covered by elementary contribution, the magnesium being actually present in all the foods in various quantities (Geormăneanu and Roşianu, 1975).

After ingestion the absorption of magnesium takes place in the intestine. The process of absorption is active it being helped along by an acid medium and the presence of vitamin D. The excess of calcium diminishes the absorption of magnesium. Once it has passed the intestine barrier, the magnesium gets into the blood protein-bound and free, ionized or combined representing the difusable fraction. Unabsorbed elementary magnesium is eliminated in the excrement. Absorbed magnesium is eliminated chiefly through the kidneys.

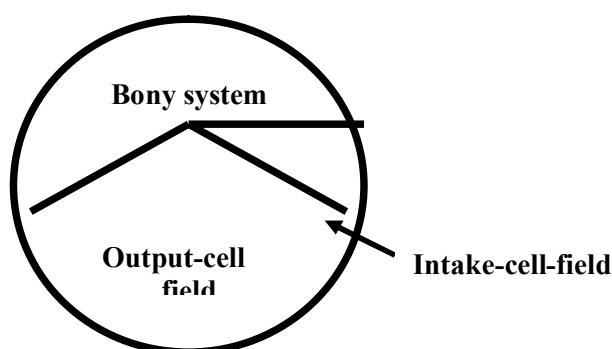


Fig.1: The repartition of magnesium in the body

The aspect related to apport-retention-elimination referring to magnesium is presented in fig. 2.

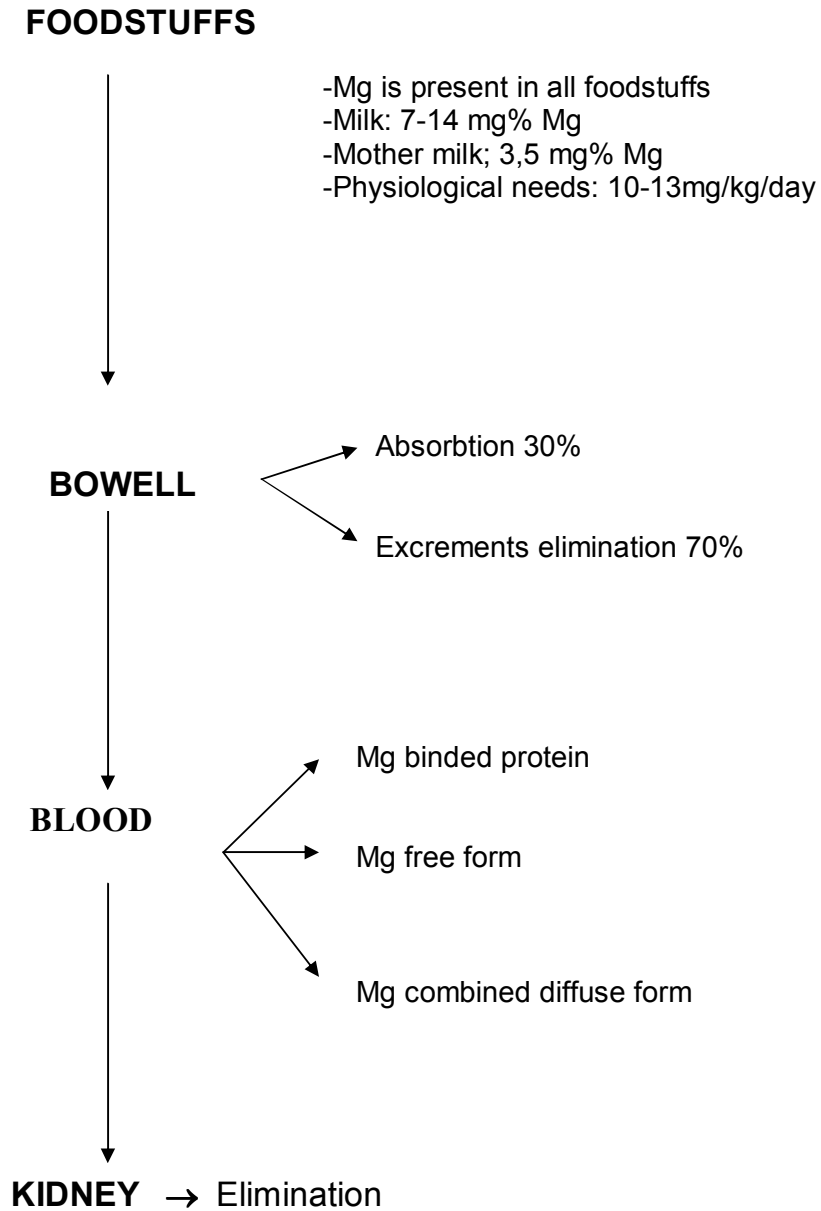


Fig. 2. The circuit of magnesium in the body

The contribution of the magnesium ion to the rickets pathogeny is conditioned by the interrelation between the metabolism of this ion and the decisive pathogen factors: the presence of the active metabolites of vitamin D, the parathormon and the amount of calcium. Besides these factors the magnesium ion itself is involved in the phospho-calcium metabolism.

According to literature data in the defective rickets sensitive to vitamin D, serum magnesium takes more often than not normal or high rates. There are also cases in which the normo- or hypocalcemia goes together with hypomagnesemia. In these cases hypomagnesemia keeps the clinical signs apparent in spite of the therapy with vitamin D (Miu et al., 1973; Prişcu et al., 1974).

In 83 of the cases under observation showed hypomagnesemia with normal or slightly low rates of calcaemia and phopsphotemia. In 38 cases the magnesemia rates ranged from 0,61 to 0,70 mmol/l, whereas in 49 cases the rates ranged from 0,72 to 0,79 mmol/l. When we had in view the type of nourishment given to these children up to the age of 4 months, the results were as follows: there were 14 cases of breast feeding, 17 cases of mixed feeding and 32 cases of artificial feeding.

As to other diseases associated with rickets there were 45 cases of acute respiratory diseases, 17 cases of acute diarrhea, 19 cases of first degree dystrophy and 2 cases of feverish convulsion

The therapy consisted of: calcium 0,5g/day oral administration; magnesium gluconolactate and B₆ - 1/2 amp/day; vitamin D – intramuscular administration 3 x 100.000U at 2 .

CONCLUSIONS

1. Determination of magnesemia in the child with clinical signs of rickets.
2. Completion of the rickets treatment with magnesium drugs when there is hipomagnesemia.
3. Avoiding calcium excess in the treatment of rickets with hipomagnesemia.
4. Prophylactic administration of magnesium in children with rickets and malnutrition in whom the food is hiperproteic.
5. Precocious variation of the baby food with vegetables rich in magnesium.

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CdSe BASED Cd – ISME AND ITS APPLICATIONS IN DETERMINING CADMIUM IN THE ENVIRONMENT

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ABSTRACT

Considering the possibilities of utilization of the CdSe based Cd-ISME, the paper aims at checking the behaviour of the electrode on determining the Cd^{2+} ion content of some unknown samples, by means of the calibration curve method.

Key words: ion-selective electrode (ISE), ion-selective membrane electrode (ISME), Cd-ISME, Cd(II)-electrode, solid-state membrane ISE, ISEs in enviromental, pollution analysis with ISEs

INTRODUCTION

At the basis of all possibilities of utilization of ISME electrodes lies the dependence relation between their potential and the activity (concentration) of the primary ion, expressed by Nernst's law.

Depending on the actual situation, one of the following determination techniques will be selected: direct potentiometry and potentiometric titration.

Although the procedure for determining the Cd^{2+} content in unknown samples by using Cd-ISME type electrodes does not compete with the STAS methods, it has to be improved, aiming at using electrodes in industrial automation processes.

Considering the higher sensitiveness, in small concentrations, of the Cd^{2+} cation of the selenide based Cd-ISME as against the sulphide based one, a series of possibilities for its application appear, of which the present paper will deal with the determination of the concentration of the Cd^{2+} cation in unkown samples by direct potentiometry, using the calibration curve method.

EXPERIMENTAL DATA

In view of drawing the calibration curve, the electrode was treated for $10 \div 15$ minutes in the most diluted solution of the standardization set. The measurements were made starting from the most diluted solution and going to the most concentrated one. After each measurement the electrodes were washed with distilled water and buffered with filter paper in order to avoid errors due to solution transport. The results obtained on the passage of electrodes through the 6 calibration solutions are given in Table 1.

Table 1: Standardization of the selenide based Cd-ISME

Sample	Cd ²⁺ concentration (moles/l)	E (mV)
1	$1.00 \cdot 10^{-1}$	-152.0
2	$1.00 \cdot 10^{-2}$	-184.0
3	$1.00 \cdot 10^{-3}$	-210.0
4	$1.00 \cdot 10^{-4}$	-237.0
5	$1.00 \cdot 10^{-5}$	-263.0
6	$1.00 \cdot 10^{-6}$	-280.0

Data processing

The data obtained on the standardization of the selenide based Cd-ISME and presented in Table 1 undergo the 4-D test. The results are given in Table 2.

Table 2: Cheking of points on the calibration curve

Checked point (pC)	a	b	R	4·D _m	A _i	Obs.
-	-130.6	-25.8	-0.9967	-	-	-
1.0	-136.8	-24.5	-0.9970	9.44	9.30	remains
2.0	-129.5	-26.1	-0.9964	14.68	2.48	remains
3.0	-130.0	-25.9	-0.9968	14.75	2.34	remains
4.0	-130.4	-25.7	-0.9971	13.99	3.77	remains
5.0	-131.3	-25.4	-0.9967	13.53	4.63	remains
6.0	-126.7	-27.5	-0.9992	5.44	11.69	is eliminated

Applying the 4-D test to the values obtained on standardizing the selenide based Cd-ISME, it results that the point corresponding to the concentration $1.0 \cdot 10^{-6}$ MCd²⁺, must be eliminated. After the elimination of the point affected by gross errors, the parameters of the calibration curve and the reliance interval are calculated. The results are given in Tables 3 and 4.

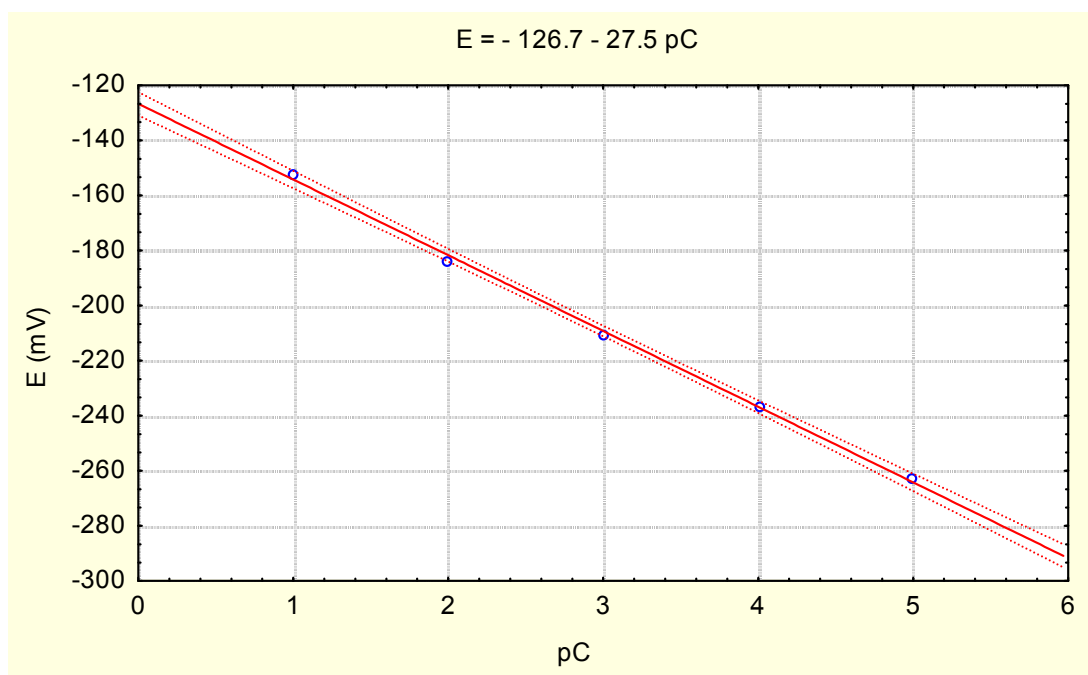
Table 3: Calculus of the calibration curve parameters

Sampl	C (moles/l)	pC (x)	(pC) ² (x ²)	E (y)	E ² (y ²)	pC·E (xy)	Parameters of the calibration curve
1	0.100	1.0	1.00	-152.0	23104.00	-152.00	a = - 209.2 b = - 27.5 R = - 0.9992
2	0.0100	2.0	4.00	-184.0	33856.00	-368.00	
3	$1.00 \cdot 10^{-3}$	3.0	9.00	-210.0	44100.00	-630.00	
4	$1.00 \cdot 10^{-4}$	4.0	16.00	-237.0	56169.00	-948.00	
5	$1.00 \cdot 10^{-5}$	5.0	25.00	-263.0	69169.00	-1315.00	
Σ	-	15.0	55.00	- 1046.0	226398.00	-3413.00	E = -209.2 – 27.5 pCd

Table 4: Calculus of the reliance interval

Sampl	pC	E_t (mV)	E_i (mV)	$ A_i $	A_i^2	I_i
1	1.0	-154.2	-152.0	2.2	4.84	$D_m = 1.36 \text{ mV}$ $S_{Em} = 0.91 \text{ mV}$ $I_{S\%} = \pm 0.33$ $I_r = \pm 2.34 \text{ mV}$ $I_{\%} = \pm 8.5$ ($t = 2.57$, for $n = 5$ și $P = 95\%$)
2	2.0	-181.7	-184.0	2.3	5.29	
3	3.0	-209.2	-210.0	0.8	0.64	
4	4.0	-236.7	-237.0	0.3	0.09	
5	5.0	-264.2	-263.0	1.2	1.44	
Σ	-	-	-	6.8	12.30	

The calibration curve drawn with the points which remain after elimination of the gross errors is given by Figure 2.

**Figure 2:** Calibration curve

In order to use electrodes in the domain of concentrations $< 1.0 \cdot 10^{-5} \text{ MCd}^{2+}$ too, the calibration curve valid on the interval $1.0 \cdot 10^{-1} \div 1.0 \cdot 10^{-5} \text{ MCd}^{2+}$ was considered and the interpolation method was applied outside this domain of linearity. Also, because, on checking the gross values, the concentration point $1.0 \cdot 10^{-1} \text{ MCd}^{2+}$ was very close to being eliminated (see Table 2), it was considered that it was necessary to apply the interpolation method on the interval of concentrations $> 1.0 \cdot 10^{-2} \text{ MCd}^{2+}$ too.

The unknown samples were of two kinds:

1. two synthetical samples obtained by:
 - a) weighing 2.000 g $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and its dissolving in 500.0 ml bidistilled water; the concentration of the sample was checked by complexonometric titration ($pC_1 = 1.89$);
 - b) dilution, ratio 1: 10, of the sample from the point 1a ($pC_t = 2.89$).
2. three samples collected from the neutralization station of S.C. AEM S.A., whose Cd^{2+} content was determined at Aquatim Timișoara:
 - a) the washing waters from the electroplating area ($pC_t = 4.67$);
 - b) the mixed waters at the entrance to the neutralization station ($pC_t = 4.81$);
 - c) the treated waters at the exit from the neutralization station ($pC_t = 5.41$).

The synthetical samples were obtained under the same conditions as the standard samples, that is, the ionic strength was adjusted to $J = 0.32 \text{ M}$ by addition of KNO_3 for the sample **1a** and dilution with KNO_3 0.32 M solution of sample **1b**; also, pH was brought to the value 4.7 addition of 15 ml acetic buffer solution.

As concerns the residual waters coming from S.C. AEM S.A., they can have either a variable pH, as in the case of sample **2a** (which results from mixing of acid and basic galvanic washing waters), or a neutral pH, as in the case of samples **2b** and **2c** (which have been neutralized in the station for residual waters treatment). The pH of these samples is adjusted to the value 4.7 with acetic buffer solution. Because these samples also contain other salts, besides those of the Cd^{2+} , and because in the previous study [1] we saw that ionic strength does not greatly influence the results, the latter was not adjusted any more.

The calibration curve method was applied to sample **1a**, **1b**, **2a** and **2b**.

Because sample **2c** is placed within the interval on $pC = 1.0 \div 2.0$, it was not determined by the calibration curve method, but by the interpolation method. Also, sample **1a**, having its $pC = 5.0 \div 6.0$ less certain, was checked by both the calibration curve method and the interpolation method.

Tables 5a and 5b give the results obtained as concerns the Cd^{2+} content determined by the calibration curve method, as well as the incertitude of reproductibility in the case of samples **1a**, **1b**, **2a** and **2b**.

Table 5a: The Cd^{2+} content in the unknown samples, expressed in pC , determined by the calibration curve method. Calculus of incertitude of reproductibility

Sample	C (moles/l)	PC_{theoret}	E_{measured} (mV)	pC_{meas}	$I_r = t \cdot S_{pC}$	$t \cdot S'_{pC}$	Reliance interv. $pC_{\text{meas}} \pm t \cdot S'_{pC}$
1a	$1.29 \cdot 10^{-2}$	1.89	-180.0	1.94	0.085	0.125	$1.81 \div 2.06$
1b	$1.29 \cdot 10^{-3}$	2.89	-209.0	3.00	0.085	0.120	$2.88 \div 3.12$
2a	$2.14 \cdot 10^{-5}$	4.67	-252.0	4.56	0.085	0.126	$4.43 \div 4.69$
2b	$1.55 \cdot 10^{-5}$	4.81	-256.0	4.70	0.085	0.128	$4.57 \div 4.83$

Table 5b: The Cd^{2+} content in the unknown samples, expressed in C (moles/l), determ. by the calibration curve method.
Calculus of incertitude of reproductibility

Sample	C	$t \cdot S_c$	$t \cdot S_{\%}$	$C \pm t \cdot S_c$
1a	$1.22 \cdot 10^{-2}$	$0.33 \cdot 10^{-2}$	26.9	$0.89 \cdot 10^{-2} \div 1.55 \cdot 10^{-2}$
1b	$1.04 \cdot 10^{-3}$	$0.28 \cdot 10^{-3}$	26.9	$0.76 \cdot 10^{-3} \div 1.32 \cdot 10^{-3}$
2a	$2.88 \cdot 10^{-5}$	$0.84 \cdot 10^{-5}$	29.0	$2.04 \cdot 10^{-5} \div 3.71 \cdot 10^{-5}$
2b	$2.18 \cdot 10^{-5}$	$0.61 \cdot 10^{-5}$	29.0	$1.48 \cdot 10^{-5} \div 2.69 \cdot 10^{-5}$

Note: Because the concentration calculated by the relation $C = 10^{-pC}$ does not give a symmetrical value as against the one calculated from $C \pm t \cdot S_{pC}$, the values $C + t \cdot S_{pC}$ and $C - t \cdot S_{pC}$ were initially determined and C was taken in the middle of the interval $(C + t \cdot S_{pC}) \div (C - t \cdot S_{pC})$.

In order to apply the interpolation method to sample **1a** and **2c**, four straight lines are considered in the intervals mentioned, delimited by the pC given in Table 6. In this case, the concentration intervals for pC_t can be restricted, namely:

- $pC_t = 1.89$ belongs to the interval $pC_1 = 1.70 \div pC_2 = 2.00$;
- $pC_t = 5.41$ belongs to the interval $pC_1 = 5.30 \div pC_2 = 5.52$.

Table 6: Standardization of selenide based Cd – ISME on the intervals $pC = 1.0 \div 2.0$ and $pC = 5.0 \div 6.0$

Sample	Concentration (moles/l)	pC	E (mV)
1	$10.0 \cdot 10^{-2}$	1.00	-148.0
01.1	$8.0 \cdot 10^{-2}$	1.10	-153.0
1.2	$5.0 \cdot 10^{-2}$	1.30	-161.0
1.3	$3.0 \cdot 10^{-2}$	1.52	-170.0
1.4	$2.0 \cdot 10^{-2}$	1.70	-175.0
2	$1.0 \cdot 10^{-2}$	2.00	-184.0
5	$10.0 \cdot 10^{-6}$	5.00	-263.0
5.1	$8.0 \cdot 10^{-6}$	5.10	-265.0
5.2	$5.0 \cdot 10^{-6}$	5.30	-270.0
5.3	$3.0 \cdot 10^{-6}$	5.52	-275.0
5.4	$2.0 \cdot 10^{-6}$	5.70	-279.0
6	$1.0 \cdot 10^{-6}$	6.00	-280.0

In this case there is only one reading for the potentials corresponding to these samples so statistical calculus cannot be used, the results and reliance interval being expressed through the medium of incertitude of determination I_d , with the remark that there are three incertitudes of determination: two belonging to the standard samples and one characteristic of the unknown sample. The results will be expressed through the medium of the highest incertitude. The values obtained are given in Table 7.

Table 7: The Cd^{2+} content in the unknown samples determ. by the interpolation method. Calculus of the incertitude of determination

Sample	pC_t			E_{meas} (mV)			pC_{calc}	I_d	Reliance interval
	pC_1	pC_2	pC_x	E_1	E_2	E_x			
1a	1.70	2.00	1.89	-175.0	-184.0	-180.0	1.87	0.034	1.84÷1.90
2c	5.30	5.52	5.41	-270.0	-275.0	-273.5	5.43	0.034	5.40÷5.46

CONCLUSIONS

The results obtained with the selenide based electrode range in the calculated reliance interval, which allows us to state that, in the liniarity zone of the standardization curve, the CdSe based Cd - ISME can be used with good results in determining the Cd^{2+} content in samples by means of the calibration curve method.

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PESTICIDES INFLUENCE ON THE ELECTRONIC STRUCTURE OF HAEMOGLOBIN ACTIVE CENTER IN EXPERIMENT

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ABSTRACT

By the gamma resonance spectroscopy method there was studied a change in the electronic structure of haemoglobin active centre (Hb) under the action of chlorophos (CHL), hexachlorcyclohexane (HCCH), and nitrates to the active organism.

Key words: haemoglobin, active centre, pesticides.

INTRODUCTION

At present the problem of understanding the influence of pesticides and nitrates on the human organism is of high importance. Research carried out on the level of the entire organism showed that the action of the mentioned toxic agents is connected to the

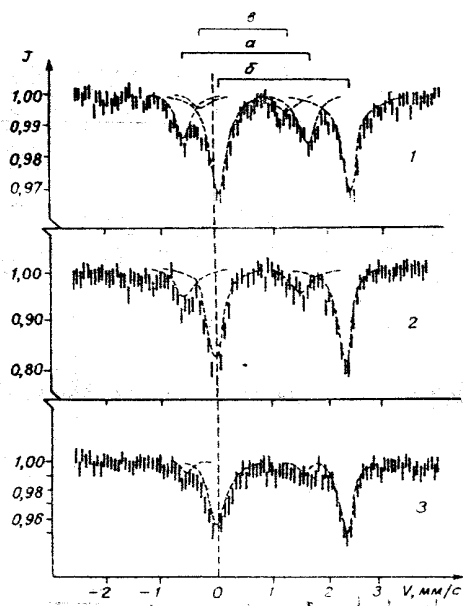


Fig. 1. Type of GR-spectra of the erythrocytes of rats under control (1), under the influence of chlorophos (2), hexachlorcyclohexane (3). Duplicate α corresponds to HbO_2 , δ to Hb, and ϵ presumably to methaemoglobin

progress of anemia symptoms (Karpenko and Voitenko, 1972). In literature there are just a few experimental works on the identification of toxic exogen substances impact on haemoglobin (Hb). The purpose of work was to study the electronic structure of haemoglobin active centre under the influence of chlorophos (CHL), hexachlorcyclohexane (HCCH) and nitrates on the alive organism by the γ -resonance spectroscopy (GRS) (Golidansky and Gerber, 1990).

MATERIALS AND METHODS

Preparation of blood samples. For iron Mossbauer effect is observed only in ^{57}Fe isotope, the quantity of which in natural compounds is 2.2%. Because of the low metal percentage in the iron-containing proteins, the investigation by GRS method of the biological objects (for example, blood) is connected with great difficulties. Usually, to study this type of systems we enrich the iron-containing proteins by ^{57}Fe isotope. For this purpose we internally introduced a solution of iron and fructose, containing 0.7 mg of ^{57}Fe (Philip et al., 1963) to the rat during 14 days on a daily basis. Within this time the process of replacement of natural Fe by ^{57}Fe in the haemoglobin occurred to be quite sufficient to erase the Mossbauer spectra, all though earlier there was proposed to carry out this procedure for a month (Grant and Cape, 1967).

The research object served the balanced blood of controlled and experimental animals. The controlled animals were introduced only ^{57}Fe isotope, but the experimental ones - also the toxic substances. CHL, HCCH and natrium nitrate were introduced per oss 5 times per week during the period of two months in the amount of 52,25 and 40mg/kg of weight respectively. Drugged by natrium thiopental, the animal's thorax was dissected and they tested big aorta blood. The blood was frozen and maintained at the liquid nitrogen temperature.

To study the resolution of $\text{Hb} \rightarrow \text{HbO}_2$ we transfused the carbogen through the blood in vitro during 30 minutes. Then those samples were frozen and investigated by GRS method. The spectra types of the investigated samples are shown in Fig. 1.

RESULTS AND DISCUSSIONS

In most cases GR-spectra of erythrocytes of animals under control represent a superposition of three duplicates. In different experiments the share of each duplicate in the spectrum is not the same (Table 1). The most accurate spectrum is shown in Fig. 1.

Table 1. Parameters of GR-spectra of rats' blood erythrocytes under the influence of pesticides

Experiment	Duplicate δ				Duplicate a				Duplicate ϵ			
	mm/s			share, %	Mm/s			share, %	mm/s			share, %
	IS _{Na+}	QS			IS _{Na+}	QS			IS _{Na+}	QS		
Control (erythrocytes)	1.17	2.33	0.41	~65	0.53	2.21	0.26	~24	0.59	1.49	0.34	~11
Erythrocytes + O ₂ (30min)	1.16	2.34	0.61	~44	0.51	2.22	0.30	47	0.51	1.86	0.31	9
Chlorophos (CHL)	1.17	2.35	0.37	86	0.52	2.15	0.38	14	-	-	-	-
Erythrocytes + CHL + carbogen	-	-	-	-	0.50	2.12	0.30	82	0.44	1.73	0.38	18
Hexachlorocyclohexane (HCCH)	1.14	2.26	0.34	86	0.54	1.92	0.55	14	-	-	-	-
Nitrates	1.20	2.37	0.37	69	0.50	2.22	0.30	20	0.43	1.82	0.34	11
Nitrates + carbogen (30min)	-	-	-	-	0.55	2.22	0.29	86	0.59	1.69	0.33	14

The main contribution into the summary spectrum (~65%) was made by δ duplicate (isomer shift (IS) = 1.17; quadropole split (QS) = 2.33 mm/s, table). The parameters of GR-spectra point out on the presence of Fe(II) ions in the high-spin state ($S = 2$) and the duplicate a corresponds to the haemoglobin (Hb). The second area-based duplicate is a (IS = 0.53; QS = 2.21 mm/s; area = 24%) that corresponds to Fe(II) ions in the low-spin state ($S = 0$) and is referred to oxyhaemoglobin (HbO₂). Along with this, one can see ϵ duplicate in the spectrum (IS = 0.59; QS = 1.49; area = 11%).

The saturation of blood by oxygen in vitro during 30 minutes leads to the fact that the biggest duplicate in the spectra is α (Fig. 2). This spectrum measurement corresponds the transition of $\text{Hb} \rightarrow \text{HbO}_2$ and demonstrates the potential possibility of using the GR-spectroscopy method to study the kinetics of the above-mentioned resolution.

According to the bibliography data (Tsai et al., 1981), GR-spectra of α - and β -chains of haemoglobin HbO_2 under 4.2 K have different magnitudes of QS ($\text{QS}_{\alpha-\text{HbO}_2} = 2.248 \text{ mm/s}$; $\text{QS}_{\beta-\text{HbO}_2} = 2.194 \text{ mm/s}$). They also have different temperature dependence of QS magnitudes of Fe ions in those hemmas.

In the work (Oistreih and Semekin, 1985) of Oistreih the duplicate HbO_2 of the natural (by isotonic content of Fe) human blood was observed as a superposition of two intensively equal duplicates belonging to α - and β -chains of haemoglobin. As it is observed in the table, in the studied blood samples the magnitudes of (G) width of HbO_2 peaks are close to the magnitude G of natrium nitropruside (0.26-0.36 mm/s), that is why HbO_2 spectrum is approximated by one duplicate.

GR-spectra of animals erythrocytes under the action of chlorophos (Fig. 1, 2) represent two duplicates. It is observed with a high degree of accuracy that a share of Hb is 6 times greater than HbO_2 (table). Because of the low intensity of the HbO_2 duplicate it is impossible to identify the presence of ϵ duplicate. It is worth mentioning that only in a single experiment the Mossbauer spectrum contains peaks corresponding to ϵ duplicate, the intensity of which is changing after the erythrocytes saturation by oxygen in vitro. Similar type of GR-spectra is obtained for erythrocytes of animals blood under the action of HCCH (Fig. 1, 3).

As a result of nitrates influence, in the GR-spectra of animals blood in experiment there were observed three states of Fe. And for this case, under the influence of carbogen the share of α duplicate increases at the account of δ duplicate which remains at a constant magnitude.

The analysis of the data in the table and their comparison with the bibliography results permit us to assume that duplicate ϵ refers to methaemoglobin. However, for the final confirmation of previously made presumption it is necessary to analyse the spectra on the Helium temperature magnitudes as well as to study haemoglobin using other independent methods.

Under the chronic influence of toxic substances, erythrocytes' haemoglobin undergoes a series of changes. First, in the GR-spectra of animals' blood in experiment

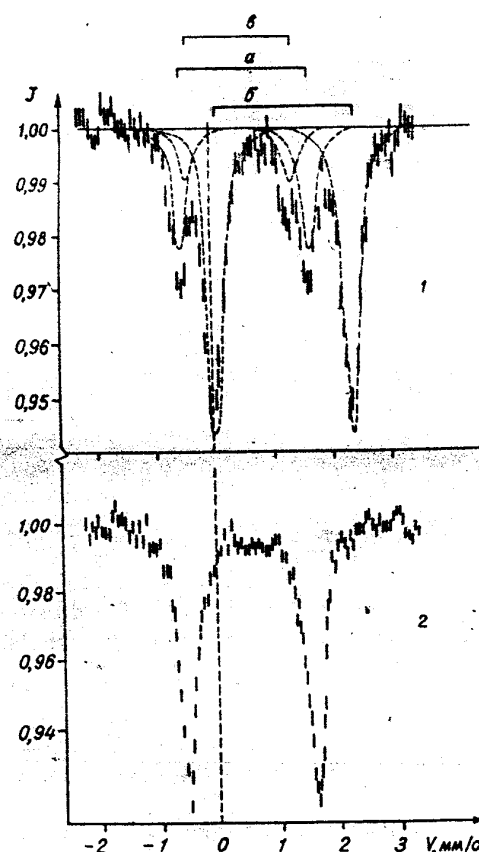


Fig. 2. Change in HbO_2 share of erythrocytes under the influence of oxygen in vitro: 1 - initial state; 2 - after 30 min.

there is no σ duplicate. Second, the share of HbO_2 in the blood considerably decreases. Third, in the series HbO_2 (control) $\rightarrow$ HbO_2 (CHL) $\rightarrow$ HbO_2 (HCCH) there is observed a step-by-step decrease of QS magnitude of HbO_2 and the width of the line somewhat grows up. One should note, however, that the parameters of Hb GR-spectra are practically unchanged.

As it was demonstrated in (Hleskov et al., 1985), big QS magnitudes of Fe (II) ions in HbO_2 are caused by the non-uniform saturation of d_{xz} , d_{xy} , d_{yz} , and in particular $Nd_{xy} > Nd_{xz, yz}$ AO Fe. The decrease of QS magnitude in the series HbO_2 (control) $\rightarrow$ HbO_2 (CHL) $\rightarrow$ HbO_2 (HCCH), is most probably the result of different saturation of $Nd_{xy} > Nd_{xz, yz}$. For the reason of tighter links Fe- N_{porf} compare to Fe-N(His) one could assume that in the observed series there are different saturation levels, mainly for d_{xz} , d_{zy} , d_z^2 AO Fe.

During the investigation of monocrystals of MbO_2 by neutronography method (Phillips and Scoenborn, 1991) it was discovered that between the group of

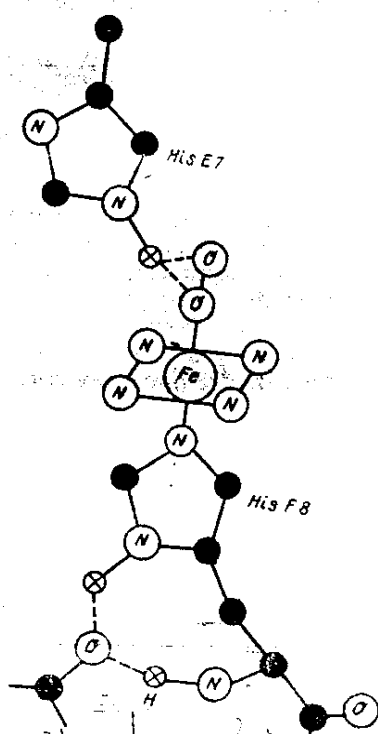


Fig 3. Structure of haemoglobin active centre

NH distilled histidine E7 and a molecule of the coordinated oxygen in oxyhaemoglobin there is a hydrogen link (Fig. 3). It is reasonable to assume that the same link is formed in the oxyhaemoglobin of rats. It is possible, that under the action of such molecules as CHL, HCCH and nitrates on the animal organism there occurs a change in the conformity of globine parts of Hb molecule and, as a result, the interaction Nh (E7)-O₂ and furthermore Fe-O₂ is different. The data obtained for GR-spectra of oxy-haemoglobins of animals' blood in experiment could be explained by assuming the intensification of N (F8) - Fe-O₂ overlapping in the above mentioned series.

CONCLUSIONS

Thus, as a result of the research carried out one could make a conclusion that the action of such agents as CHL, HCCH on rats' organism does not lead to the change of closely surrounding metal ions in Hb, however, the conformity of globine part of haemoglobin molecule is changed and this fact leads to the intensification of AO Fe along the axis N (F8) - Fe - O₂.

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A POSSIBLE VALORIFICATION OF MINE STERIL FROM COPPER MINES

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ABSTRACT

The steril material resulted from copper mines exploitation in Moldova Nouă and Sasca Montană and stored in large warehouses of thousands of tones of steril material, contains important quantites of micro and macroelements, which can be used in agriculture. This paper presents the effect of direct mine steril application on pea, wheat, and maize crops.

Key words: mine steril, microelements, macroelements

INTRODUCTION

Researches have been initiated by Goian M., Borcean I. and Vîlceanu in 1989 and continued after that by a large team, including the initial authors, between 1991 and 1993. These researches pointed out that the use of mine steril as fertilizer on crops like wheat, barley, maize and soia bean bring about an increase of yield situated between 5 and 12 %.

This increase due to the content of macroelements (phosphorus, magnezium, sulphur, calcium, etc), and microelements (manganese, bor, molibden, copper, zinc) of the mine steril in accesible form for the plants.

It must be mentioned that, because of the high calcium content as well as of the ore process is made with basic elements, the mine steril presents an alkaline pH, and can be used with succes as amendment on acid soil.

MATERIALS AND METHODS

Researches have been caried out on a brown luvic pseudo–gleyzed soil, from the western part of Romania, located Caras river and the Danube. The fertilizer amount was of $N_{50}P_{70}K_{70}$ at peas and $N_{100}P_{70}K_{70}$ at wheat and maize.

RESULTS AND DISCUSSIONS

The content in heavy metals are between the international limits. Reffering to this data table 1 presents some data.

A special interest represents the evaluation of the admitted concentrations for heavy metals. Analysing the data recorded in table 2 and comparing them with the maximum admissible concentration of some heavy metals according to international standards we point out that all four tests are between international limits.

Table 1 : The content of some elements from some tests of mine steril carried out in the western part of the country (oxides or elements)

Element	Content from mine steril (%)			
	Test number			
	1	2	3	4
Si	32.00	43.00	51.00	47.00
Al	2.00	1.00	13.00	13.00
Fe	2.00	1.50	6.00	3.00
Ti	0.30	0.10	0.27	0.10
P	0.07	0.03	0.05	0.05
Ca	27.00	15.20	33.00	18.00
Mg	2.30	1.50	1.80	2.20
Mn	0.10	0.05	0.12	0.09
S	0.32	0.81	0.53	0.33
Co	traces	traces	traces	0.001
B	traces	0.001	traces	traces
As	0.001	traces	0.005	0.004
Bi	0.02	0.005	0.006	0.006
Mo	0.003	0.005	0.004	0.005
Pb	traces	traces	traces	0.001
Cu	0.10	0.07	0.09	0.09
Zn	0.06	0.03	0.01	0.05
Cd	traces	traces	0.001	traces
Cr	0.002	0.005	0.003	0.006
Ni	0.001	0.001	0.002	0.001
Se	traces	traces	traces	traces
Ag	traces	traces	traces	traces
H ₂ O (105°C)	7.00	8.30	10.00	9.30
Other elements undetermined			100%	

Table 2: The maximal admissable concentration in heavy metals mg/kg (p.p.m.) on different muds which can be used in agriculture (Dumitru et al..1996)

Element	International limits	Recomended
1	2	3
As	10 – 75	-
Cd	3 – 30	20
Co	20 – 150	-
Cr	200 – 1200	750
Cu	500 – 3000	1000
Mg	5 – 25	16
Mn	500 – 3000	-
Mo	20	-
Ni	30 – 500	300
Pb	300 – 1200	750
Se	14 – 100	-
Zn	1000 – 10000	2500

Results pointed out that all three crops present a favorable reaction at mine steril application (table 3), the increase of the yield at once with the increase of the amount in the research field.

Table 3: The effect of mine steril on the yield of peas, wheat and maize

Crop	Mine steril amount (kg/ha)	Yield (kg/ha)	Differences		Significance
			%	kg/ha	
Peas	0	2335	100	-	Mt.
	500	2616	112	281	XX
	1000	2939	125	604	XXX
	1500	3155	135	820	XXX
	DL 5% =175		DL 1% =216	DL 0.1% =375	
Wheat	0	3849	100	-	Mt.
	500	4027	104	178	X
	1000	4189	109	340	XX
	1500	4326	112	477	XX
	DL 5% =127		DL 1% =316	DL 0.1% =719	
Maize	0	4765	100	-	Mt.
	500	5020	105	255	X
	1000	5216	109	451	XX
	1500	5405	113	640	XX
	DL 5% =144		DL 1% =327	DL 0.1% =918	

The best use was recorded at peas, which can be explained by the necessity of the leguminous plants in general for oligoelements and especially for molybdenum, boron and manganese, because these elements contribute to the improvement of nitrogen fix and certainly to a better plant growth.

More detailed data referring to the topic of this paper are mentioned in literature – predilectly in the references presented.

The yield increase at peas with 12% for a dose of 500 kg/ha mine steril, increase at 15% for a dose of 1000 kg/ha of mine steril and respectively with 35% for a dose of 1500 kg/ha of mine steril.

Both cereals, wheat and maize, have the same reaction at mine steril application, the wheat yield increasing between 4 and 12 % and maize yield increasing between 5 and 13%.

CONCLUSIONS

1. Mine steril result from the western part of Romania cooper mines are a very important source of macroelements and microelements for agriculture.

2. The yield increase after mine steril application was between 12 – 35 % at peas between 4 – 12 % at wheat and 5 – 13 % at maize.

3. Mine steril application is recomandable on acid soils because of its alkaline reaction.

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MATHEMATICS RELATION BETWEEN TRANSITIONAL METALS AND SOME OXIDASE ACTIVITY

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ABSTRACT

We established the correlation between some transitional metals, Mn, Fe, Cu, Zn and the activity of catalase and peroxidase in "tropical spinach" leaves (Basella alba grown in field and greenhouse conditions), by mathematics regression method.

We obtained in this way one mathematics equation and applied it at all investigations performed in our laboratory.

So we could emphasize that the catalase activity is strictly dependent on iron quantity. But the activity of peroxidase seems be dependent on manganese, not on iron.

Key words: mathematics model, transitional metals, oxidase.

INTRODUCTION

"Tropical spinach" is the sub-name of *Basella alba* native from tropical India and is one of the most appreciated vegetables for its edible leaves.

The metabolic features of *Basella alba* grown in our temperate zone conditions represent a study domaine important for biochemistry, vegetal physiology and it can be evaluated by mathematical methods.

Correlation between the quantity of iron, manganese, copper, zinc and the catalase and peroxidase activity could be made using the mathematics regression model.

MATERIALS AND METHODS

Experiments were performed on fresh biological material consisted on *Basella alba* leaves from field and greenhouse.

Content in Mn, Fe, Cu, Zn was established by PIXE (Particular Induced X-Ray emission) method and the results were expressed in mg / 100g fresh substance.

Catalase and peroxidase activity was made by the titration method and the results were expressed in μM / ml.

RESULTS AND DISCUSSIONS

Mathematics regression of relation between transitional metals and enzymes activity emphasize some interesting aspects (Somnea et al., 1993; Somnea and Calciu, 1994; Winter and Winter, 1996).

The catalase activity strictly depends on iron quantity in *Basella alba* leaves from field (equations 1 and 3).

But in the plants leaves from greenhouse it is influenced also by manganese, the reason being the values obtained from the regression model. (Table 1 and 2; fig. 1 and 3).

Aspect of the diagram and the values of the mathematics regression coefficients (equations 2 and 4) show us that the peroxidase activity depends not strictly on the iron quantity (fig. 2 and 4), but it seems that only the iron presence is necessary for it.

The values of the zinc regression show that is involved in the peroxidase activity in the plants leaves from greenhouse.

Table 1: Corelation between transitional metals and oxidases activity (1998) – *Basella alba* field

No. crt.	Specification	Mn (mg/100g)	Fe (mg/100g)	Cu (mg/100g)	Zn (mg/100g)	Catalase (μM/ml)	Peroxidase (μM/ml)
1	June	1.3200	5.2000	0.0310	1.5000	0.0750	30.0000
2	July	1.3500	5.0900	0.1200	1.2450	0.3902	6.5000
3	August	0.9655	11.5550	0.0340	0.7500	2.2500	3.0000
4	September	0.0520	10.5000	0.2900	0.1000	0.8100	14.5000

CATALASE MODELLING				
Regression Output:				
Constant		3.929471		
R Squared		0.9999		
No. of Observations		4		
X Coefficient(s)	2.022893	-0.023003	-8.877473	-4.08658
PEROXIDASE MODELLING				
Regression Output:				
Constant		-117.5785		
R Squared		0.9999		
No. of Observations		4		
X Coefficient(s)	-73.38932	7.410345	154.0573	134.09524

$$\text{Catalase} = 3,9294 + 2,0228\text{Mn} - 0,0230\text{Fe} - 8,8774\text{Cu} - 4,0865\text{Zn}$$

Equation 1

$$\text{Peroxidase} = -117,579 - 73,3893\text{Mn} + 7,4103\text{Fe} + 154,057\text{Cu} + 134,0952\text{Zn}$$

Equation 2

Table 2: Corelation between transitional metals and oxidases activity (1998) – *Basella alba* greenhouse

No. crt.	Specification	Mn (mg/100g)	Fe (mg/100g)	Cu (mg/100g)	Zn (mg/100g)	Catalase (μM/ml)	Peroxidase (μM/ml)
1	June	0.5800	4.2000	0.0380	1.2500	0.1525	40.5000
2	July	1.0600	4.6250	0.0240	1.1700	0.1800	4.2500
3	August	0.6995	10.8500	0.0260	0.7150	1.9112	3.0000
4	September	0.0610	5.5000	0.0200	0.0600	1.5500	18.5000

CATALASE MODELLING				
Regression Output:				
Constant		0.457772		
R Squared		0.9999		
No. of Observations		4		
X Coefficient(s)	-0.178504	0.198849	3.258166	-0.928571
PEROXIDASE MODELLING				
Regression Output:				
Constant		-5.88111		
R Squared		0.9999		
No. of Observations		4		
X Coefficient(s)	-18.76563	-2.233484	1904.213	-4.571429

$$\text{Catalase} = 0.4577 - 0.1785\text{Mn} + 0.1988\text{Fe} + 3.2581\text{Cu} - 0.9285\text{Zn}$$

Equation 3

$$\text{Peroxidase} = -5.8811 - 18.7656\text{Mn} - 2.2334\text{Fe} + 1904.213\text{Cu} - 4.5714\text{Zn}$$

Equation 4

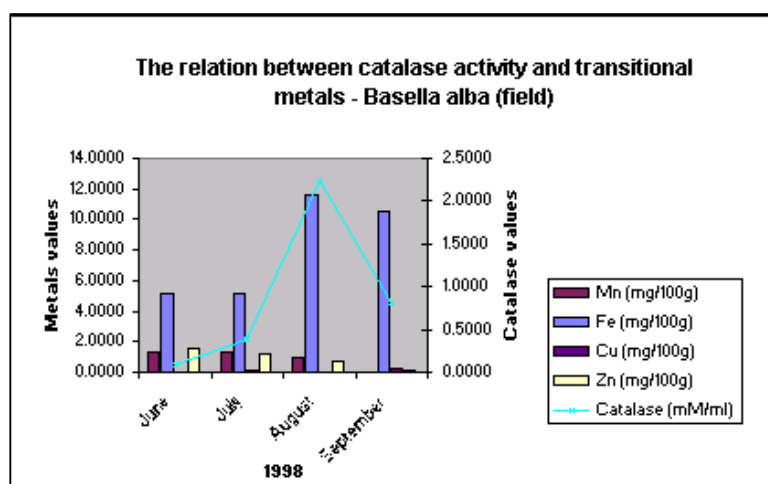
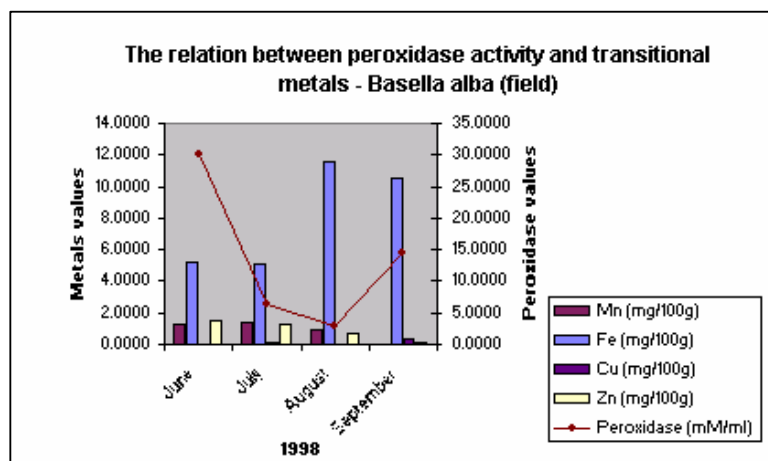


Fig. 1



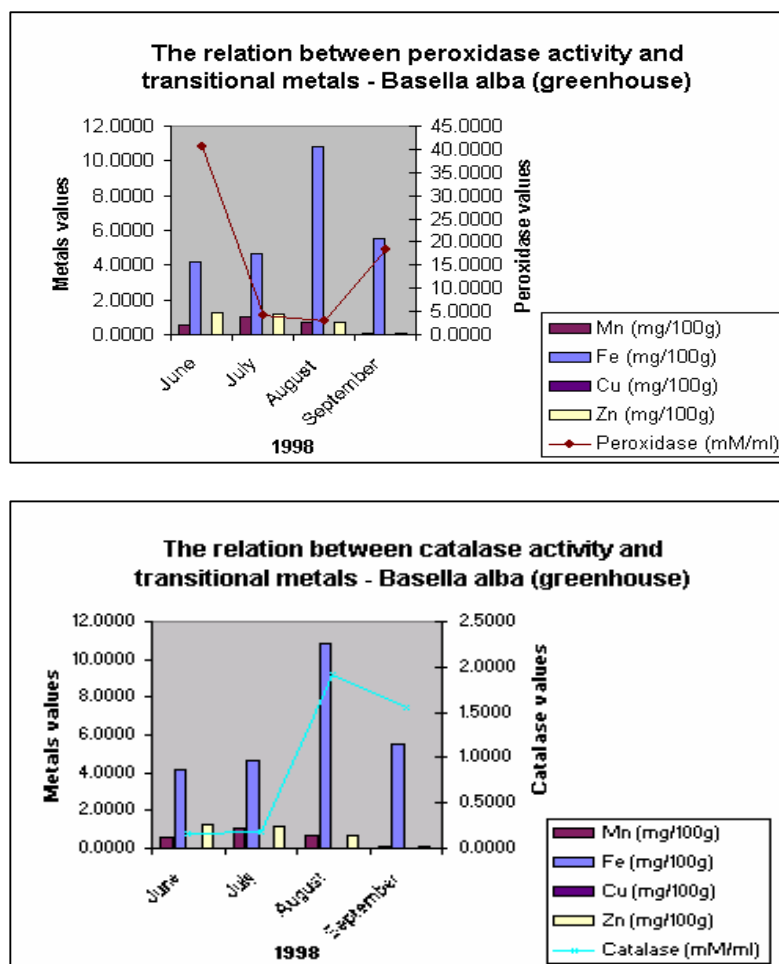


Fig. 4

CONCLUSIONS

1. The mathematics regression method emphasizes the correlation between transitional metals and the enzyme activity in our case.
2. The catalase activity depends strictly on the iron quantity.
3. The peroxidase activity depends not on the iron quantity, but its presence is absolutely necessary.

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ACCUMULATION IN SOILS OF METALLIC TRACE ELEMENTS WITH TOXICOGEN POTENTIAL: FROM PEDOGENESIS TO CONTAMINATION

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ABSTRACT

According to soil sciences the problem of metallic trace elements with toxicogen potential may be approached from two points of views: metal sources originated in the existing primary geochemical substance (rocks which constitute the origin of pedogenesis) and secondary metal sources resulting from contaminated processes. This work proposed to present a review of the metallic trace elements with toxicogen potential which are not radioactive (i.e. radionuclids were excluded). In the literature these groups of metals are known also as "heavy metals".

In the first part this work deals with the peculiarities of pedogenesis, the relationship of geochemical aspects with minerals and rocks which are at the origin of soils. In this context various geological compounds which contain metallic trace elements, such as: lead, cadmium, zinc, manganese etc. are discussed.

Aspects related to the chemical contamination of the soils are presented in the second part of the work. There are described the pollutants, meaning the contamination sources having at their origin: industrial residues, agricultural residues, atmospheric relapse a.o.

Approaching the problem in the context of the soil-plant-animal-human relationship it could be observed that some of the discussed metals are considered biometals, e.g. Zn, Mn. Their potential toxicity is given by their high concentration in soil, which can influence their concentration in plants and the possibility of translocation.

Key words: pedogenesis, soil contamination, metallic trace elements

INTRODUCTION

Generally, in biology as well as in vegetal and animal biochemistry, there are a lot of metallic trace elements considered to be bioelements. These are currently part of the composition of organisms, plants, animals and man. This group includes Fe, Cu, Zn, Co etc.

In the acceptance of biology and vegetal and animal biochemistry, was accredited the term trace elements with toxicogen potential, or as a usually term: heavy metals for some trace elements, i.e. Pb, Cd, Hg, Ni, etc.

We mention that having in view the contamination from soil, our work also discusses about Cu, Zn, Mn. These are soil contaminants and, generally, contaminants of the habitual area due to the fact that they are in much increased concentrations in soil, which can determine an increasing of their concentration over the physiological limits in plants.

Pedogenesis related to the geochemical composition

As defined, the geochemical composition represents the natural content in microelements that are present in soils, without human intervention. It usually depends on the content that is present in the rock that was at the origin of the soil, but also on the processes that contributed at the formation of soils (Table 1).

Table 1. Natural content of microelements in soils

Metallic elements	Magmatic (eruptives) rocks			Sedimentation rocks		
	Basic rocks	Neutral rocks	Acid Rocks	Argilos and sand sedim. rocks	Gresia	Carbonates (calcars)
Cd	0.13-0.22	0.13	0.09-0.20	0.22-0.30	0.05	0.035
Co	35.0-50.0	1.0-10.0	1.0-7.0	11.0-20.0	0.3-10.0	0.1-3.0
Cr	170.0-200.0	15.0-50.0	4.0-25.0	60.0-100.0	20.0-40.0	5.0-16.0
Cu	60.0-120.0	15.0-80.0	10.30	40.0	5.0-30.0	2.0-10.0
Mg	0.0x	0.0x	0.08	0.18-0.40	0.04-0.10	0.04-0.05
Mn	1200.0-2000.0	500.0-1200.0	350.0-600.0	500.0-850.0	100.0-500.0	200.0-1060.0
Mo	1.0-1.5	0.6-1.0	1.0-2.0	7.0-2.6	0.20-0.80	0.16-0.40
Ni	130.0-160.0	5.0-55.0	5.0-15.0	50.0-70.0	5.0-20.0	7.0-20.0
Pb	3.0-8.0	12.0-15.0	15.0-24.0	18.0-25.0	5.0-10.0	3.0-10.0
V	200.0-250.0	40.0-100.0	40.0-60.0	80.0-120.0	15.0-30.0	10.25
Zn	80.0-120.0	40.0-100.0	40.0-60.0	80.0-120.0	15.0-30.0	10.25

It may be observed that the clay rocks are richer in trace elements as for the magmatic rocks, the content of these trace elements increases from acid rocks to basic and ultrabasic ones. Things can be more complex if metamorphism or vulcanic activity intervenes.

Another important aspect represents the localisation of heavy metals in minerals. Often, in the magmatic crystalline rocks, trace elements spread in the crystal network of the magma and in the crystal network of the minerals, replacing some major elements. There are crystallo-chemical rules which permit that chromium, nickel, cobalt (so called lithophile elements) can substitute iron, in silicates for example. A large number of trace elements are found in ferromanganese minerals. Other elements (Cu, Zn, Mn) are also called calcophiles or siderophiles, have the tendency to concentrate in sulphures.

A factor that gives heterogeneity to the rocks is given by the fact that trace elements precipitate in the last stage of crystallization or metamorphism, being found in their cracks. During the process of pedogenesis, primary minerals (ferromanganese minerals) are partly destroyed or replaced in the sandy fractions, sulphures can be oxidated and the trace elements redistributed.

As a general remark the content of trace elements in soils is higher as compared with that of the rocks they originate from. This content depends on the type of pedogenesis and time. Their mobility is high in the less evolved soils (brown soils) or the carbonated ones (rezins) where free elements accumulate.

Migration or redistribution of elements on the profile intervene in washed soils, hydromorphic soils where the migration is toward the base of the profile (with clay and iron); in podsoils, deep, aluminium and iron accumulate and at the surface, lead.

Metallic ions retention in soil.

Heavy elements are grouped under the denomination of “heavy methods” due to the special chemical properties which make them susceptible to incorporate in the biomass, perturbing the enzymatic system. These are indispensable elements and also easy for dosage: copper, molybdenum, zinc, manganese, selenium and also cadmium, mercury, nickel, lead, chromium and less arsenium and titanium.

Heavy methods are retained in the colloidal complex of the soil; because there is an equilibrium between the absorbed forms and the soil solution, often they are released in assimilated forms only in a less amount that is taken over by the plants.

Their immobilisation and retrogradation takes place under organic and mineral form. Immobilisation is obtained by the help of polymerised humic compounds which are stable and retrogradation by embodying in the network of silicates and unsoluble oxydes (Fe_2O_3 , MnO_2 , Al_2O_3). Retrogradation is more rapid and complex as compared with that of nutritive elements, when the pH is high.

Table 2. Microelements content – total forms – in the main soils from Banat
Soil type and the localisation. Microelements – total forms – in A **Orisont** (ppm)

Soil type – localisation	Cu	Pb	Zn	Co	Ni	Mn	Cr	Cd	Fe
Gleised chernozems – Lovrin	23.5	18.5	56.5	20.0	44.5	685.0	101	1	25000
Gleised cambic chernozems – Comloș	44.0	21.0	49.5	18.5	25.5	575.0	68.5	1.9	17400
Gleised cambic chernozems – Jebel	19.5	30.5	42.0	17.5	38.0	575.0	84.5	1.0	19000
Argillic chernozems – Fântânele	24.0	20.0	53.0	23.5	47.5	760.0	133.0	1.5	29500
Brown soil – Fibiș	22.5	25.0	59.5	22.5	46.5	1150.0	100.0	1.5	23600
Brown soil – Șagu	25.5	31.0	85.0	27.5	27.5	955.0	131.0	1.0	35600
Brown soil – Moldova Nouă	25.0	35.5	97.0	23.0	48.5	650.0	166.0	1.0	27700
Luvic brown soil – Coșova	9.0	17.5	22.5	13.5	21.0	375.0	69.0	1.5	10100
Luvic brown soil – Prigor	17.0	31.5	170.5	20.0	42.0	530.0	146.0	1.0	21600
Luvic brown soil – Darova	16.5	27.5	44.5	18.0	32.0	490.0	113.0	1.0	16900
Albic luvisol soil – Șiștarovăț	19.0	34.0	47.5	21.0	29.5	1015.0	97.0	1.0	19600
Luvisol albic soil – Fârdea	15.0	17.5	34.5	13.0	31.0	360.0	485	1.5	11200
Brown soil – Receaș	18.5	30.0	103.5	23.0	43.5	560.0	10.7	1.0	30300
Brown soil – Gătaia	22.5	34.0	69.0	18.5	45.0	540.0	96.0	1.0	23300
Gley soil – Jimbolia	19.5	12.0	67.0	26.0	29.0	420.0	79.0	1.5	23500
Salined Solonetz – Foeni	45.0	28.0	37.5	10.0	28.5	265.0	84.0	1.0	12600
Gleised Vertisol – Cheglevici	62.5	41.0	101.0	27.0	69.0	320.0	84.5	1.5	36000
Aluvial soil – Coștei	26.0	29.5	64.0	20.0	52.5	710.0	115.0	1.5	35300
Aluvial gleised soil – Dalbești	22.0	31.0	112.0	21.5	49.0	510.0	150.0		26200

In acid medium, the mobility of toxic metals, thus the danger of absorption by plants, increases considerably. Toxic metals mobility in soil is different: an increased mobility have Pb, Cu, Zn, Cd.

Lead is 91% of atmospheric origin and is generated by the gases emitted by vehicles; the lead content of soils is higher along the highways and in urban centers. For example, around the industrial centers of Germany, the lead content of soil can reach 3g/kg; in the coniferous forests acid rains depose yearly 500 g lead on each hectare of soil. Immobilisation and retrogradation of lead both in organic and mineral form is very rapid in basic medium; it is not easily absorbed by plants, neither transported into the deep horizons of the soil nor in the freatic waters.

Copper appears in soil from atmospheric fungicides and amendments. It is very well retained by humus (moll). In podsoils there is accumulated in the Bh horizon and in the pseudogleic soils.

Zinc reaches the soil from the atmosphere; as a mean value 800g/ha/year, reaching also 4kg/ha/year in industrial zones. In acid, levigated soils, zinc migtares together with clay particles and accumulates in the Bt horizon.

Cadmium. Cd content varies in a large range from one region to another, from 3g/ha/year in rural zones to over 500g/ha/year in industrial zones. If well retained in the soils saturated with basic ions, under pH=6 it passes easy into the solution of soil, becoming toxic for the soil microorganisms, begining with the dosis of 0,1 mg/l.

Manganese arrives in soil after applying phosphorus fertilizers, and also from the atmosphere. The annual deposit of manganese in soil can rise at 10 Kg/ha. In neutral and well-aired soils, Mn is retained as insoluble MnO. In acid medium (pH=5) appear the changeable forms Mn^{2+} , which are easy absorbed by plants, but in excess can become toxic. This happens in agricultural soils due to the equipments used. In forest soils Mn^{2+} is easy recycled and accumulates in the surface horizon as Mn_2O_3 .

CONCLUSIONS

The main conclusion is the following: a well structured soil, with a saturated colloidal complex, plays the role of a filter for heavy metals, when these are not recycled by vegetation.

Soil pollution is dangerous when the supply of nutritive substances is over the normal limits:

- soil present an acid pH
- the soil structure is destroyed, the inclination is high favourising the leaking of surface waters and the erosion of fine particles, these ones having absorbed heavy metals which can contaminate the waters.

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CISPLATIN: THE FIRST 25 YEARS

NOTE I. GERM CELL TUMORS

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ABSTRACT

The Oncological Institute of Cluj is the first in Romania where high dose Cisplatin (100-120 mg/m²) was routinely administered (since 1982) in testicular germ cell tumours (GCT) and undifferentiated nasopharyngeal carcinomas (UCNT).

Administration in GCT: Between 1982-96, 203 pts entered Cisplatin based protocols. Stage I vs IIA vs IIB vs III: 15% vs 15% vs 34% vs 36%; risk group low vs medium & high 45% vs 55%. Results: 57% CR and 31% PR (=88% OR). 5y survival (S): 67%, influenced by the response to platinum combination (CR vs PR+NC+PD: 89% vs 26%, $p < .01$), the Indianapolis risk category (low vs medium & high: 91% vs 43%, $p < .01$)

Key words: cisplatin, testicular germ cell tumor.

INTRODUCTION

The optimal use of Cisplatin (CDDP=Cis-diammine dichloro platinum) represented the most important advance in the chemotherapy of the 80's. Platinum is up to now the only metallic element used for cytotoxic properties, whereas most drugs are purely organic. Its antineoplastic activity was demonstrated by Rosenberg, after the serendipitous observation that *E. coli* under electric currents with platinum electrodes had DNA alterations (Rosenberg, 1965).

Activity. CDDP (in combination) has a major activity with >70% objective responses (OR), and >50% complete responses (CR) in: germ cell tumours of the testis (GCT), undifferentiated carcinomas of nasopharyngeal type (UCNT), ovarian carcinomas, small cell lung cancer, trophoblastic gestational disease. Considerable activity (OR>30%, CR>10%) was demonstrated in epidermoid carcinomas, non-small cell lung cancer, bladder carcinoma, osteosarcoma (Table 1).

Mechanism of action: The main target is the DNA. The monoaquo and diaquo derivatives (substitution of chlorine) bind to the hydroxyl or amine group of nucleosides and macromolecules and to the thiol groups of proteins. It forms interstrand and intrastrand crosslinking of DNA and therefore behaves like a bifunctional alkylating agent and an intercalating agent. The drug behaves predominantly in a non-phase-specific manner. There is evidence implicating an action on DNA repair phenomena and an apoptotic (programmed cell death) pathway in cell killing by CDDP (Barry et al., 1990).

Mechanisms of resistance: Cellular resistance to platinum compounds is the major limitation to the successful treatment of malignancies, is multifactorial and include: (1) decreased cisplatin accumulation, (2) inactivation by metallothionein,

Table 1: CDDP combinations: spectrum of activity
(after Cvitkovic 1993, modified)

Major efficacy >70% OR, including >50% CR	Testicular cancer Placental choriocarcinoma Ovarian cancer Small cell lung cancer UCNT
Considerable efficacy >30% OR including >10% CR	Bladder cancer Non-small lung cancer Epidermoid cancers Stomach cancer Osteosarcoma
Moderate efficacy <20% OR	Soft tissue sarcomas Thyroid medullary cancer Neuroblastoma Breast cancer Melanoma
Ineffective	Leukemias Colorectal adenocarcinomas

(3) inactivation by glutathione conjugation, (4) enhanced platinum-DNA adduct repair, and (5) platinum-DNA damage tolerance pathways, which influence cell death (O'Dwyer, 1997)

Toxicity. CDDP is the most emetogenic of all anticancer drugs. Dose limiting toxicities are represented by nephrotoxicity (manifested by electrolyte disturbances like hypoMagnesemia, hypoKalemia, hypoNatremia, through tubular damage, acute or chronic renal failure), neurotoxicity (proprioception) and ototoxicity. Myelosuppression is mild, but a post CDDP chronic anaemia may occur, in respect with the cumulative dose.

The solutions found this decade to overcome the toxicity of this very effective drug may represent a paradigm for successful research programmes in oncology. The anti-HT3 antiemetic agents (ondansetron, granisetron, tropisetron, dolasetron) developed for highly emetogenic agents are now the most potent antiemetic drugs, controlling this side effect in >70% of the patients (Berger AM et al., 1997). Besides the protocol of administration with saline hyperhydration and mannitol diuresis, the recently FDA approved amifostine is providing efficient prophylaxis for the Cisplatin induced nephrotoxicity (Patterson W.P and Reams G.P., 1997). This cytoprotective agent reduces also the neurotoxicity of Cisplatin (Macdonald D.R., 1997). The rHu- erythropoietin proved useful in Cisplatin - induced anemia and also in the chronic form of "anemia of cancer" .

Analogues. One of the problems encountered with CDDP is the toxicity profile, especially nephro and neurotoxicity. It was found that substitution of more stable ligands for the chloride leaving group did diminish the renal side effects, while retaining activity. Carboplatin is such an analogue, replacing Cisplatin in many tumour types, such as ovarian carcinomas or lung cancer (not in testicular cancer), due to its ease in administration (no saline hyperhydration and mannitol diuresis needed) and different toxicity spectrum (more myelosuppression, but no nephro and neurotoxicity). Carboplatin is unfortunately more expensive than CDDP.

Unlike CDDP, another new analogue, Oxaliplatin, proved active in colorectal adenocarcinomas.

Oral platinum (such as JM 216) is currently in phase II trials (O'Dwyer et al, 1997)

The Oncological Institute of Cluj is the first in Romania where the high dose Cisplatin (100-120 mg/m²) was routinely administered (since 1982) in a variety of tumor types, such as germ cell testicular tumours (GCT), and undifferentiated nasopharyngeal carcinomas (UCNT).

GCT represent the most common malignancy in men from the ages of 15 to 35 years. Dramatic improvements in survival, with an almost total reversal from a 10% survival rate in the 1970s to a 90% survival rate in the 1990s, has resulted from effective multidrug chemotherapeutic regimens (Cebotaru, 1997). The promising results published in 1982 by the investigators of Memorial Sloan-Kettering Cancer Center with the VAB 6 regimen (78% durable complete responses on a series of 166 patients), who applied the dose-intensity concept in the testicular tumors, determined the introduction of this protocol as the standard therapy at the Oncological Institute of Cluj since 1982. Since 1992, consecutively to the international trials results and the availability of Etoposide on the Romanian market, the BEP and EP protocols have been introduced. The aim of this study is an analysis of the results of chemotherapy in GCT patients. From 1982 to 1996, 203 patients presenting a testicular tumor have been treated. The endpoints were: the objective and complete response rate, the overall survival and the univariate analysis of prognostic factors. We also performed a comparison between the results of VAB 6 and the new Etoposide-containing chemotherapy regimens.

PATIENTS AND METHODS

Patients with histologically documented newly diagnosed GCT, with no prior radiation, chemotherapy or surgery (except orchiectomy), were eligible for study entry. Each patient was required to meet the following criteria: age < 70 years, seminoma: clinical stage II (with bulky lesions > 5 cm) or III and all nonseminomatous tumor patients (stage I, II and III- according to the International Classification Staging- with the exception of stage I marker-negative patients amenable to regular follow-up), an adequate bone marrow reserve, (i.e., WBC count > 3,000/microL with neutrophil count > 1,500/ μ L, platelet count >100,000/ μ L, and hemoglobin level >8 g/dL), adequate liver function (i.e. total bilirubin level < 1.5 mg/dL, serum AST and ALT levels <2x N), and adequate renal function (i.e., serum creatinine < 110 mmol/L and calculated creatinine clearance > 70 mL/min).

Patients with other histological findings (metastases, lymphoma, rhabdomyosarcoma), with age > 70, active infection or with myocardial infarction < 6 months, cardiac, renal and respiratory failure or active concomitant malignancy were excluded.

Pretreatment and Follow-Up Evaluations

Pretreatment investigations consisted of a routine history and physical examination, WHO performance status, weight loss, complete blood cell counts, routine chemistry measurements, serum tumor markers (AFP, β -HCG), chest radiograph, abdominal and pelvic computed tomographic scan /ultrasound and isotope bone scan (for the patients with bone pain, elevated serum alkaline phosphate, hypercalcemia or ESR > 30/ first hour). To define the risk categories to adjust the treatment, the Indianapolis Staging System for Metastatic Testicular Malignancies was adopted (Table 2).

Table 2: Indiana University Staging System for Disseminated Testicular Cancer**Minimal extent**

1. Elevated markers only
2. Cervical nodes (+/- nonpalpable retroperitoneal nodes)
3. Unresectable, nonpalpable retroperitoneal disease
4. Fewer than five pulmonary metastases per lung field *and* largest < 2 cm (+/- nonpalpable retroperitoneal nodes)

Moderate Extent

1. Palpable abdominal mass only (no supradiaphragmatic disease)
2. Moderate pulmonary metastases: 5-10 metastases per lung field and largest < 3 cm *or* solitary pulmonary metastases of any size >2 cm (+/- nonpalpable retroperitoneal disease)

Advanced Extent

1. Advanced pulmonary metastases: primary mediastinal nonseminomatous germ cell tumor *or* >10 pulmonary metastases per lung field, *or* multiple pulmonary metastases with largest >3 cm (+/- nonpalpable retroperitoneal disease)
2. Palpable abdominal mass plus supradiaphragmatic disease
3. Liver, bone, or CNS metastases

Complete blood cell counts, differential and routine chemistry measurements were performed on days 1, 21, 42, 63. Tumor measurements were made and recorded before the first course of therapy and before each subsequent drug administration.

Follow-up evaluation was performed every other month in the first year, every three month during the next two years, then twice per year until the fifth year, and once per year thereafter.

Treatment Schedule

Patients were admitted to hospital during the chemotherapy courses. Patients received 3 or 4 cycles of chemotherapy (VAB 6, EP or BEP protocol). The chemotherapy regimens were given at 21 days interval.

The VAB 6 protocol consisted of:

Vinblastin (VBL) = 4 mg/m² administered intravenously (IV) on day 1,
Dactinomycin (Dact) = 1 mg/ m² IV on day 1,
Bleomycin (BLE) = 30 mg IV bolus on day 1 and
20 mg/ m² daily IV continuous infusion on days 1, 2 and 3,
Cyclophosphamide (CTX) = 600 mg/ m² IV on day 1,
Cisplatin (CDDP) = 120 mg/ m² IV on day 4.

The BEP protocol included:

Etoposide (VP-16) = 100 mg/ m² IV on days 1-5,
CDDP = 100 mg/ m² IV on day 2,
BLE = 30 mg IV bolus on days 1,8,15.

The EP protocol consisted of:

VP-16 = 100 mg/ m² IV on days 1-5,
CDDP = 100 mg/ m² IV on day 2.

The Indianapolis low risk patients received 4 cycles of EP or 3 cycles of BEP. The intermediate and high risk patients received 4 cycles of BEP. The marker negative complete responders (CR) had no further treatment. The patients with CR and positive Mk had 2 supplementary chemotherapy cycles. The patients with a partial response (PR) and positive Mk had a salvage chemotherapy.

In partial responders with negative Mk, a secondary resection (retroperitoneal lymphadenectomy or pulmonary metastasectomy) was performed 4-6 weeks after the completion of the treatment. In patients where histology was necrosis, fibrosis or mature teratoma, the treatment was considered sufficient. Patients with active tissue and complete resection had 2 supplementary chemotherapy courses and those with incomplete resection had a salvage chemotherapy. For patients with stable or progressive disease, salvage chemotherapy was delivered.

Response Evaluation

Tumor response was assessed according to WHO criteria. Patients were evaluated at the end of 3-4 cycles of chemotherapy. Complete or partial response and stable disease had to be maintained for 4 weeks, otherwise it was judged as progressive disease. Duration of response was calculated as the time from the first documentation of major response to first documentation of progressive disease. Survival time was calculated by the Kaplan-Meier method. All *p* values cited are two-sided.

RESULTS AND DISCUSSIONS

Patient Characteristics

From January 1982 to September 1996, 203 patients entered this study. Characteristics of the 203 patients are listed in Table 3. The age ranged from 14 to 53 years (median, 30). Thirty-one patients had stage I disease, 30 had stage II A, 69 had stage II B and 73 had stage III disease. Histology showed seminoma in 40, nonseminomatous in 125 and mixed in 38 patients. According to Indianapolis risk group: 57 had high risk, 54 intermediate risk and 92 patients had low risk. A hundred thirty-five patients had a Zubrod (WHO) performance status of 0 or 1 and sixty-eight of 2, 3 or 4. Forty-seven patients had weight loss greater than 5% of normal body weight during the 6 months before beginning the treatment.

Response

Responses were determined after 3-4 cycles of first line chemotherapy. All 172 stage II- III patients were assessable for evaluation of response after chemotherapy. There were 151 objective responders in 172 patients (88%), (CI 95%: 83-93%), and 98 (57%) achieved a complete response. Twenty-one patients (12%) were refractory to first-line therapy. There was one toxic death due to acute renal failure. Twenty nine partial responders with negative markers, Indiana low risk 6, intermediate & high risk 23 underwent secondary resection (retroperitoneal tumor 28 patients, pulmonary 1 patient) to remove postchemotherapy residual masses visualized on radiological imaging studies. Complete secondary resection was possible in 16 patients (55%). The pathological findings at secondary surgery specimen were: necrosis in 14 (48%), active tumor in 11 (38%), teratoma in 4 patients (14%). Viable tumor was not found in low risk patients, and was more frequent after VAB 6 than BEP&EP regimen (88% vs 25%, *p*<.01). Initial histology and dimension of the residual mass after CT did not influence histological findings at secondary resection.

Table 3. Patient Characteristics (N = 203)

Characteristics	No of Patients	%
Age, years		
Median	30	
Range	14-53	
	< 35	72
	> 35	38
Performance status		
0,1	135	66
2,3,4	68	34
Weight loss (%)		
< 5%	156	77
> 5%	47	23
Cell type		
Seminoma	40	20
Nonseminomatous	125	61
Mixed	38	19
Stage		
I	31	15
II A	30	15
II B	69	34
III	73	36
Risk group		
Low risk	92	45
Intermediate	54	27
High	57	28

Survival

All patients were assessable for survival. With a median follow-up of 60 months [6+...144+], the 5 years overall survival was 67% (fig 1). According to the chemotherapy protocol, there was a 3 years survival advantage for the BEP &EP regimens (95% vs. 68%, $p=.004$). When examining survival rate using univariate analysis, prognostic factors ($p<.01$) were: weight loss : <5% vs. >5%: 76% vs. 14%; performance status: 0-1 vs. 2-4: 81% vs. 31%; stage: I vs. IIA vs. IIB vs. III: S= 90% vs. 96% vs. 64% vs. 46%, Indiana low risk vs. pooled intermediate & high risk: 91% vs. 43%. Achievement of a complete response (CR vs. others: S= 89% vs. 26%-fig2) and the chemotherapy regimen (BEP&EP vs. VAB 6: 95% vs. 61%- fig 3) also appeared to be significant prognostic markers. Survival was not influenced by the initial histological type, the postorchiectomy value of AFP, HCG, LDH. For the 29 patients undergoing secondary surgery for residual masses after chemotherapy, survival for totally resected vs. unresectable was 100% vs. 26% ($p<.01$).

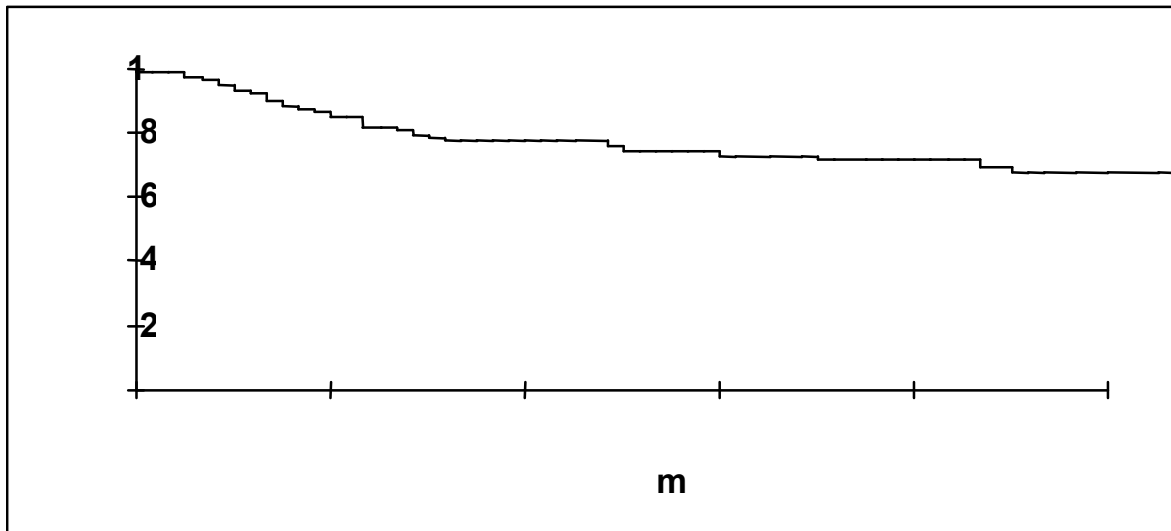


Fig. 1- Five years overall survival in testicular tumors

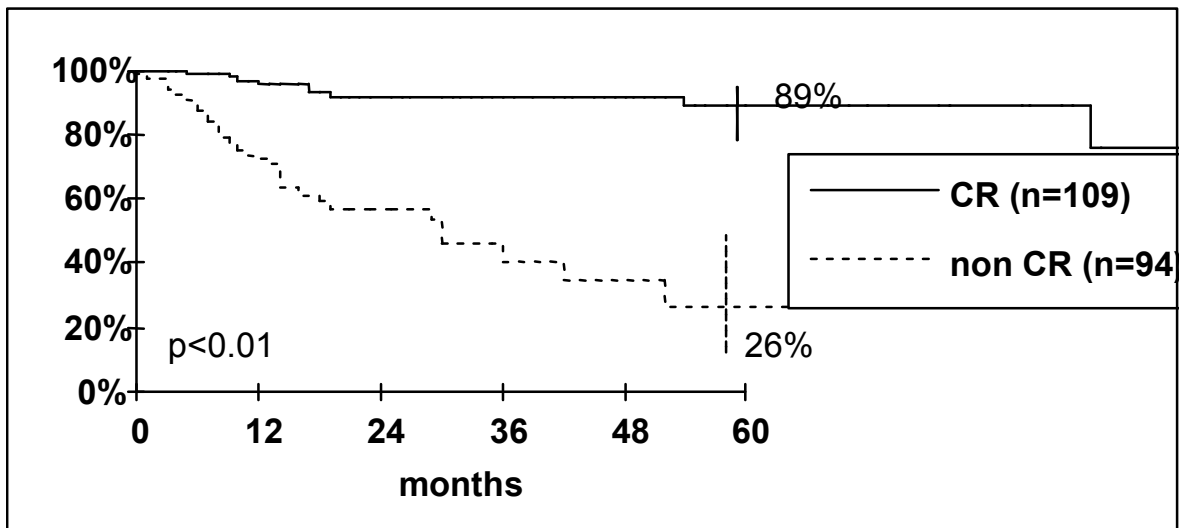


Fig. 2- Survival by chemotherapy response

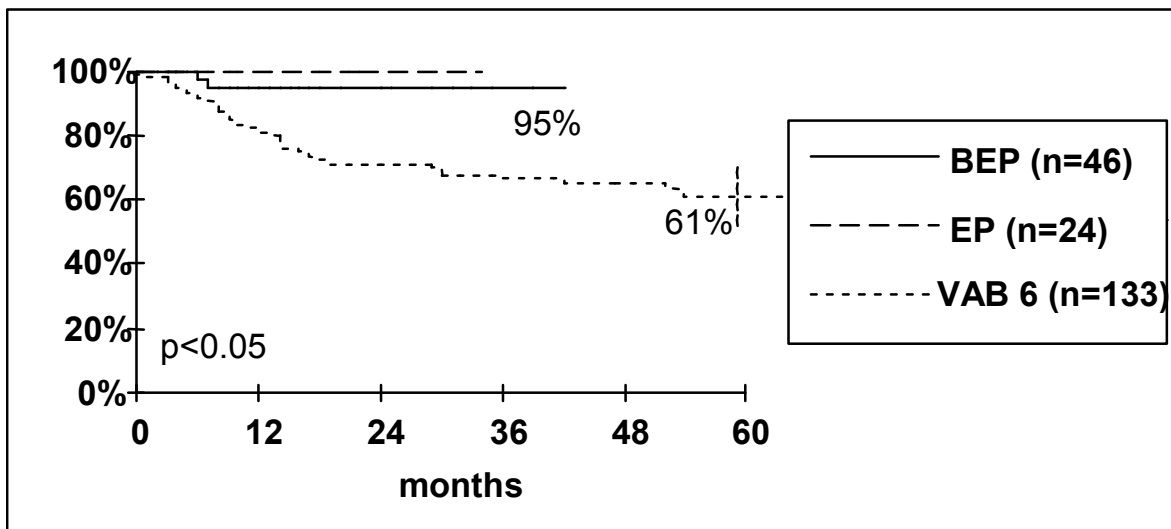


Fig. 3- Survival by chemotherapy regimen

As of September 1996, 158 patients (78%) are alive, with 112 complete responders and 28 partial responders, and 45 (22%) have died: 43 patients had disease progression: 7 (15%) regional adenopathies, 11 (24%) metastases only and 25 (50%) patients regional and distant failure, one patient had a treatment-related death: acute renal failure and one patient died of other disease: acute myocardial infarction. In two patients there was a bilateralization of the testicular tumor, associated with a regional relapse in one patient and with a regional and metastatic relapse in the other one. The relapse patterns were: metastatic disease in 80%, retroperitoneal lymph node relapse in 71% and bilateralization in 2% of patients.

DISCUSSION

Cancer of the testis, a relatively uncommon disease, is an important neoplasm as it represents a highly curable tumor and one in which the incidence is focused on young patients. Modern multiagent chemotherapy has had a significant impact on the treatment of testicular tumors, with a rate of 90% durable complete responses in the good risk group patients.

The VAB 6 regimen results (56% CR and 82% CR+PR, with a 5 year actuarial survival of 60%) were satisfactory for the Oncological Institute of Cluj team, being nearly close to those reported by the investigators at MSKCC with this protocol (78% durable CR) (Barry et al., 1990). With a number of 130 patients and a median of 5 years follow-up (range 6+...144+ months), our series represents an important study concerning the analysis of either the VAB 6 regimen and the cisplatin-based chemotherapy with a dose intensity close to the maximum tolerated dose. The randomized trial in good risk patients comparing the five-drug VAB 6 regimen with the two-drug EP regimen concluded that cisplatin plus VP-16 is equally effective but considerably less toxic than the VAB 6 protocol (Bosl, 1988). Since 1993, we started the evaluation of EP protocol in good-prognosis patients.

In this study, we performed a univariate analysis for prognostic factors for survival. The 5 years overall survival was significantly influenced by: factors concerning 1) the patient: performance status and weight loss; 2) the disease extension: stage and Indiana risk category; 3) the treatment: achievement of a complete response and the chemotherapy regimen.

This analysis demonstrates the advantage of the Indiana University Risk System, elaborated on a multivariate analysis, for the management of testicular tumors: the poor-risk categories mandates a more aggressive approach. The actual standard strategy in our Institute is based on the literature guidelines as well as on our own results. The BEP protocol became the standard first-line therapy for disseminated testicular cancer. In our study, the 3 years survival rate with BEP & EP vs. VAB 6 was 95% vs. 68%.

A prospective study conducted in the cohort of patients defined as good-risk, randomized four courses of BEP as a control arm versus three courses of identical BEP regimen. This study confirmed the high cure rate in favorable-prognosis disease and documented that the optimal cure rates in this patient population could be attained with merely three courses of cisplatin combination chemotherapy (Einhorn LH et al., 1989). The already cited study comparing the five-drug VAB-6 regimen with four courses of cisplatin plus VP-16, demonstrated that EP protocol was equally effective but significantly less toxic than the VAB-6 schedule. Our results showed even a survival advantage with the etoposide-containing

regimens (BEP & EP) as compared with VAB 6 (survival at 3 years: 95% vs. 68%). These studies demonstrated that the current standard therapy in favorable-prognosis disease is either three courses of BEP or four cycles of EP.

It seems that in nonseminomatous tumors, carboplatin is less effective than cisplatin. The substitution of carboplatin for cisplatin has shown therapeutic inferiority for the noncisplatin arm, with 30% of unfavorable events (Bajorin D, 1993). On the other hand, carboplatin successfully replaces adjuvant radiotherapy for stage I seminomas (O'Dwyer et al., 1997).

According to the results of these studies, the Oncological Institute of Cluj current standard therapy for good risk disease is four courses of EP and for the poor risk disease is four cycles of BEP regimen.

CONCLUSIONS

Our results with dose-intense platinum regimens confirm the superiority of etoposide containing regimens over older protocol VAB 6, in terms of improved survival and less frequent viable tumor in secondary surgery specimens. There was no difference in survival between Indianapolis moderate and advanced risk disease categories, but distinction between this mixed group and minimal risk disease patients remained predictive for the individual outcome.

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CISPLATIN: THE FIRST 25 YEARS

NOTE II. UNDIFFERENTIATED CARCINOMA OF THE NASOPHARYNX

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ABSTRACT

The Oncological Institute of Cluj is the first in Romania where high dose Cisplatin (100-120 mg/m²) was routinely administered (since 1982) in testicular germ cell tumours (GCT) and undifferentiated nasopharyngeal carcinomas (UCNT).

Administration in UCNT locoregionally advanced. Between 1988-95, 35 pts had neoadjuvant platinum based chemotherapy. Results: 94% OR, 40% CR after chemotherapy, and 75% CR after completion of radiotherapy. 2 y survival: 80%. These results illustrate the tremendous progress brought in by the use of full dose platinum regimens in medical oncology.

Key words: cisplatin, undifferentiated nasopharyngeal carcinoma

INTRODUCTION

Undifferentiated nasopharyngeal carcinomas (UCNT) constitutes a well defined entity among head and neck cancers. The classical etiological association between tobacco and alcohol use and head and neck cancers is not important in the cancerogenesis of the UCNT, where a certain relation with the Epstein Barr virus was demonstrated (Liebowitz D, 1994).

UCNT is usually a locoregional disease at presentation, with a high propensity for systemic dissemination. Although UCNT is highly radiosensitive, the 5-years survival is less than 40% due, mainly, to the occurrence of hematogenous metastases. The use of chemotherapy in a neoadjuvant setting is logical, it's aim being to eradicate the distant micrometastases, and to improve local control. Planning of radiotherapy is easier after chemotherapy, as a consequence of a reduced tumour bulk.

PATIENTS AND METHODS

From 11/90 to 12/93, 35 consecutive patients with histologically confirmed locoregionally advanced UCNT entered this study. There were no concurrent trials studying this patients' population during that time.

Eligibility criteria included: histologic confirmation of disease (WHO type II and III); locoregionally advanced tumour (T3-4 and/or N2-3- UICC); a performance status (WHO) = <2; age between 10 and 70 years; normal renal, hepatic, cardiac functions (creatinine <1,5 mg/dl, bilirubin <3mg%, FEV> 50%); no prior therapy, patients to be amenable to regular follow-up; informed consent.

In addition to full clinical examination, all patients had the following procedures: CT scan of the nasopharynx and the cervical region, liver ultrasound, blood count and standard biochemical parameters. Bone marrow biopsy and/or scintigraphy was performed in patients with unexplained fever, elevated leucocytes ($>13000/\text{mm}^3$), ESR >30 mm during the first hour.

Data referring to patients characteristics are presented in table 4.

Table 4: UCNT. Patients characteristics

Number of patients	35 (Inclusion: 11/90-12/93)		
Age	38 (11-64)		
Sex		Number of patients	%
	M	20	57
	F	15	43
Performance status Zubrod			
	1	29	83
	2-3	6	17
Weight loss			
	< 5 %	28	79
	5 %	7	21
Nasopharynx		30	86
Oropharynx		3	9
Ethmoido-maxillary		2	5
T N M Distribution			
	T2-3	20	57
	T4	15	43
	N0-2	19	54
	N3	16	46

There were 20 males and 15 females. Median age was 38 years (11-64). WHO performance status was 1 in 29 pts and 2 in 6 pts. Histology showed type III (undifferentiated) in 32 and type II (squamous nonkeratinizing) in 3 patients. There were 15 (43%) patients with involvement of the base of the skull (T4) and 16 (46%) patients with massive adenopathy (N3). We included in our study 3 patients with oropharyngeal and 2 patients with ethmoido-maxillary UCNT.

Chemotherapy protocol:

Bleomycin 10 mg day 1- IV push + 12 mg/m² days 1-4 in 12 hours infusion

Epirubicin 70 mg/m² day 1 -IV

Cisplatin 100 mg/m² day 5 - IV 1-2 hours infusion, with hydration and mannitol diuresis

The protocol was repeated every 3 weeks, for 3 cycles.

Radiotherapy was started 21 days from the end of chemotherapy. Radiotherapy consisted of 1,7-2 Gy/fraction, one fraction every day, 5 days a week, for an overall total duration of 49 days. A dose of 64 - 76 Gy was delivered to the primary tumour, 64-66 to clinically affected nodes and 44-50 Gy to all other cervical lymph nodes.

Evaluation of response

Patients were evaluated for chemotherapy response after the third cycle (before the start of RT) by clinical evaluation and radiological evaluation. Evaluation of response to the primary treatment was performed 2 months after completion of radiotherapy, with a clinical and biological evaluation, chest X-rays and CT scan of the nasopharynx, liver ultrasound. In case of ESR>35 at the first hour, leucocytosis, fever, bone pain, bone marrow biopsy and scintigraphy were mandatory. Next evaluations were performed at 3 months interval for 2 years and yearly thereafter. Response duration and survival were calculated from the first day of chemotherapy. All patients were evaluable for response, toxicity and survival. Responses (complete, partial, no change, progressive disease) were defined by the WHO criteria.

RESULTS AND DISCUSSIONS

Toxicity with BEC regimen (107 cycles/ 35 pts) was moderate and there were no toxic deaths. A grade 4 anaemia persisted at day 21 in 6/107 cycles (3 pts) and was correlated with the occurrence of bone metastasis in 2 of these patients. Grade 3-4 leukopenia, which occurred in 20/67 pts (=30%) in IGR study, was less frequent in our study (4/35 pts - 11%), but day 15 blood count was not available in 6 pts. Nausea and vomiting gr 3-4 occurred in 75% of patients with Metoclopramide 0,5mg/kg qid, d 1-5 vs only 20% of patients with Zophran 8mg/ day 1 and Metoclopramide 0,5 mg/kg qid, d 2-5.

Activity: There were 33/35 (=94%) objective responses to chemotherapy (CI = 76-99%) ; 14 (=40%) complete responses (CI = 24-56%) and 19 (=54%) partial responses.

Compliance to chemotherapy: 3 patients (=9%) refused the third cycle of chemotherapy and subsequent irradiation because of grade 3 nausea/ vomiting.

After completion of radiation therapy, complete response was achieved in 24/32 pts (=75%, CI95%: 60-90%) and objective response rate was 30/32 pts (=94%, CI95%: 76-99%). 32 patients completed the planned radiotherapy.

Survival. Median follow-up is 20 months (3+, 40+). Actuarial survival at 2 years was 67% (fig 4). Two-years survival for chemotherapy complete responders versus all other patients is 80% vs 54%, but the difference was not significant ($p=0,15$) (see fig 5).

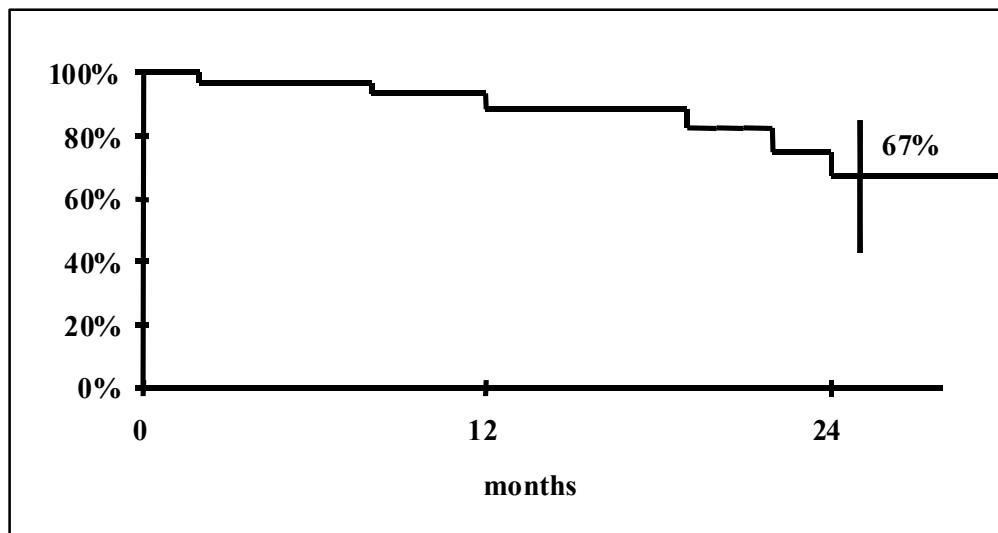


Fig. 4 Overall survival (n=35 patients)

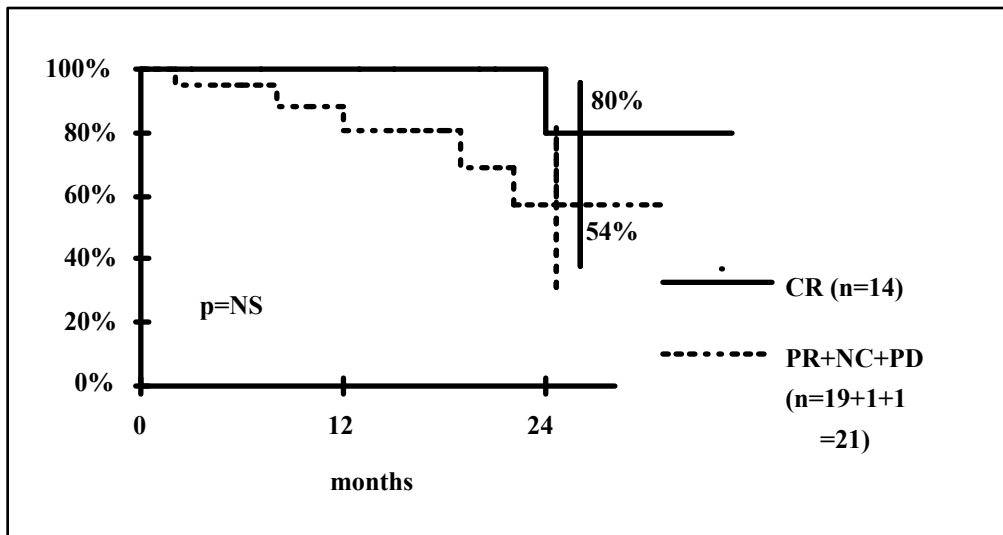


Fig.5. Survival-Chemotherapy CR versus other patients

Median survival has not yet been reached. Median duration of response was 26 months (2+,40+). Patterns of failure: local 2 patients, locoregional 5 patients, metastatic 4 patients, locoregional and metastatic 1 patient. Bone was the main site of metastasis (4/5 patients).

DISCUSSION

For years, the standard treatment for undifferentiated carcinoma of nasopharyngeal type consisted in radiotherapy alone, but 5-year survival was less than 40% in published series, mainly due to metastatic spread (Fandi 1994, Cappelaere 1993, Ghilezan 1983). Theoretically, neoadjuvant chemotherapy could improve results 1) by decreasing systemic dissemination 2) by increasing locoregional control.

Induction chemotherapy studies show that UCNT is a highly sensitive tumor, with complete response rates ranging between 10% and 66%, and CR+PR rates between 75-98% (Azli, 1994). Several studies are listed in table 5 (Azli, 1992, Cvitkovic 1994, Boussen 1991, Onat H 1992, Fountzilas 1991, Rahal M, 1994). Complete response rates reported are generally the highest between head & neck cancers at similar volume (Recondo, 1994). BEC regimens yielded the best published results in 67 patients with locoregionally advanced UCNT in a phase II study at Institute Gustave Roussy La Grange (Azli, 1992), with 66% CR and 98% CR+PR after neoadjuvant chemotherapy and 94% CR+PR at the end of all the primary treatment, and a 64% overall survival rate with a median follow-up of 4 years. These results were superior to the previous PBF regimen (CDDP, Bleomycin, Fluorouracil) where 10% CR and 75% CR+PR were obtained in 30 patients, and a 47% overall survival and 35% DFS, with a median follow-up of 65 months (Azli 1992). Our results (40% CR; 94% CR+PR) are confirming the high objective response rates reported by the French investigators, with a complete response rate situated between that reported with PBF and that originally obtained with BEC

Table 5. Neoadjuvant chemotherapy studies in UCNT

<i>Author</i>	<i>No.</i>		<i>Median</i>			
	<i>Pts</i>	<i>Regimen</i>	<i>CR+PR</i>	<i>CR</i>	<i>surv.</i>	<i>follow up</i>
Institut Gustave-Roussy (Armand, Cvitkovic)	67	BEC	98%	62%	64% OS	47 mths
Cluj (Ciuleanu, Ghilezan)	30	PBF	75%	10%	35% DFS	65 mths
INCSG (Cvitkovic)	35	BEC	94%	40%	67% OS	20 mths
	339	BEC+RT vs RT	-	-	47 vs 30% DFS	24 mths
AOCOA	-	HD Epi+ CDDP	80%	-	-	ongoing
Tunis (Boussen)	30	HD Adr+ CDDP	87%	30%	53% DFS	24 mths
	30	PAB	87%	27%	46% DFS	28 mths
Constantine (Rahal)	48	HD Epi+ CDDP	98%	58%		18 mths
Istanbul (Onat)	21	CDDP+ 5FU	94%	33%	73% PFS	17 mths
Thessaloniki (Fountzilias)	39	CDDP or Carboplatin	82%	33%	63% OS	36 mths

Abbreviations: OS - overall survival; DFS - disease-free survival; PFS – progression - free survival; HD: high - dose; AOCOA: Asian - Oceanian Clinical Oncology Association; INSG: International Nasopharyngeal Cancer Study Group

Clear conclusions regarding the real impact of the neoadjuvant chemotherapy on survival can be drawn only for randomized trials. The first results of the International Nasopharynx Cancer Study Group (INCSG) randomized trial, which included 339 pts and compared 3 courses of induction BEC followed by standard radiotherapy at 70 Gy, vs radiotherapy alone, showed a significant difference in disease-free survival favouring the chemotherapy arm (47,1% vs 30,5%), after a median follow-up of 24 months. More follow-up is needed to establish the impact of neoadjuvant chemotherapy on overall survival (Cvitkovic 1994). An excess of treatment related morbidity and mortality in the chemotherapy arm attributed to Bleomycin toxicity with an impaired clearance due to a transient Cisplatin-induced renal toxicity was reported in IGR study. In our series, we did not have important toxicities (excepting for gr. 3 nausea/vomiting and 3 cases with gr 4 anemia) and no toxic death, demonstrating once again the feasibility of this protocol. First results in phase II studies with regimens including high-dose epirubicin and CDDP (without Bleomycin) were promising (Boussen 1991, Onat 1992). A study reported 58% CR, 98% CR+PR in 48 pts treated with high-dose Epirubicin and CDDP, with a different toxicity profile as compared with BEC (a tendency to more mucositis and leukopenia, but no pulmonary toxicity - (Rahal 1994). From January 1994 we are also evaluating the "EC" regimen (Epirubicin 60mg/m² day 1 and 8 + CDDP 100 mg/m²) in our institution. This protocol has theoretically the advantage to avoid Bleomycin toxicity and is less expensive than BEC.

CONCLUSIONS

- Our results (94% objective responses and 40% complete responses after neoadjuvant chemotherapy and 75% complete responses after the end of radiotherapy sequence), are confirming BEC as a highly active regimen for undifferentiated nasopharyngeal carcinomas. The benefit in terms of survival needs to be defined within prospective randomised trials.

- To date, taking into account reported results with BEC in phase III trials, we believe this regimen must be considered as standard initial chemotherapy for undifferentiated carcinoma of nasopharyngeal type.

- The results obtained in our institution in GCT and UCNT with dose intense Cisplatin based regimens illustrate the safety of the administration and the tremendous progress (high response rates, prolonged survival, and even cure), brought in by the use platinum in medical oncology.

- In the Oncological Institute of Cluj, along with the clinical use of the standard high dose Cisplatin protocols in various other tumour types, clinical trials are ongoing to evaluate its efficacy in combination with the new generation of drugs such as Taxol, Gemcitabine, Navelbine.

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EFFECTS OF THE DAY ADMINISTRATION IN PROPHYLAXIS AND THERAPY OF IRON DEFICIENCY ANAEMIA PIGLETS WITH IRON-DEXTRAN PREPARATIONS

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ABSTRACT

Two iron-dextran preparations: Ursoferran R 100 and Ferodex R 75 were used for ascertaining their capability to prevent the piglet's iron-deficiency anaemia. Another aim of this paper was to follow the degree in which the administration of the two preparations on different days after birth influenced the levels of sideremy, haemoglobin content and erythrocyte number.

Key words: iron-deficiency anaemia of piglets, iron-dextran, effects

INTRODUCTION

The iron-deficiency anaemia in piglets prevention and therapy are among the obligatory means for avoiding the economic losses due to not achieving the anticipated and normally gains with the conventional fodder doses and also mortalities encountered in populations in which the treatments are not effected or are not accurately performed (Miloș and Drinceanu, 1980).

Iron is located in the middle of many biomolecules with functional role in vital processes as well in erythropoiesis and in numerous coenzymes and prosthetic groups of heteroproteins (Cucuianu et.al., 1979; Eder and Kirchgessner, 1996; Gürtler et.al., 1997).

The iron-deficiency anaemia in piglets occurs in the first 2-3 weeks of life, in the suckling period, predominantly in specialised breeds, more frequently in the cold seasons of the year. They are clinically characterised by the skin and mucosal paleness, cardiac respiratory insufficiency and by sideremy, haemoglobinemia and by erythrocyte number decrease, as well by pitting in of hypochromic microcytary anaemia.

At present, the most efficient method for iron-deficiency anaemia control is the injection of the iron-dextran preparations, which presents many benefits such as: hydrolysables in organism, doesn't form deposit complexes, are sterilisable by autoclaving .

If the intramuscular injections of the iron-dextran efficiency is unanimously accepted, the optimal time for administration is controversial until now (Ghergariu, 1980). So, some authors (Bader, 1971; Biederman, 1972) observe that the smallest losses are found in piglets which received the injections in the fourth day of life, even if the iron alone or associated with vitamins does not affect their weight gain (Danielson and Nonan, 1975), when others (Panič, 1970; Cotruț et.al., 1976) found that the highest efficiency after preparations administration are on the third day.

The aim of this paper is to establish the optimum time of the iron-dextran preparations, by determining the sideremy, haemoglobin content and the number of

erythrocytes, as well the possible local or general effects occurring after the administration.

MATERIALS AND METHODS

The trials were made at S.C. COMTIM S.A., unit Birda, Timiș district. The maternity halls were provided with cement board boxes, which did not allow any iron supplementation of the piglets.

Groups of five piglets were formed, as well as a control group for estimating the value of blood levels in the absence of iron preparations administration, and experimental groups, in which iron was administered 2, 3, 4 and 5 days after their birth.

Two iron-dextran preparations were used, Ursoferran R 100, an iron-carboximetildextran aqueous solution with iron concentration of 100 mg Fe/mL, injected intramuscularly in each 1.5 mL doses and Ferodex R 75 (Polish preparation), a complex poly-carbohydrate (dextran)-iron with 75 mg Fe/mL administered also intramuscularly in doses of 2 mL each.

The blood drawing was undertaken on the 1, 2, 3, 7, 10 and 14 days after birth, from the jugular confluence, with thin syringe needles sterilised previously by boiling.

For sideremy determinations, 5 mL blood was drawn, from which, after clotting, 2 mL serum was obtained. For the haemoglobin and erythrocyte number, the blood was drawn with the anticlotting agent (sodium citrate 4% in ratio of 1:9 v/v). The drawings were undertaken simultaneously for the three determinations.

The serum iron was dosed according to the modified Heylmeyer method (Manta et.al., 1976). For the erythrocyte counting, the Türk chamber was used, Mercano dilution solution and Pottain pipette, and the haemoglobin was determined according the Sahly method (Cucuianu et.al., 1979).

RESULTS AND DISCUSSIONS

Analysing the development of the resulting values of the three blood determination values in the control group shown in Table 1, one can see that the piglets are born with rather high values: 230 μg Fe/100 mL, for sideremy, 39% haemoglobin and $3.54 \cdot 10^6$ erythrocyte number, but which in the first three days undergoes a sudden diminishing, reaching to 116.5 μg Fe/100 mL for sideremy, 32.6% for haemoglobin and $2.7 \cdot 10^6$ for erythrocyte number, without recording clinical cases or mortalities. The decreasing of the three values for the blood determinations continues, but moderately and one can observe a definite differentiation between the weight gains for the piglets of the experimental groups and the control group.

Table 1: The blood indices for the control groups

Days	Sideremy $\mu\text{g}/100$ mL	Haemoglobin %	Erythrocyte number $\cdot 10^6$
1	230.0 ± 28.8	39.0 ± 12.6	3.54 ± 0.102
2	175.3 ± 12.8	35.0 ± 2.2	2.82 ± 0.097
3	116.5 ± 7.7	32.6 ± 2.4	2.70 ± 0.07
7	105.7 ± 4.3	29.2 ± 2.1	2.56 ± 0.06
10	102.9 ± 5.2	30.4 ± 2.3	2.50 ± 0.03
14	96.9 ± 2.0	29.4 ± 0.13	2.68 ± 0.13

Comparative results of the determination for the three blood indices, and the two iron-preparations registered at various time intervals of administration after birth are shown in tables 2-5.

Table 2: The blood indices for administration two days after birth

Days	Preparation	Sideremy $\mu\text{g}/100\text{ mL}$	Haemoglobin %	Erythrocyte number $\cdot 10^6$
1	Ursoferran	115.6 ± 5.6	35 ± 2.24	3.38 ± 0.15
	Ferodex	136.2 ± 12.9	35 ± 2.24	2.90 ± 0.13
3	Ursoferran	147.4 ± 11.0	36 ± 1.83	3.27 ± 0.18
	Ferodex	199.5 ± 27.9	30 ± 0.70	3.03 ± 0.08
7	Ursoferran	228.8 ± 32.2	51 ± 1.02	4.13 ± 0.06
	Ferodex	279.3 ± 19.5	44 ± 6.04	3.90 ± 0.28
10	Ursoferran	294.4 ± 44.0	55 ± 2.97	4.70 ± 0.19
	Ferodex	283.5 ± 9.3	53 ± 4.13	4.47 ± 0.37
14	Ursoferran	174.4 ± 19.0	58 ± 2.07	4.80 ± 0.11
	Ferodex	176.3 ± 20.1	54 ± 2.15	4.85 ± 0.15

The two iron-dextran preparations in doses of 150 mg Fe/piglet, prevent the iron-deficiency anaemia, without showing statistically significant differences between Ursoferran R 100 and Ferodex R 75, regarding their prophylactic capability.

Both Ursoferran and Ferodex administered 2-3 days after birth, accomplished beginning with the seventh day after their administration increased values of the erythrocyte number (4.13 and 3.91 respectively), with significant differences, and at the end of the experimental period (14 days after birth), higher values (4.8 and 5.11 respectively) with very significant differentiation.

Table 3: The blood indices for administration three days after birth

Days	Preparation	Sideremy $\mu\text{g}/100\text{ mL}$	Haemoglobin %	Erythrocyte number $\cdot 10^6$
1	Ursoferran	129.8 ± 13.6	28 ± 1.3	2.58 ± 0.07
	Ferodex	129.6 ± 10.9	27 ± 1.1	2.74 ± 0.16
3	Ursoferran	138.3 ± 32.5	32 ± 0.92	2.86 ± 0.11
	Ferodex	321.9 ± 33.8	29 ± 3.9	3.03 ± 0.24
7	Ursoferran	221.9 ± 17.1	49 ± 1.53	4.04 ± 2.27
	Ferodex	247.2 ± 19.1	49 ± 2.35	3.91 ± 0.30
10	Ursoferran	181.1 ± 32.6	51 ± 1.49	4.59 ± 0.23
	Ferodex	161.9 ± 15.6	53 ± 3.08	4.24 ± 0.36
14	Ursoferran	126.4 ± 32.8	58 ± 2.64	5.11 ± 0.14
	Ferodex	142.3 ± 10.7	57 ± 2.29	4.49 ± 0.10

Table 4 : The blood indices for administration four days after birth

Days	Preparation	Sideremy $\mu\text{g}/100 \text{ mL}$	Haemoglobin %	Erythrocyte number $\cdot 10^6$
1	Ursoferran	203.7 ± 15.7	29.8 ± 0.58	2.86 ± 0.33
	Ferodex	270.7 ± 21.3	26.8 ± 0.58	2.89 ± 0.05
3	Ursoferran	229.2 ± 18.4	34.2 ± 0.86	2.78 ± 0.06
	Ferodex	233.1 ± 32.1	25.5 ± 0.68	2.91 ± 0.03
7	Ursoferran	297.1 ± 23.6	34.7 ± 0.77	3.15 ± 0.04
	Ferodex	325.4 ± 46.4	36.2 ± 4.95	3.29 ± 0.23
10	Ursoferran	244.1 ± 21.3	53.0 ± 4.47	4.07 ± 0.29
	Ferodex	184.9 ± 26.0	35.2 ± 3.69	3.26 ± 0.76
14	Ursoferran	173.7 ± 8.7	43.2 ± 3.99	3.39 ± 0.34
	Ferodex	143.3 ± 18.9	36.2 ± 3.27	3.39 ± 0.21

In case of the groups with treatment 4-5 days after birth, one can not observe significant differences of the values registered in the control group at the respective age, although the differences are slightly increased as compared to the corresponding values for the control group. Therefore, irrespective of the administration day for the two iron preparations, in the 2-3 days period, the piglet's sideremy registered an increase, reaching maximum values 7-10 days after birth, followed by a slow, but constant diminishing till the age of 14 days, and reaching values as high as the initial ones or even lower.

Between the dynamics of the sideremy values and the haemoglobin and erythrocyte number, there was not registered any correlation, but one could observe a positive correlation between the variation of the haemoglobin values and that of the erythrocyte number.

The administration of the two iron-dextran preparations on the third day after birth causes an increase of the sideremy, haemoglobin and erythrocyte number values as compared with the initial values, as well as with those of the control group.

Table 5 : The blood indices for administration five days after birth

Days	Preparation	Sideremy $\mu\text{g}/100 \text{ mL}$	Haemoglobin %	Erythrocyte number $\cdot 10^6$
1	Ursoferran	227.3 ± 18.4	30.4 ± 1.03	2.85 ± 0.254
	Ferodex	168.5 ± 7.9	33.4 ± 1.03	2.98 ± 0.063
3	Ursoferran	219.0 ± 24.2	26.4 ± 1.03	2.86 ± 0.156
	Ferodex	252.1 ± 28.1	25.8 ± 0.68	2.58 ± 0.128
7	Ursoferran	299.9 ± 29.2	37.2 ± 2.41	3.11 ± 0.149
	Ferodex	255.8 ± 29.8	31.8 ± 6.67	3.28 ± 0.658
10	Ursoferran	237.6 ± 15.6	36.2 ± 2.33	3.00 ± 0.05
	Ferodex	263.0 ± 27.5	33.8 ± 2.54	3.42 ± 0.34
14	Ursoferran	165.7 ± 18.13	38.0 ± 2.23	3.16 ± 0.07
	Ferodex	182.9 ± 12.25	36.0 ± 2.64	3.43 ± 0.246

The administration of the iron preparations on the fourth day after birth although bringing about noticeable increase of the blood index values, their evolution show no uniformity, showing variable values during the two weeks.

The two iron-dextran preparations administered on the fifth day after birth do not cause remarkable increase of the sideremy, haemoglobin or erythrocyte number, however they maintain their initially registered level.

Without some local momentary and transitory disturbances for 15-20 minutes (walk hindrance), there were not observed any negative effects following administration of the two iron-dextran preparations.

CONCLUSIONS

The pursued blood indices: sideremy, haemoglobin percentage and erythrocyte number registered in untreated with iron-dextran preparation piglets shows a sudden diminishing in the first three days of life, followed afterwards by a moderate but constant decrease till the 14-th day of age, getting to much lower values proper for this age.

The two iron-dextran preparations used in this study, Ursoferran R 100 and Ferodex R 75, administered in doses with 150 mg Fe/piglet, prevent the iron-deficiency anaemia.

Irrespective of the administration day of the two iron-dextran preparations, in the 2-3 day period after birth, the piglet's sideremy registered an increase, reaching maximum values 7-10 days after birth, followed afterwards by a slow decrease till the age of 14-th day.

The highest values for the blood indices haemoglobin and erythrocyte number were registered when the administration was undertaken 3 days after birth. In the case of Ursoferran R 100, the highest value registered at the end of the experimental period was obtained when the injecting was undertaken at two days and in the case of Ferodex R 75, when the injecting was undertaken at three days after birth.

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CHANGES IN THE CALCIUM AND MAGNESIUM CONTENTS OF WHEAT, RYE AND TRITICALE GRAINS DEPENDING ON THE VARIETIES

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ABSTRACT

Calcium and magnesium contents of the wheat, rye and triticale samples were investigated depending on the varieties. Triticale grains were grown by the cross-breed of the wheat and the rye. According to our measurements the magnesium contents were the same in the wheat and in the rye grains, but the calcium contents were found to be 50% more in the rye than in the wheat samples. Amounts of both two elements were two-fold higher in the triticale than in the two parents (wheat and rye). These findings led us to show the transgression, which is the increasing in the mineral (e.g. magnesium and calcium) contents compared to the parents (plant improvement).

Key words: wheat, rye, triticale, calcium, magnesium, transgression

INTRODUCTION

There is significant role of the wheat, rye and triticale in the calcium and magnesium supply used them in the human and animal alimentation. According to Zook and Green,1970; Davis and Lous,1984, the magnesium content is 1.17 more in the durum wheat than in the bread wheat.

Our former studies revealed (Csikkel-Szolnoki and Kiss, 1996; Kiss et al., 1998), that the mineral composition of the wheat depends on the variety in the same soil and decreases with the increase of the ploidity. The ploidity usually increases after the cross-breed. These findings lead us to investigate the question, whether the decrease in the mineral composition (calcium and magnesium) of triticale true or not after the cross- breed compare to the wheat and rye grains (parents).

MATERIALS AND METHODS

We thank the Cereal Research Institute in Szeged for the wheat samples, the Research Centre in Tápiószele for the rye samples and the Research Centre in Iregszemcse for the triticale samples.

The different grains were powdered and dried at 105°C for 3 hours. Then approximately 0.2 g of sample was digested with 5cm³ of concentrated nitric acid in Berghof B type microwave apparatus (Csikkel-Szolnoki, 1995). The elemental analyses (calcium and magnesium) were performed by means of ICP-atomic emission spectrometer (Winge et al., 1991).

*Parameters for digestion:**Warming:*

- power supplied 80% (850W)
- temperature 140 °C
- time 2 min 30 sec
- P-range 5°C

Digestion:

- power supplied 80%
- temperature 190 °C
- time 7 min 30 sec
- P-range 20°C

Cooling:

- power supplied 0%
- temperature 100 °C
- time 10 min.
- P-range 5°C

Experimental parameters for ICP-AES measurements:

Frequency of Rf generator: 40.68 Mhz

Power supply of Rf generator: 0.8 kW

Flow rate of plasma gas (argon):
12 dm³/minFlow rate of sheath gas : 0.37 dm³/minFlow rate of aerosol carrier gas: 0.2 dm³/minFlow rate of nebulization (Babington):
1.4 cm³/minWavelengths: Ca 393.366 nm,
Mg 279.553 nm**RESULTS AND DISCUSSIONS**

The experimental results are shown in the Table 1. The calcium and magnesium contents are the same both in the wheat and the rye varieties, but significantly less than in the triticale varieties. Consequently the calcium and magnesium contents are increased by the cross-breed. This symptom can be known as the transgression, when after the cross -breed an increase occurs in the variability of the hybrids (F_1) compared to the parents. The transgression is very common in the plant improvement. We can observe the transgression in case of the triticale, in which the calcium and magnesium content increases compare to the wheat and rye (parents).

Table 1: Calcium and magnesium contents and their ratio in the wheat, rye and triticale grains depending on the varieties (mg/kg)

Plant	Ca	Mg	Ca/Mg
WHEAT			
GK-Öthalom	85	1060	0.08
GK-Góbé	288	1110	0.25
GK-Csűrös	152	1180	0.12
GK-Olt	288	1340	0.21
J-50	259	814	0.31
<i>Average:</i>	<i>214</i>	<i>1100</i>	<i>0.19</i>
RYE			
BO-151	393	937	0.42
Caracurz	250	1050	0.24
Hankija 6901	375	1110	0.34
Hohenthurm	242	1100	0.22
Maton	372	1610	0.23
Saratovskaja 4	349	1030	0.34
<i>Average:</i>	<i>330</i>	<i>1140</i>	<i>0.29</i>
TRITICALE			
Monico	814	2050	0.39
Presto	721°	1930	0.37
Tewo	925	2070	0.45
<i>Average:</i>	<i>820</i>	<i>2016</i>	<i>0.40</i>

Our experimental data show, that the magnesium content is the same in the wheat and in the rye (1100 mg/kg), so these cereal varieties have with identical diatetical importance.

But if we see the ratio of calcium and magnesium, this is lower (0.19) in the wheat than in the rye (0.29). This fact has diatetical importance in consequence of the antagonism in the calcium and magnesium uptake.

CONCLUSIONS

The magnesium content is almost two times more in the triticale than in the wheat and in the rye. At the same time the calcium content becomes manifold,

so the ratio of the calcium/magnesium is disadvantageous (0.40) in the triticales. In spite of this ratio the triticales is a good magnesium supply as a fodder-plant for animals.

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ESSENTIALITY OF METALLIC BIOELEMENTS AND THE IMPORTANCE OF FOOD SUPPLEMENTS INTAKE

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ABSTRACT

Minerals are essential nutrients to life. In comparison with carbohydrates, proteins, lipids - the so-called macronutrients, they are needed in small amounts and beside vitamins they constitute the class of micronutrients. After they had been absorbed become a part of the structure of the organism (bones, muscles, blood a.o.). They can be utilized immediately or stored and utilized over a period of time. For the human organism there are essential about twenty six elements, e.g.: Ca, Mg, Na, K, Zn, Fe, Cu etc. It is known that the nutrient content of foods, especially of minerals fluctuates widely, depending on the growing conditions. The using of artificial fertilizers for increase crop yields deplete often the soil of essential minerals. These fertilizers are based solely on plant needs and overlook human requirements. Fruits and vegetables lose nutrients from the moment they are picked, then stored, then shipped. Also, food processing determine the lose of mineral and other nutrients. It is known that various diseases also reduce the mineral nutrients intake (e.g.: Ca, Mg, Zn, Fe etc.), that in stress conditions the organism need more special nutrients (e.g.: Mg, Zn, Cu etc.). In this context the importance of food supplements intake with mineral content is discussed. The quantity of the nutrients ingested daily which would cover the normal requirements of 97.5% of the population is called "Recommended Daily Allowance (RDA). In a recent report the Scientific Committee of EEC – based on "Codex Alimentarius", called this quantity "Population Reference Intake" (PRI) in order to emphasise that this figure is applicable to populations rather than to individuals and it should be used accordingly.

Key words: essential minerals, nutrient intake, food supplements

INTRODUCTION

Besides carbohydrates, proteins, lipids and vitamins (organic substances) the humans organism need certain chemical elements in their inorganic form. These chemical elements in their organic forms are classified as biominerals.

Biominerals, essential micronutrients to life, are found as cations (e.g. Ca, Mg, Na, K, Zn, Cu etc.) or anions (e.g. phosphates, selenate etc.). In comparison with macronutrients (i.e. carbohydrates, proteins, lipids), micronutrients (i.e. vitamins, biominerals a.o.) are needed in small amounts.

After they had been absorbed become part of the structure of the organism (bones, muscles, blood a.o.). They can be utilized immediately or stored and utilized over a period of time. For the human organism there are essential about twenty eight elements, among with the most important are: Ca, Mg, Na, K, Zn, Fe, Cu (Mertz, 1989; Hedler, 1991)

These nutrients participate in a multitude of biochemical and physiologic processes necessary for health maintenance.

FOOD AND MINERAL CONTENT

It is known that the nutrient content of foods, especially of minerals fluctuates widely, depending on the growing conditions. Because of differing geological conditions, minerals may be scarce in the soils of certain regions and rich in other regions.

Referring to the main metallic trace elements, in order to have a general view on their status, in table 1 there are presented synoptic data upon their total quantity in the organism, the daily allowance and their daily intake by food.

Table 1. Status of metallic trace element in organism, their daily allowance and their intake by food.

No.	Trace metals	Total quantity in organism (mg)	Daily allowance (mg/day)	Food intake (mg/day)	Quantity absorbed by diet (%)
1.	Fe	4.200	10	13	7
2.	Zn	2.300	3	13	30
3.	Cu	75	2	5	5
4.	Mn	15	2.5	4	3
5.	Mo	9	0.1	0.2	50
6.	Cr	6	0.2	0.1	10
7.	Co	1.5	0.0004	0.3	80
8.	Ni	10	0.0023	0.4	5
9.	Sn	17	3	3	1

Environmental pollution (artificial fertilizers used in agriculture, pesticides, industrial pollutants) deplete often the soil of essential minerals. These fertilizers are based solely on plant needs and overlook human requirements.

Food processing, use of various food additives, e.g.: artificial colorants, stabilizing agents, emulsifying agents, preservatives etc. may determine the loss of minerals and other nutrients.

Any sort of stress deplete the vitamin and mineral stores of organism more rapidly. With age the organs tend to function less efficiently: the metabolism slows, physical activity diminishes, food intake goes down. Various diseases reduce the mineral nutrients intake and especially those that interfere with the ingestion, digestion, absorption and requirement of nutrients: celiac disease, Crohn disease, irritable bowel syndrome, lactose intolerance, bacterial, viral and parasitic infections.

Many drugs interact with nutrients in the body, often causing depletion: tetracycline a widely used antibiotic interferes with the absorption of calcium; neomycin interferes with iron, calcium, potassium absorption; anticonvulsants (dilatrin, phenobarbital), digoxin, furosemide oral contraceptives may cause magnesium and calcium depletion; hydralazine may cause magnesium deficiency; penicillamine interferes with iron, copper, zinc absorption; cisplatin may cause magnesium deficiency.

SCIENTIFIC BACKGROUND

For 50 years the basic point of reference for nutrition requirement for prevention of malnutrition and disease has been the “Recommended Daily Allowance (RDAs)” , first issued by the Committee on Food and Nutrition of the US National Research Council. RDAs were periodically reviewed (Liebermen and Bruning, 1990; Hatchcock, 1993).

This is similar in concept with the Recommended Daily Nutrient Intakes in Canada, Recommended Daily Amounts and Recommended Nutrient Intakes in United Kingdoms and the Population Reference Intakes of the European Union.

The Codex Guidelines on Nutrition labeling recommended that nutrient reference values should as far as possible based on nutrient intakes recommended by FAO and WHO. There is a list of values for ten vitamins and six minerals.

Today it is considered that RDA or PRI prevent only deficiency but cannot assure an optimal intake. Mineral insufficiency and trace element insufficiency states are actually more likely to occur than are vitamin insufficiency. Those at increased risk of such insufficiencies include people who eat low-calories diet, the elderly, pregnant women, people taking certain drugs (e.g. diuretics)., vegetarians and those living where the soil is deficient in certain minerals. Because of differing geologic conditions minerals and trace elements may scarce in soil of certain region and rich in those of other regions.

FOOD SUPPLEMENTS – CHARACTERISTICS

Food supplements are generally products marketed as concentrated sources of vitamins and minerals, alone or in combination, which purpose is to supplements the intake of these nutrients from the normal diet.

Data on the main mineral micronutrients (cationic and anionic) are presented in table 2.

Table 2: Synoptic data upon mineral micronutrients

Mineral micro-nutrients	UM	PRI	Median daily intake	Upper safe level for daily supplementation	Risk domain
Ca	mg/day	700	917	1500	< 2500
Mg		150	308	350	< 700
Cu		1.1	1.5	5.0	< 10
Cr	µg/day	50	30	200	< 200
Fe	mg/day	9	13.2	15	< 60
Mn		1	4.6	15	< 20
Zn		15	11.1	15	< 30
P		550	1417	1500	< 3000
I	µg/day	130	225	500	< 1000

Evidence from recent studies reveal that mineral trace element supplementation may help to prevent various forms of cancer, heart disease and some other degenerative processes.

In table 3 there are given data concerning some food supplements, produced in Farmington-USA, with metallic and non-metallic elements content (Daranyi, 1997).

Table 3. Food supplements with macro- and trace elements

Product name	Metallic elements	Non-metallic elements
Advanced Fat Burner	Cr, Fe	-
Arginine Plus	Mn, Co	-
Chelated Zinc	Zn	-
Citrimax and chromium	Cr	-
Coenzyme Q10	-	Se
Full Spectrum	Ca, Fe, Mg, Cu, Zn, Cr, Mn, K, Mo	I, Se
Green Care	Mg	-
Iron Plus	Fe	-
Lion Kids	Ca, Fe, Mg, Cu, Zn, Cr, Mn, K, Cr	P, I, Se
Liver Aid	Cr	-
Mega Chrom	Cr	-
Mega Zinc	Zn	-
Muscle Nitro	K	
New Life	Ca, Mg, K, Zn, Mn, Fe, Cu, Cr	I
Power Mins	Ca, Mg, K, Zn, Mn, Fe, Cu, Cr, Mo	I, Se
Pro Selenium	-	Se
Protect 4 Life	-	Se
Stress Management B-Complex	Mg	-
Super Fat Burners	Cr	-
Strong Bones	Ca, Mg	
Super Mega 50	Mg, Ca, K, Zn, Mn, Fe, Cu, Cr, Mo, B, V	I, Se

These food supplements contain, beside the mentioned bioelements, other organic compounds such as: proteins, carbohydrates, vitamins etc. assuring a better nutritional support. Food supplements are recommended for various deficiencies according their composition.

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PECULIARITIES OF THE RELATIONSHIP NUTRITION-METAPHYLAXY OF UROLITHIASIS II. ROLE OF SOME METALLIC MICRONUTRIENTS

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ABSTRACT

In the last decades the frequency of urolithiasis increased. About 5% of population present renal stones and about 40-60% of these patients make recurrence. As to the qualitative composition of urolithiasis there were found: oxalates 75%, phosphates 15% and uric acid-urates 8%. Today the essential role of food consumption is recognized in the prophylaxy and metaphylaxy of some types of urolithiasis, especially of oxalic and uric acid ones. It is known that an excess of calcium or certain other minerals in the diet (sometimes due to local geographic water or soil condition), a diet of fruits and vegetables high in oxalate, an excessive uric acid intake lead to urinary microcrystals (i.e. "primers" or "starters") and then to uroconcrements formation. It has been discovered that avoiding calcium may do more harm than good. It seems that high dietary calcium actually blocks a chemical action which causes the formation of kidney stones by binding with oxalates (from foods) in the intestine which then prevents both being absorbed into the blood stream and later transferred to the kidneys. Persons with highest consumption of potassium from eating fruits and vegetables had only half the risk of stones. The role of magnesium in preventing urolithiasis formation is well known. So, one can recommend to stone former patients foods rich in this mineral or a supplementation, because a normal diet does not assure an efficient concentration for metaphylaxy and prophylaxy.

Key words: urolithiasis - metal ions - metaphylaxy

GENERAL CONSIDERATIONS

Metals are important components of the living matter being present in the environment (food, water, air, soil). Taking into account their concentration they are classified in: macro-, micro- and trace metallic elements and play an important role in the human organism as bioconstituents or as biochemical effectors being involved in hydro-electrolytic metabolism.

They are essential for many metabolic processes (Underwood, 1981; Gonzales-Revelderia et al., 1990; Anonymous, 1997). Metallic electrolytes are not produced or destroyed in the body. Thus, in a balanced system, the amounts taken in excess (not necessary for organism) are excreted, mostly in the urine.

METAL COMPOUNDS AND TRANSLOCATION

Studies dealing with the presence of metals in food, air, soil and water are of great importance in order to understand the possibility of their translocation in human organism. In this context it is necessary to evaluate the absorption, retention and elimination of metals in relation with urolithogenesis.

Metallic micronutrients and especially transitional metals play an important role in metabolic processes involved in translocation and uroconcrements formation. In table 1 there are presented the concentrations of some trace metals in various foods (Steinmassl, 1987).

Table 1. The natural distribution of some transitional metals in feed

Feed	Trace Metals			
	Fe (%)	Cu (mg/Kg)	Mn (mg/Kg)	Zn (mg/Kg)
<i>Animal origin</i>				
Buttermilk	-	-	3.5	-
Carcass residues ^{1,2}	-	38.7	19.1	-
Casein	-	-	4.4	-
Calm shells	0.46	-	335.7	-
Crab process residue	0.44	32.8	133.8	-
Fish, herring	-	-	9.9	-
Salmon	0.02	11.9	7.9	-
Sardine	0.03	20.2	22.2	-
Tuna	-	-	-	-
Viscera ¹	0.05	97.0	7.3	-
Pork cracklings	-	-	14.3	-
Whey	0.02	43.1	4.6	-
<i>Plant origin</i>				
Apple	0.03	-	7.3	-
Citrus seeds ³	0.03	6.6	7.5	7.5
Coconut meat ⁴	0.19	18.7	55.4	-
Palm seeds ⁴	0.02	67.5	272.1	-
Peanuts kernels ⁴	-	-	25.5	-
Shells	0.03	16.2	62.7	-
Potato process residue ^{5,6}	0.04	15.4	31.5	-
Rice grains	-	3.3	17.6	1.8
Bran with germ ⁶	0.02	13.0	417.8	29.9
Rice white	-	2.9	10.9	1.8
Soybean, aerial parts	0.01	2.1	27.5	-
Sunflowers, aerial parts	-	-	233.9	-
Seeds with hulls ⁷	-	-	22.9	-
Tomato pomace ^{5,6}	-	-	47.3	-
Wheat, aerial parts	0.01	-	10.5	-
Germ	0.01	8.8	134.9	-
Yeast ⁵ , <i>Saccharomyces</i> brewer's	0.01	33.0	5.7	38.7
Primary	0.03	-	3.7	-
<i>Torula</i>	0.01	13.4	12.8	99.2

Note: 1 – animal not specified; 2 – with blood; 3 – Solvent – extracted, ground; 4 – Mechanically extracted, ground; 5 – Dehydrated; 6 – Ground; 7 – Mechanically extracted, dehydrated, ground.

An excess of metallic micronutrients or certain other metals in the diet (sometimes due to local geographic water or soil condition), a diet of fruits and vegetables high in oxalate, an excessive uric acid intake lead to urinary microcrystals (i.e. “primers” or “starters”) and then to uroconcrements formation.

It has been discovered that avoiding calcium may do more harm than good. It seems that high dietary calcium actually blocks a chemical action which causes the formation of kidney stones by binding with oxalates (from foods) in the intestine which then prevents both being absorbed into the blood stream and later transferred to the kidneys.

NUTRITION AND UROLITHOGENESIS

The kidneys play a major role in maintaining balance with respect to water and mineral electrolytes: cationic, e.g. Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Zn^{2+} etc. and anionic, e.g. $(\text{COO})^{2-}_2$, HPO^{2-}_4 , Cys^{2-} . They accomplish this by adjusting the output of these substances in the urine to match the rate of addition to the body. Renal excretion of water is under the continuous control of the antidiuretic hormone and is normally adjusted to maintaining water balance. The kidneys are the primary site of control of electrolyte output.

In the organism the mineral electrolytes are distributed by blood in the liver, kidney and other organs, a part of them is accumulated and the remainder is excreted in the urine. Minerals keep in normal range the parameters for an optimum physiological status of the human organism. In Table 2 there are presented the normal concentrations of metals in human urine (Altman and Dittmer, 1968).

Tabel 2. Concentration of metals in human urine – in $\mu\text{g/L}$

Metal	Medium values $\bar{X} \pm \text{SD}$	Range
Al	10.90 ± 1.060	2.300 – 19.50
Cd	0.860 ± 0.060	0.380 – 1.340
Co	0.570 ± 0.100	0.180 – 0.960
Cr	0.610 ± 0.110	0.040 – 1.600
Cu	23.00 ± 6.900	4.200 – 50.00
Fe		60.00 – 1000
Hg	3.500 ± 0.200	0.100 – 6.900
Mn	1.020 ± 0.050	0.120 – 1.900
Mo		10.00 – 16.00
Ni	0.900 ± 0.110	0.060 – 1.740
Pb	17.00 ± 0.460	12.00 – 27.00
T	0.420 ± 0.090	0.010 – 0.280
Zn	456.0 ± 58.00	266.0 – 846.0
V	0.800 ± 0.080	0.200 – 1.000

Today it is unanimously recognized the role of nutrition in the appearance of renal calculus. So, it is known that a low liquid intake, excessive alcohol consumption, excessive intake of food rich in oxalates or urates etc. lead to urinary crystals and then to uroconcrements formation (Guthrie, 1975; Daranyi et al., 1997a). The metals play an important role in urolithogenic mechanisms.

Qualitative analysis of uroconcrements revealed the existence of various metals in their composition (Rannert and Chan, 1981; Daranyi, 1997b).

METAPHYLAXY OF UROLITHIASIS

The management of renal lithiasis has a series of general principles of prevention, applicable to each patients but also specific measures, according to the peculiarities of the urolithiasis type. It is well known the role of nutrition (especially the qualitative role) in the appearance of renal calculus.

Persons with highest consumption of potassium from eating fruits and vegetables had only half the risk of stones. The role of magnesium in preventing urolithiasis formation is well known (McNeely, 1986; Garban et al., 1995).

Today, it is recognized that a low liquid intake, excessive alcohol consumption, excessive intake of food rich in oxalates or urates lead to urinary crystals and than to uroconcrements formation. When the diet has to be controlled it is necessary to know the concentrations of purines in food. Special dietetic recommendations to patients with different types of urolithiasis may assure a good metaphylaxy.

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ANTIBACTERIAL ACTIVITY OF SOME QUATERNARY PHOSPHONIUM SALTS GRAFTED ON SOLUBLE MACROMOLECULAR SUPPORTS

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ABSTRACT

In this paper we present an experimental study of some polymer – bound phosphonium salts grafted on soluble macromolecular supports: poly(ethyleneglycole)s - PEG for antibacterial activity against Staphylococcus aureus and Escherichia coli. Compiled data showed that these insoluble bactericides can be easily separated and used with enhanced ease and repeatedly, with only a sterilisation prior to use required.

The studies of such polymeric disinfectants had in view the possibilities of important applications e.g.: water and air disinfection, antifouling coating and fiber finishing, drugs with a longer activity and less toxicity a.o. Our results indicate the best antibacterial activity against the gram-negative bacteria (Escherichia coli) using tributylphosphonium chloride (P_3C) grafted on PEG.

Key words: quaternary phosphonium salts, Staphylococcus aureus, Escherichia coli.

INTRODUCTION

Soluble, low molecular weight disinfectants frequently give rise to problems (Ikeda and Tazuke, 1984; Senuma et al., 1993) like toxicity and formation of unwanted residual derivatives, especially when applied in water treatment, foodstuffs and packaging materials.

Such problems can be solved in many cases, if the bactericide is covalently grafted on polymeric carriers or other insoluble support materials (Ikeda et al., 1986, 1990; Tashiro, 1993). These carrier-bound disinfectants retain, in many cases, their antimicrobial activity in bound state. Advantageously, these insoluble bactericides can be easily separated, used with enhanced ease and repeatedly, with only a sterilisation prior to use required.

Many insoluble disinfectants reported are polymer-bound "onium" salts (Kanazawa et al., 1993a, 1993b, 1994a, 1994b; Davidescu et al., 1996). Polymeric disinfectants have received considerable attention in recent years with respect to important applications, such as: drugs with prolonged activity and less toxicity, antifouling coatings and fiber finishing, water and air disinfection.

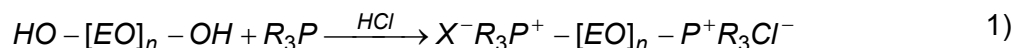
In a previous paper, the antibacterial activity of quaternary phosphonium salts grafted on insoluble macromolecular supports by polymer-analogous reactions was studied (Davidescu et al., 1996). Such products were proved to have inhibitory, bacteriostatic activity against *Staphylococcus aureus*. As a great advantage, the

active species grafted on insoluble carriers can be used in repeated cycles, with only a sterilization prior to use needed.

In this paper we report some preliminary results obtained in the study of the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* of quaternary phosphonium salts grafted on soluble macromolecular supports, thus overcoming some of the problems observed with low molecular weight disinfectants.

EXPERIMENTAL PART

The polymeric disinfectants used in the present study were polymer-bound phosphonium salts grafted on soluble, poly(ethyleneglycole)s (PEG) through the polymer-analogous reaction:



where: $[EO]_n = -CH_2CH_2-[OCH_2CH_2]_{n-1}-$
 $R = -C_6H_5$; Product code: P₁C
 $R = -C_2H_5$; Product code: P₂C
 $R = -C_4H_9$; Product code: P₃C

The following starting materials were used: polyethyleneglycole (PEG1000, Merck, p.a.), triethylphosphine (Aldrich, 68% in isopropanol), tributylphosphine (Fluka, 85%, $d=0.822 \text{ g/cm}^3$), triphenylphosphine (Merck, p.a.), benzene (Merck, p.a.), hydrogen chloride (Reactivul, 33% (w), $\rho=1.167 \text{ g/cm}^3$)

The synthesis and characterization of the polymeric disinfectants were reported elsewhere (Popa, 1998). Some characteristics of the polymer-bound phosphonium salts are given in Table 1.

Table 1. Characteristics of the poly(oxyethylene)s functionalized with quaternary phosphonium end groups.

Product Code	Phosphorus (% weight)	Yield of functionalization η_F	Degree of functionalization mmoles $R_3P^+Cl^-$ /g. of polymer
P ₁ C	2.75	59.0	0.44
P ₂ C	4.85	99.3	0.78
P ₃ C	2.11	40.0	0.34

The yields of the quaternization reactions are ranging between 40 - 99% being convenient for a polymer - analogous reaction.

The degrees of the chemical modification with quaternary phosphonium salts are relatively high, ranging from 0.34-0.78 mmoles of functional groups/g. of polymer, ensuring a sufficient concentration of active centers per unit mass of polymer.

ANTIBACTERIAL ACTIVITY OF POLYMER-GRAFTED PHOSPHONIUM SALTS

As the functionalized PEG's are soluble the antibacterial activity was assessed by the standard dilution series method (Trif and Popa, 1996; Daniels, 1980). Starting

solutions of P_1C , P_2C and P_3C with the concentrations of 30000 $\mu\text{g/mL}$; 17000 $\mu\text{g/mL}$ and respectively 39000 $\mu\text{g/mL}$ were prepared, ensuring the same concentration of phosphonium active groups: 13.26 $\mu\text{moles } P^+R_3Cl^-$. Test solutions were then prepared at the dilution ratios of 1/2, 1/10, 1/20, 1/40, 1/50, 1/80, 1/100, 1/150, 1/200, 1/250 and 1/300 with nutrient broth.

Then, each of the resulted solutions was seeded with a 24 hours culture of *Staphylococcus aureus* or *Escherichia coli* (containing 10^7 - 10^8 cells/mL). The test tubes were then incubated at 37°C for 24 hours.

As a measure of the bacteriostatic activity, the *minimum inhibitory concentration* (**MIC**) was determined by standard procedures as the maximum dilution (minimum concentration) where no development of the bacterial culture was observed.

As a measure of the bactericide activity, the *minimum bactericide concentration* (**MBC**) was determined on seeds on nutrient agar by standard procedures. The test tubes were incubated at 37°C for 24 hours.

The experimental results showed that triphenylphosphonium chloride (P_1C), triethylphosphonium chloride (P_2C) and respectively tributylphosphonium chloride (P_3C) grafted on PEG exhibit both bacteriostatic and bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* (Figure 1).

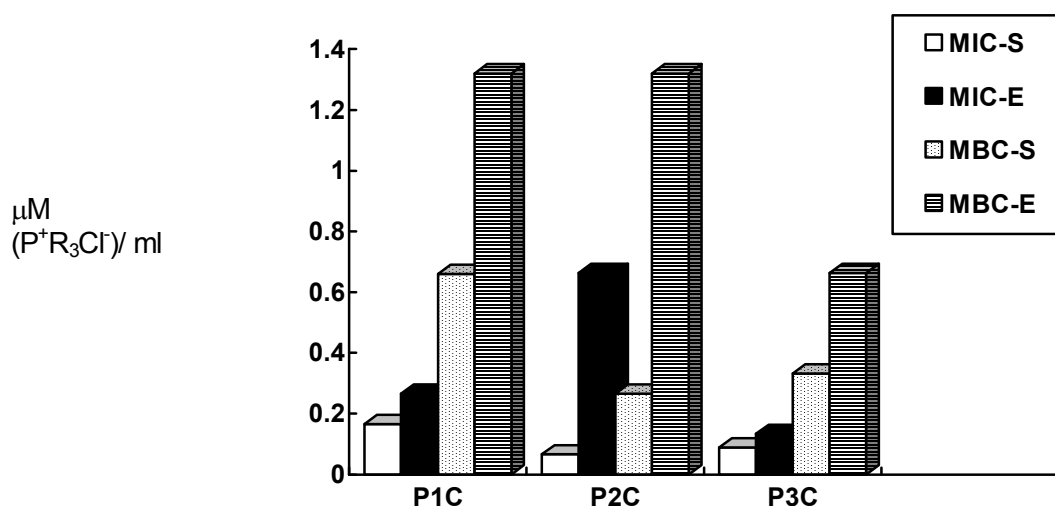


Figure 1. Antibacterial activity of phosphonium salts grafted on PEG against *Staphylococcus aureus* and *Escherichia coli*.

The antibacterial activity of the phosphonium salts grafted on PEG can be discussed in direct relationship with the hydrophilic and respectively hydrophobic structural balance of the products in which the polyoxyethylene main backbone is essentially hydrophilic and the functionalized end-groups are containing alkyl or aryl substituents mainly hydrophobic.

The hydrophilic/hydrophobic balance could probably affect the fixation of the bacteria cells at the "onium" active centers but the major factor seems to be the electrostatic interaction between the positive charge of the phosphonium ions and the negative charge of the bacteria cells surface.

The triethylphosphonium chloride P_2C has the highest antibacterial activity against *Staphylococcus aureus*, as indicated by the lowest MIC and MBC values for the bacteriostatic and respectively bactericide activity (0.066 $\mu\text{M/mL}$ and respectively

0.265 $\mu\text{M/mL}$). For the same product the activity against *Escherichia coli* is reverted. The values for MIC and MBC are the highest (0.663 and respectively 1.33 $\mu\text{M/mL}$).

The best antibacterial activity (both bacteriostatic and bactericide) against *Escherichia coli* was observed with the tributhylophosphonium chloride P_3C grafted on PEG with the lowest values for both MIC and MBC (0.133 $\mu\text{M/mL}$ and respectively 0.633 $\mu\text{M/mL}$).

The presence of the phenyl radicals in the structure of the phosphonium ion as in P_1C lowers both the bacteriostatic and bactericide activity against *Staphylococcus aureus* and *Escherichia coli*.

The lower activity observed against the gram-negative bacteria (*Escherichia coli*) as compared with the gram-positive species (*Staphylococcus aureus*) could be most likely explained in terms of a different structure and complexity of the cell walls (Daniels, 1980). *Escherichia coli* has a supplementary outer membrane composed mainly of lipopolisacharides, phospholipids and proteins outside the peptidoglycane layer acting as a barrier and affecting the elementary events in the lethal action of the quaternary phosphonium salts.

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MOTHER ZINC DEFICIENCY AND NEUROLOGICAL MALFORMATIONS OF THE FETUS

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ABSTRACT

Besides genetical factors, zinc deficiency must be taken into account in the aethyology of the neurological malformations. In animal experiments, severe zinc deficiency determined an increased incidence of fetal malformations but in human beings some authors found relationships between zinc levels of mother or foetal organism and foetal malformations, whereas the other ones found no such connections. We determined serum zinc and alkaline phosphatase in a group of 11 pregnant women, antepartum diagnosed, by echographyc exam, having malformed foetuses. They were compared to a control group of pregnant women with normal evolution of pregnancy. The two groups were significantly different. This means that there an association between serum zinc deficiency and malformations. So, zinc deficiency could be a cause of the detected malformations.

Key words: zinc deficiency, neurological malformations

INTRODUCTION

Besides genetical factors, nutrition may be an important cause of neurological malformations. Among the nutritional factors, the deficiencies of folate and zinc must be taken into account first of all. These factors must act very early during pregnancy, because the neural tube formations occurs in the first 28 days of intra uterus development.

In animal experiments, severe zinc deficiency determined an increased incidence (over 90% - Da Cunha Ferreira et al., 1989) of foetal malformations. Its effect is very rapid and becomes apparent in the third day on fertilized eggs. This can be explain by the dependence of ovum during the first few days on the uterine fluid as a source of zinc.

A connection between maternal zinc deficiency and congenital malformations in human has been also postulated (Halsted, 1973). Few pregnancies in women suffering from acrodermatitis enteropatica have been reported and resulted in high incidence of malformed infants (Hambidge et al., 1975). Pregnant women suffering from untreated celiac disease, diabetes, or were on treatment with some drogs (thalidomide analogue, diphenylhidantoin, valproate, etc.) had low serum zinc and gave birth to infants with congenital malformations (Jameson, 1983).

Maternal serum zinc levels and umbilical blood serum zinc levels were significantly lower in mother who gave birth to anencefalic fetuses, compared to normal controls (Cavdar et al., 1980).

A study performed on pregnant teenagers showed that mothers who gave birth to malformed infants has lower serum zinc compared with the mean serum zinc of the whole group investigated (Cherry et al., 1981). Milunsky et al. (1992) found an association between increased toenail zinc level of the pregnant women in the

second quarter and the risk of neural tube malformations, probably to zinc sequestration in neural tubes. Sharma et al. (1994) found significant lower zinc level in mother cord blood serum congenital malformed babies and significantly higher level in the amniotic fluid, compared to healthy controls. Other authors (Bower et al., 1992-93; Hambridge et al., 1993; Mahomed et al., 1993) showed that there is no relation between zinc level from serum, hair or amniotic fluid and neural tube defects and McMichael et al. (1994) found that high serum zinc levels in midpregnancy may rise the risk of neural tube defect, consequently of a deficient maternal to fetal transfer of zinc.

The purpose of the paper was to find possible associations between serum zinc deficiency and malformations.

MATERIALS AND METHODS

During two years (1996 and 1997), in the IVth Clinic of Obstetrics and Gynaecology of Iași, 11 pregnant women with neurological malformations were antepartum diagnosed. This was done by echography with Toshiba Sonolayer apparatus and was confirmed by anatomo-pathological exam of the pregnancy outcome after delivery.

The studied group was compared with a control group with normal evolution of pregnancy (Table 1).

Table 1. Data regarding the subjects

Specification	Studied group	Control group
Number of pregnant women	11	68
Age (years)	26.0 ± 5.6	25.4 ± 4.3
Gestational age (weeks)	31.3 ± 5.2	34.4 ± 2.6

Serum zinc levels by atomic absorption spectrophotometric method and alkaline phosphatase by colourimetric method (with p-nitro phenyl phosphat) were determined. For the statistical comparison of the groups, the unpaired t test, with significance at $p < 0.05$, was used.

RESULTS AND DISCUSSIONS

There were detected, by echography exam, the next malformations:

- spina bifida - 4 cases,
- hydrocephaly - 3 cases,
- anencephaly - 4 cases.

The laboratory determinations showed statistically significant lower zinc levels in pregnant women which gave birth to malformed infants comparing with the control group. These levels were associated with significantly decreased alkaline phosphatase levels (Table 2).

Table 2. Serum mean values of the assessed indicators

	Studied group	Control group	t	p
Zinc (µg/dl)	56.2 ± 11.2	64.9 ± 12.9	2.33	<0.02
Alkaline phosphatase (U.I./l)	55.3 ± 18.5	70.3 ± 32.4	2.19	<0.05

Our results support the findings of Favier A. and Favier M. (1992) and Jameson (1983). In order to explain zinc deficiency action, there were elaborated some hypothetical mechanisms, as follows (Favier, 1992):

- the decreasing of the activity of numerous enzymes in the brain (thymidine kinase, 2'3' cyclic nucleotidyl phosphorylase, lactate dehydro-genase, L-glutamate dehydrogenase),
- the disturbance of the expression of certain genes through the zinc finger protein mechanisms,
- the diminution of cell differentiation zinc dependent factors,
- the upset of the cytoskeletal assembly of brain cells,
- the hyperproduction of free radicals that are liable to damage DNA.

Some authors (Favier, 1990; Jameson, 1993) proposed zinc therapy in order to avoid zinc deficiency syndrome in pregnancy, including fetus malformations.

Folate deficiency is also considered to be a teratogenic factor, but the folate uptake from the proximal gut is impaired by zinc deficiency and it is suggested that folate conjugase, the enzyme which hydrolyzes complex folates to monofolate is a zinc dependent enzyme (Tamura et al., 1978). So, it was proposed that zinc therapy be accompanied by folate therapy.

CONCLUSIONS

Zinc deficit was associated with the detected malformations, meaning that it could be an incriminate cause, as was demonstrated in animal experiments.

Starting from this premise, we think that zinc supplementation in periconceptional period has at least as much reasons existing for the prevention of malformations recurrence.

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SELENIUM ENRICHMENT OF CROPS

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ABSTRACT

This paper reviews progress that has been made in the development of foliar supplementation practices, and compares responses to those from other forms of selenium (Se) use. By application of new, environmentally safe formulations and technological processes on were produced crops with optimal content of Se. Benefit from increased natural Se content in crops were tested on volunteer that consumed wheat naturally enriched with Se and its products. Obtained data showed that increased average daily Se intake of 18 µg induce after 6 week in blood and hair of volunteers, due to high availability of Se from wheat, increase of Se concentration that is comparable or even higher than increase obtained after consumption 100 µg Se/daily as Se-enriched bakery yeast with vitamins A, C and E for 2 months.

Key words: Selenium enrichment, crops, foliar sprays, selenium availability

INTRODUCTION

Before 40 years Klaus Schwarz identified that selenium (Se) protects against hepatic necrosis. This led to the demonstration that Se along with vitamin E protected against white muscle disease in sheep and goats, exudative disease in poultry, mulberry heart disease in pigs and many other disease problems in domestic animals and fowl, and to the understanding that it was an essential micronutrient (Oldfield, 1990).

Subsequently, Se was utilized in China to prevent the endemic cardiomyopathy, Keshan disease (KD), bone and joint disease, Keshan-Beck-Syndrome (KBS), although other factors, including viral agents, play a role in KB and KBS pathogenesis (Tan and Huang, 1991; CAS, 1990). Recent research indicates that Se is a versatile protecting agent against a variety of biologic and abiologic pathogenic agents, including certain viruses, toxic heavy metals, ozone, ionizing radiation, mycotoxins and industrial cancer causing compounds. It also positively affects the functions of the cellular or humoral immune system. Se exerts its protective effects by direct or indirect mechanisms. A direct mechanism of protection is the detoxification of mercury through, for example the formation of mercury selenide-protein complexes. Indirect protective mechanisms operate by way of inducing the biosynthesis of glutathione peroxidases, thioredoxin reductases or the other selenoenzymes in target organs. Se in appropriate genetic forms and dosages is also effective in the treatment of a variety of chronic and life-threatening acute conditions (Schrauzer, 1998).

The latest results shows that Se is also essential for some micro-organisms and plants. In plants such is wheat, maize, rape, soybean, garlic and mushrooms it has an antioxidant role, by the enzymatic mechanism of glutathione peroxidase,

which is similar to that in human and animal organisms (Hou et al., 1994; Xue et al., 1993a; Xue et al., 1993b, Wagner et al., 1998).

Worldwide millions of acres of land have been identified as too low in Se concentration to produce crops containing adequate levels to support normal animal and human health. Therefore Se supplementation has become widely adopted as a livestock production practice and partially in a human health improvement.

Selenium may be given orally or parenterally, and in the case of ruminants, it can be placed in the fore-stomach in heavy, slow-release boluses. It has been clearly shown that an appropriate increase of natural Se content in crops food/feed is the best way to avoid Se deficiencies. Techniques available for this are: soil application, foliar sprays and seed treatment Se techniques (Kivisaari and Vermeulen, 1994).

The dominant forms of Se in most soils and waters are selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}). Most crops take up Se from soil more easily in the selenate form than in the selenite form, whereas leaves absorb more easily the selenite form of Se. Other factors relevant for their absorption level are crop species and part of the plant sampled (Djujic et al., 1998).

Se fertilization has been effectively employed in two countries, Finland and New Zealand, and has been carefully monitored there for over a decade. In Finland, this practice was adopted because of concerns for human health, whereas in New Zealand it was motivated by deficiency problems with livestock, which are an important part of the country's economy. Even soil applications are not equally a good method as foliar spraying, due to better fitting with actual agricultural practice and due to problems with variable results in practice, fertilization is today the most popular method for improving Se status in crops.

In our studies the main emphasis was placed upon the effect of selenite form of Se foliar sprays on crop Se uptake.

MATERIALS AND METHODS

To increase the Se content of crops produced in low Se areas, combinations for foliar spraying with selenite-Se forms that has proved to be effective in raising Se concentration in crop species was used alone or together with urea (Djujic et al., 1998). Foliar sprayed crops with Se were: wheat (*Triticum spelta*), maize (*Zea mays*), barley (*Hordeum sativum*), oats (*Avena sativa*), rye (*Secale cereale*), soybeans (*Glycine hispida*), sunflower (*Helianthus annuus*), beans (*Phaseolus vulgaris*), livestock peas (*Pisum sativum*), garlic (*Allium sativum*), onions (*Allium cepa*), potatoes (*Solanum tuberosum* var. *white*), cabbage (*Brassica oleracea*) and spinach (*Spinacia oleracea*).

The stages of plant development that were, on the basis of our previous results (Milovac et al., 1998) and unpublished data, considered as suitable for foliar enrichment with Se were used for crop treatments and their efficiency tested in field experiments at various localities from 1995 to 1998. After harvesting, treated and non-treated (control) plant samples were dried, thoroughly ground and mixed to obtain representative samples for investigation. Samples of cultivated topsoil (0-30cm) were before planting and after harvesting collected from the fields and used for ecological effects studies.

By means of resin fractionation technique the water soluble Se amino acids were separated from selected crops and in them Se determined (Gissel-Nielsen G. and Gissel-Nielsen M., 1973).

Crop and soil samples were wet ached and in them Se was determined by vapor hydride generation atomic absorption spectrometry (Djujic, 1996).

RESULTS AND DISCUSSIONS

The results obtained for total Se content in different types of cultivated topsoil (Table 1) showed that average Se content for all soil types of 176 $\mu\text{g/kg}$ is within the range for Se in the Serbian soils: 39 - 440 $\mu\text{g/kg}$ (Maksimovic and Djujic, 1998), about 50 % less than average Se content in plow layer: 400 $\mu\text{g/kg}$ (Kabata-Pendias and Oendias, 1984), and more than 60 % less than upper limit of 500 $\mu\text{g/kg}$ for Se deficient soils (Mayland et al., 1989). Values obtained for total Se content are close to the lower value of 200 $\mu\text{g/kg}$ that separates low Se from Se sufficient soils in the USA (Leveskue, 1964) and the reference value for risk assessment of soil Se potential deficiency of 125 - 175 $\mu\text{g/kg}$ for the Keshan disease and Keshan Back disease affected areas in China (Tan et al., 1998).

Table 1. Total and available Se content in different top soils

Top soil type	Total Se, $\mu\text{g/kg}$	Available Se, $\mu\text{g/kg}$
Hydromorphic black soil	160 - 175	18 - 28
Chernozem salinized	134 - 188	22 - 27
Chernozem limeless	110 - 220	21 - 26
Chernozem calcarous	120 - 220	16 - 26
Chernozemlike meadow soil	140 - 265	18 - 28
All soils	110 - 265	16 - 28

Data obtained for amount of available Se in analyzed top soils (Table 1) showed that percentage of Se extractable with 1M NH_4OAc varied from 16 - 28 % of the total Se. The pH of these soils which is from 7.4 - 7.7 explain the relatively high amounts of extractable Se. Tan et al. (1998) found that water soluble Se in different top soils varies from 1.07 - 6.69 %. Although methods used for "available" Se determinations are not the same, evidently the content of available Se in our topsoils are higher. Perhaps the most important reason for explaining why endemic diseases do not occur in some areas of Serbia with low total soil Se and why we have endemic disease called "Balkan endemic nephrophaty" in some areas with acidic soil is lower extractable Se content.

The Se concentration in crops grown on the previously analyzed topsoil (Table 2) illustrate that Serbia is a low selenium area (Table 2). The mean Se content in wheat and soybean was close to the values that has been suggested for distinguishing Se-deficient from Se-sufficient areas (EGAS, 1981), whereas the obtained value in maize was about 50% lower. A comparison of our results for Se level in wheat with other results showed that Se content in wheat is higher than in Se deficient area in China: $0.012 \pm 0.008 \mu\text{g/kg}$ (Tan and Huang, 1991) with Keshan and Keshan-Beck diseases, or in Finland before addition of Se to multiminerall fertilizers: 9 $\mu\text{g/kg}$ (Kumpulainen, 1989), similar as in Belt with low Se in Central China: 20.1 $\mu\text{g/kg}$ (Tan & Huang, 1991), Switzerland (Kumpulainen, 1989), Germany

(Bruggemann et al., 1996), Scotland (Bruggemann et al., 1996) and Sweden (Kumpulainen, 1989). Se content in maize is slightly higher than in Se-deficient area in China: $0.008 \pm 0.003 \mu\text{g/kg}$ (Tan and Huang, 1991) with Keshan and Kashin-Beck diseases, lower than in Belt with low Se in Central China: $16.1 \mu\text{g/kg}$ (CAS, 1990) and in low-Se areas in USA: $25\text{-}38 \mu\text{g/kg}$ (Combs G.F. and Combs S.B., 1986).

Table 2. Se concentration in Serbian-grown crops

Crop	Part	Se ($\mu\text{g/kg}$)	Crop	Part	Se ($\mu\text{g/kg}$)
Wheat	grain vegetative tissue	20 - 30 11 - 16	Beans	seed vegetative tissue	5 - 9 3 - 6
Maize	kernel vegetative tissue	9-14 7 - 17	Livestock Peas	peas pod	18 - 23 12 - 16
Barley	grain vegetative tissue	7 - 9 4 - 8	Garlic	corm vegetative tissue	23 - 41 14 - 25
Oats	grain vegetative tissue	8 - 13 6 - 11	Onions	corm vegetative tissue	6 - 23 3 - 21
Rye	grain vegetative tissue	10 - 14 6 - 11	Potatoes	tuber vegetative tissue	4 - 9 6 - 10
Soybeans	seed pod vegetative tissue	9 - 15 5 - 14 9 - 18	Cabbage	leaves	5 - 11
Sunflower	peeled seed vegetative tissue	4 - 11 5 - 10	Spinach	leaves	3 - 7

Our previous investigation showed that fertilization with selenate, although inducing higher rising of Se content in crops than in the case of fertilization with selenite, was, in comparison with foliar application of selenite, a less efficient method of adding Se compared to soil application. These results indicate that, in our case, to raise Se concentration in grain of wheat and maize to the required level, we need about 4 times more Se/ha as selenate or 10 times more Se/ha as selenite than for foliar application (Milovac et al., 1998). The undesired effect is that fertilization with selenite and selenate induced changes in the extractibility of some essential elements from soil (Djujic et al., 1995), whereas foliar application induce significant changes, only in plant Se content. Having in mind the obtained efficiency by foliar application and facts that Se is absorbed very easily through the leaves, as well as that time of foliar application was important for Se level and distribution in the plant, we continued with testing of foliar application of Se on Serbian crops important for human and animal nutrition in field experiments on various localities. The main results are given in Table 3.

Field studies have shown that just a few grams (6 to 12) of Se/ha were sufficient to reach the desired Se level in most crops. Spraying of wheat, maize, barley, oats, rye, sunflower, beans, livestock peas, cabbage and spinach with selenite resulted in greater deposition of this element than application to the soil (Djujic et al., 1998; Whanger et al., 1998). The obtained Se concentrations were higher in the grain, seed or peas of crops than in the vegetative tissues, what is opposite when fertilization with selenate is applied. In that case Se concentrations were generally higher in the vegetative tissue than in

grain (Milovac et al., 1998; Djujic and Milovac, 1998; Gupta and MacLead, 1998). Spraying of cereals such as wheat, maize, barley, oats or rye with selenite in combination with urea (Djujic and Milovac, 1998) contributed that grain enrichment became higher than in those sprayed only with selenite.

The spraying leaves with Se was not effective in enriching root crops such as potatoes, onions or garlic. In them the Se concentrations were higher in the vegetative tissues. Thus for most efficient enrichment of these bulb plants Se must be added to the soil.

Table 3. Concentrations of Se ($\mu\text{g/kg}$) in Serbian-grown crops after foliar application of selenite with or without urea

Crop	Part	Se ($\mu\text{g/kg}$)	Crop	Part	Se ($\mu\text{g/kg}$)
Wheat	grain	91-126	Beans	seed	85-109
	vegetative tissue	62-81		vegetative tissue	53-64
Maize	kernel	86-110	Livestock peas		88-123
	vegetative tissue	54-72	peas	pod	62-86
Barley	grain	97-131	Garlic	corm	38-47
	vegetative tissue	78-118		vegetative tissue	123-156
Oats	grain	88-106	Onions	corm	32-41
	vegetative tissue	55-87		vegetative tissue	78-114
Rye	grain	90-114	Potatoes	tuber	26 -38
	vegetative tissue	76-102		vegetative tissue	98-108
Soybeans	seed	112-134	Cabbage	leaves	87-114
	pod	65-84			
	vegetative tissue	59-75			
Sunflower	peeled seed	66-79	Spinach	leaves	98-122
	vegetative tissue	54-72			

Experiments have shown that foliar sprayed selenite is metabolized practically immediately to Se-amino acids and translocated as such in wheat, maize, barley, oats, rye, soybean seed, beans seed and livestock peas. In sunflower, onions, garlic, cabbage and spinach the mayor seleno-compound is Se-methyl-selenocysteine (Wanger et al., 1998).

Our further investigation selenium naturally enriched weat showed that: main form of Se in grain is Se-methionine, as well as that applied treatment lessen moderate Cu deficiency in grain and increase yield by about 10-15% (Djujic et al., 1998). The wheat products obtained after milling contained from 3,5 to 6 times more Se and same or even better other quality parameters than products from non supplemented wheat (Fig. 1).

On the basis of the obtained results we expected that the average daily intake of by wheat products in Serbian population, instead 7 μg /daily (10% of the RDA) would became about 4 times higher than in 1992-1997 period (30% of the RDA). To confirm our calculations We tested the efficiency of diet which contain Se naturally enriched wheat and its products on young healthy volunteers. Results of this testing are presented in Figure 2 (Djujic et al., 1998).

Analysis of average weekly Se intake in duplicate diet samples showed that diet contains from 12 - 35 μg /person per day more Se., as well as that Se

concentrations in plasma, erythrocytes, hair and urine indicates that availability of Se from diet is high in all volunteers. Comparison of obtained results for Se in volunteers that consumed during 6 week food naturally enriched with Se that contained about 18 μg Se more daily, with results obtained for Se in volunteers that for 2 month consumed 100 μg Se daily as Se-enriched bakery yeast in combination with vitamins A, C and E ("Ferxevit"), showed that similar, even higher Se increase in obtained in investigated tissues. The value of Se naturally enriched wheat and its products on the basis of obtained results can be judged as the highest.

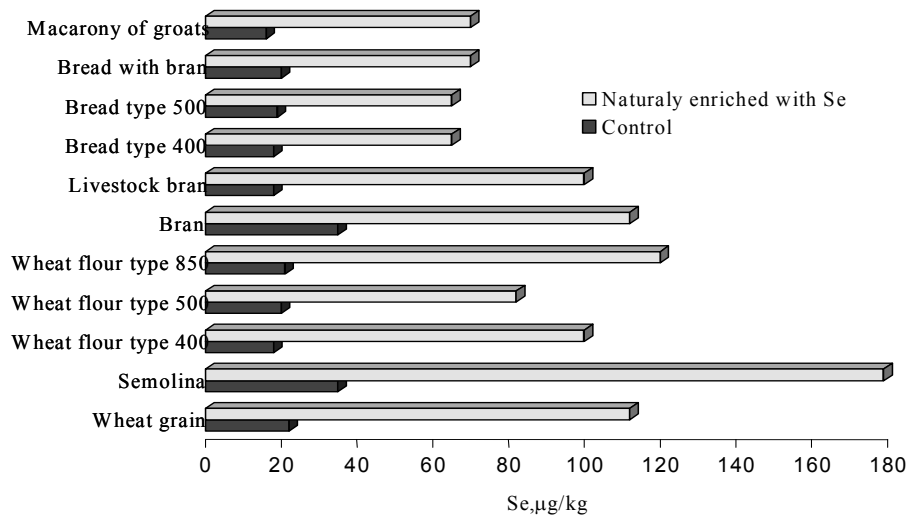


Figure 1. Se concentration in wheat and its products

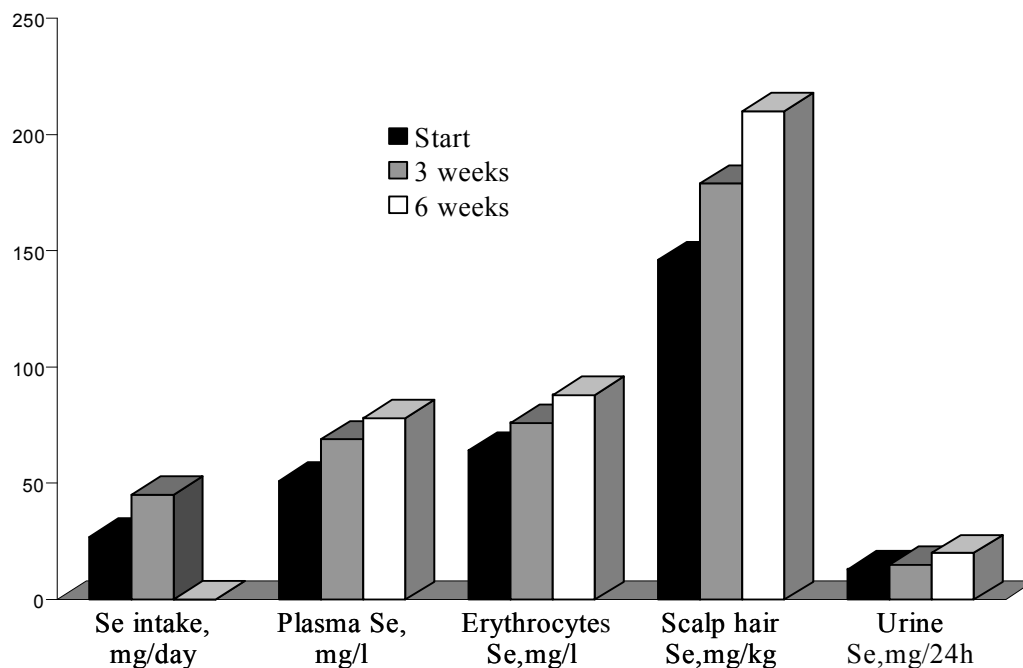


Figure 2. Dietary Se intake and its levels in volunteers

Having in mind that Se-methionine is the most available and less toxic form of Se for man and animals, as well as that average consumption of wheat and other cereals by our population and livestock is high, and discovering that dietary Se-methionine was effective as hemopreventive agent in colon cancer induction, reduction of the metachronous adenomas and many other pathological stages, we recommend foliar application of crops that as main form contains Se-methionine, as safe, economically acceptable and the most effective way of increasing the Se level in humans and animals.

Se-methylselenocystein, that is main Se form in vegetables has been shown that is the most effective in the reduction of chemically induced mammary tumors in rats. These results suggest that the consumption of Se enriched vegetables by humans will also reduce the incidence of cancer and that vegetables enriched with Se in close future we should consume to reduce our chances for cancer.

CONCLUSIONS

The application of new, environmentally safe, formulations and technological processes for production of highly valuable agricultural crops - bio-food with optimal content of selenium (YU patents application No. 483/97 and 484/97) and other trace elements in order to regulate their balance in humans and animals in endangered areas (areas with confirmed deficiency of Se or other trace elements on the basis of previous investigation), gave expected results.

Enriching of crops with Se through addition of selenite to the foliar sprays is a cost-effective way to improve the nutritional value of crops. Se enriched crops may possibly create new markets and improve the profitability for crops growers by providing a value-added product for the consumers to purchase.

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PLASMA AND INTRACELLULAR MAGNESIUM STATUS IN ALCOHOLISM AND ESSENTIAL HYPERTENSION

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ABSTRACT

In 30 healthy subjects, 19 patients with a steatosis and 14 patients with a cirrhosis of the liver due to chronic alcoholism plasma and total intracellular magnesium concentrations were determined. Cellular measurements were performed in red blood cells. In controls and patients with steatosis there was no significant difference in plasma magnesium concentrations (0.98 ± 0.12 mmol/l versus 0.98 ± 0.18 mmol/l). Plasma magnesium concentrations in patients with cirrhosis of liver were measured 0.85 ± 0.22 mmol/l which was significantly decreases as compared to healthy subjects ($p < 0.05$). Intracellular magnesium concentrations were not found of significant difference in controls versus patients with a steatosis due to alcoholism (1.86 ± 0.14 mmol/l versus 1.83 ± 0.24 mmol/l). In drinkers with cirrhosis of liver intracellular magnesium levels were significantly lowered as compared to healthy subjects or to the steatosis patients (1.31 ± 0.23 mmol/l. $p < 0.001$). A subgroup ($n = 5$) of drinkers with cirrhosis of liver and essential hypertension showed plasma Mg^{2+} concentrations of 0.79 ± 0.31 mmol/l and erythrocyte Mg^{2+} concentrations of 1.25 ± 0.25 mmol/l being significantly reduced as compared to controls ($p < 0.001$) but being not significantly different to the cirrhosis of liver patients without essential hypertension (1.33 ± 0.25 mmol/l). The data presented here show that a cellular magnesium deficiency depends on the degree of the liver disease in chronic alcoholism. Furthermore cellular magnesium content is a better parameter when determining alterations in magnesium status in alcohol induced diseases of the liver than plasma magnesium concentrations, which can still remain in the normal range even in severe forms of cirrhosis.

Key words: magnesium status, alcoholism, essential hypertension

INTRODUCTION

Severe chronic alcohol ingestion is known to cause hypomagnesemia (Hsieh et al., 1995). Many attempts have been made to clarify the nature of the underlying defects. Typical reasons may be a magnesium deficient nutrition, malabsorption, increased intestinal or sweat excretion, hyperaldosteronism or disorder in catecholamine metabolism. Alcohol ingestion can also cause increased urinary excretion of magnesium and zinc in humans and animals (Lindeman et al., 1970) and may lead to tissue depletion of magnesium (Prasad et al., 1963).

Furthermore it is known that moderation of alcohol intake reduces blood pressure and leads to an increase in lowered cellular magnesium stores (Hsieh et al., 1995; Puddey et al., 1987; Saunders et al., 1981).

To test the hypothesis that depending on degree of the liver insufficiency the most severe forms of hypomagnesemia can be observed, in the present study the magnesium status in controls and drinkers with a steatosis and cirrhosis of liver was investigated, additionally a hypertensive subgroup cirrhosis of liver patients was studies.

As plasma magnesium concentrations in alcoholism have often been described, but constitute only less than 3 % of total body magnesium measurements were performed in red blood cells additionally in each patient.

Patients and methods

A number of 30 patients - non drinkers - served as controls (C) and 19 drinkers with a steatosis (S) and 14 drinkers with a cirrhosis of liver (CL) were studied. The diagnosis of liver disease was obtained from bioptic puncture. The mean alcohol consumption was 15.2 ± 2.8 g/day in the steatosis patients and 22.3 ± 3.4 g/day in the patients with cirrhosis of liver ($p < 0.05$).

Additionally a subgroup ($n = 5$) of essential hypertensive alcoholic patients with cirrhosis of liver was studied (blood pressure syst./diast.: $172.5 \pm 11.3/102.7 \pm 7.8$ mm Hg. means $\pm$ S.D.). The clinical data are shown in table 1.

Table 1: Clinical data of 30 healthy subjects (C), 19 patients with a steatosis (S) and 14 patients with cirrhosis of liver (CL) due to chronic alcoholism (means $\pm$ SD)

Specification	C	S	CL
BP (mm Hg. systolic/ Diastolic)	$115.1 \pm 9.7/82.7 \pm 7.6$	$128.6 \pm 10.8/90.5 \pm 6.1$	$148.7 \pm 6.6/94.3 \pm 8.4^*$
Serum creatinine (mg%)	1.04 ± 0.12	1.01 ± 0.14	1.09 ± 0.11
Age (yr)	47.5 ± 13.1	45.7 ± 11.9	50.8 ± 10.7
Sex (m/f)	15/15	11/8	8/6
Renal disease	-	-	-
Hyperlipidemia	-	13*	11*
Smoker	-	4	2

* As compared with C. $p < 0.05$

Plasma and total erythrocytic magnesium concentration were measured as Titanic yellow method (6). Statistical analysis was performed using ANOVA. Data are mean $\pm$ SD, p values below 0.05 were considered significant. The reported p value are 2 - tailed.

Results

The results are shown in table 2.

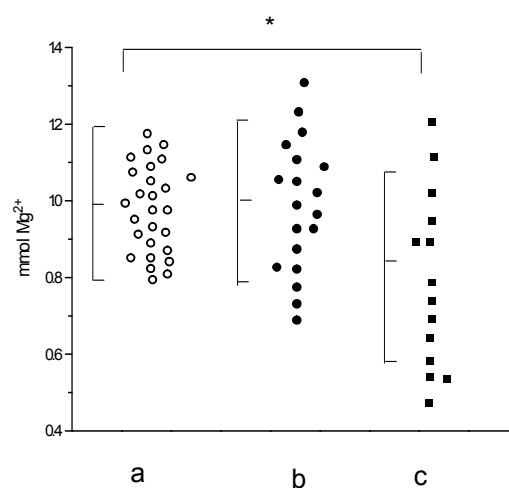
Table 2. Plasma and intracellular magnesium content in 30 controls, 19 patients with steatosis and 14 patients with cirrhosis of liver due to alcoholism (means $\pm$ SD)

Specification	Plasma Mg ²⁺ (mmol/l)	Intracellular Mg ²⁺ (mmol/l)
Healty subjects	0.98 $\pm$ 0.12	1.86 $\pm$ 0.14
Patients with steatosis	0.98 $\pm$ 0.18	1.83 $\pm$ 0.24
Patients with cirrhosis of liver	0.85 $\pm$ 0.22*	1.31 $\pm$ 0.23**

* As compared with healty subjects. $p < 0.05$

** As compared with healty subjects and patients with steatosis. $p < 0.001$

In controls and patients with steatosis there was no significant difference in plasma magnesium concentrations. But to chronic alcoholism plasma magnesium concentrations. but in patients with cirrhosis of liver due to chronic alcoholism plasma magnesium was significantly lowered as compared to the control group ($p < 0.05$). Nearly all plasma magnesium values were found in the reference range (0.8 - 1.2 mmol/l) (fig.1).

**Fig.1:** Plasma Mg²⁺ concentrations in controls (a), drinkers with steatosis (b) and cirrhosis of liver (c) (means $\pm$ S.D. * = $p < 0.05$).

Intracellular magnesium concentrations were not found of significant difference in controls versus patients with steatosis due to alcoholism. In drinkers with cirrhosis of liver intracellular magnesium levels were significantly lowered as compared to controls or steatosis patients; all magnesium values in this group were found below the reference range for red blood cell magnesium content (1.6-2.2 mmol/l) ($p < 0.001$) (fig.2).

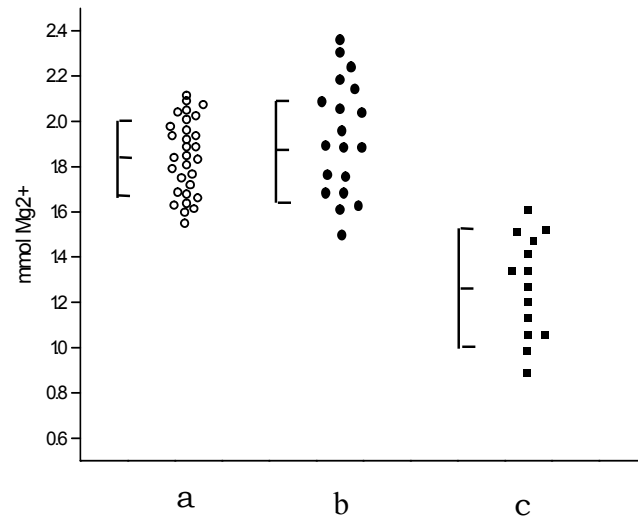


Fig.2: Intracellular Mg^{2+} concentrations in controls (a), drinkers with a steatosis (b) and cirrhosis of liver (c) (means $\pm$ S.D. * = S.D. $p < 0.001$).

In figure 3 plasma and intracellular Mg^{2+} concentrations of essential hypertensive and 9 normotensive drinkers with cirrhosis of liver are shown.

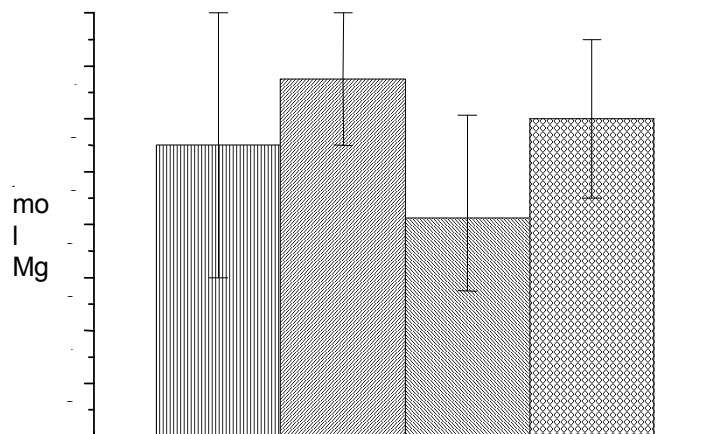


Fig. 3: Plasma (a,b) and intracellular (c,d) Mg^{2+} content in 5 hypertensive (b,d) and 9 nonhypertensive drinkers with cirrhosis of liver (a,c) (means $\pm$ S.D.).

Plasma Mg^{2+} concentrations were found in the normal range in both groups (0.79 ± 0.31 versus 0.87 ± 0.20 mmol/l).

Intracellular Mg^{2+} content was significantly lowered in both groups as compared to controls ($p < 0.001$) but being not significantly different in the hypertensive versus the nonhypertensive drinkers with cirrhosis of liver (1.25 ± 0.20 versus 1.33 ± 0.25 mmol/l).

There was no correlation between plasma or intracellular Mg^{2+} concentrations or blood pressure values in the studied groups.

DISCUSSIONS

Severe chronic alcohol consumption is known to cause hypomagnesemia (Hsieh et al., 1995) and is often associated with arterial hypertension. Many attempts have been made to clarify the nature of the underlying defects.

Typical reasons may be a magnesium deficient nutrition, malabsorption, an increased intestinal or sweat excretion, hyperaldosteronism or disorder in catecholamine metabolism. Alcohol ingestion can also cause increased urinary excretion of magnesium and zinc in humans and animals and may lead to a tissue depletion of magnesium (Prasad et al., 1963; Saunders et al., 1981).

In a recent study Martell et al. (1997) investigated the alcohol intake in 1024 normotensives and essential hypertensives and its relation with plasma lipids (Martell et al., 1997; Puddey et al., 1987).

They concluded in the population studies both the alcohol intake and the proportion of heavy drinkers were significantly higher in the hypertensive versus the normotensive group. Total cholesterol and triglyceride were also higher in the hypertensive versus the normotensive group.

It is known that the moderation of alcohol intake reduces blood pressure, although the exact mechanism has not been yet established.

Hsieh et al. (1995) studied the effects of alcohol moderation on blood pressure and intracellular cations in mild essential hypertension and documented a fall in mean blood pressure correlated positively with the reduction in weekly alcohol consumption (Hsieh et al., 1995).

They described also an increase in intraerythrocyte Mg^{2+} concentration after a reduction in alcohol consumption. In the presented study here, we found plasma Mg^{2+} concentrations in the normal range in steatosis and liver cirrhosis patients due to a chronic alcohol consumption. Intracellular Mg^{2+} concentrations were found significantly decreased in the drinkers with a cirrhosis of liver as compared to controls, the normal range or patients with a steatosis ($p < 0.001$).

Additionally we found significantly decreased intracellular Mg^{2+} values as well in the hypertensive as in the nonhypertensive drinkers with cirrhosis of liver. But we could not find any significant difference between these two groups (figures 2 and 3).

For assessing total body magnesium status intracellular magnesium levels are a better parameter than plasma magnesium levels.

CONCLUSIONS

The data presented here show that an intracellular magnesium deficiency depends on the degree of the liver disease in chronic alcoholism.

Furthermore intracellular magnesium content is a better parameter when determining alterations in magnesium status in alcohol induced diseases of the liver than plasma magnesium concentration, which can still remain in the normal range even in severe forms of liver cirrhosis. A further negative effect of essential hypertension in drinkers with cirrhosis of the liver on Mg^{2+} status was not noted in our

study. The negative Mg^{2+} status in hypertension with alcoholic diseases of the liver seems to be dependent of the severity of the liver damage and the daily amount of alcohol.

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METALLIC COMPOSITION OF UROCONCREMENTS AND CRYSTALLOGRAPHIC ASPECTS

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ABSTRACT

Metals are important constituents of the human body and participate in many essential metabolic processes. They enter in the organism by absorption from intestine, respiratory tract and skin. A part of them is accumulated and the remainder is excreted in the urine. In the lithogenic mechanism and especially of the mechanism of uroconcrements formation the concentration of urinary metal cations influences the crystallization process. In the microcrystals (called also "starters" or "primers") appearance mostly calcium ions are involved. Crystallographic analysis of uroconcrements permitted to found out the main metallic ion in various urolithiasis type. It was established that mainly six metallic ions (i.e. Ca, Mg, Na, K, Zn, Fe) are present in the composition of various crystals. Calcium was found in whewellite, weddellite, brushite, monetite, whitlockite crystals. Magnesium is the main metal ions in struvite, newberryite, bobbierite, hannayite, dittmarite crystals. Sodium is a component of sodiumdihydrogenurate, monohydrate, potassium of potassium-hydrogenurate. More rare are the hopeite crystals with zinc and humboldtin crystals with iron. Crystallographic studies on uroconcrements revealed similitude with mineralogical data and in this context the present paper approaches the problem of urolithiasis from mineralogical point of view.

Key words: uroconcrements – crystallographic peculiarities

INTRODUCTION

In the last decades the frequency of urolithiasis increased both in adults and in children. More than 80% of them are idiopathic and only a small proportion have recognisable abnormalities (e.g. hyperparathyroidism, renal tubular acidosis, disorder of oxalate metabolism).

Nowadays is known that metals play an important role in the etiopathogeny of urolithiasis. The human urine, considered as a metastable solution, contains beside nitrogenous compounds, proteins, polysaccharides, organic acids, inorganic anions and inorganic cations (Na, K, Ca, Mg, Zn, Cu, Mn, Fe). All these ions participate in the lithogenesis processes which, initially, lead to the microcrystals and thenafter to the uroconcrements formation. The concentration of metals in uroconcrements is different dependent on the type of urolithiasis (Eusebio and Elliott, 1967; Drăgan et al., 1981; Garban et al., 1981; Kohri et al., 1988; Daranyi et al., 1995; Massey and Whiting, 1995).

Animal models demonstrated an interaction between cells and crystals and they have suggested a correlation between cellular damage and crystal interaction. It is considered that the crystals preferentially attach to the cells (urothelium) that

have lost their integrity and is one of the major events in the formation of the kidney stones (Mandel, 1994).

1. BIOGENESIS OF MICROCRYSTALS AND URINE CONCENTRATION

The identification of the uroconcrements composition is of interest both for the fundamental researches and for the applicative ones (Williams and Chisholm, 1976; Gârban et al., 1981). The most frequently used methods in the study of urolithiasis composition are: X-ray diffraction, infrared spectroscopy (IRS); thermal analysis (TA); X-ray spectroscopy; atomic absorption spectroscopy (AAS) a.o. (Smith et al., 1981; Dragan et al., 1981; Achilles et al., 1995).

As the material present in urolithiasis is mostly crystalline, crystallographic analysis may give more information. Crystals have typical morphologies and therefore they can be recognized and identified by means of polarizing microscope (an optical examination). But, the most satisfactory technique for identification of crystalline components is that of X-ray diffraction which permits to determine the structure of crystal, to recognize their textures and to establish the growth direction of crystals etc.

2. MINERALOGICAL PECULIARITIES OF CRYSTALS

The formation and growth of crystals and, in time, of that of concrements in the urinary tract is not yet elucidated. In this context crystallographic studies of urolithiasis may bring information about some involved processes.

2.1. Texture of crystalline compounds

The texture of urolithiasis is variable. This may be conglomerates of fine- or coarse-grained powders, aggregates of single crystals or mixtures of several textures. In many there are laminated bands of fine-grained material often showing radial striations. Cystine, weddellite, brushite and struvite often crystallise as single crystals, while whewellite, uric acid, uric acid dihydrate, ammonium acid urate, apatite and xanthine occur frequently as fine-grained powders (Sutor, 1984).

Studies on the texture of urolithiasis by means of X-ray diffraction had shown an orientated growth of most constituents of calculi, whatever their texture is a common phenomenon. The crystals of a component are not randomly arranged but have grown with a certain amount of regularity so that they have an orientation relationship with respect to one another. This orientated growth is such that they have the same prominent crystallographic axis approximately along the radial direction of the stone, perpendicular to the preceding band of material, but they are randomly orientated about this axis. Orientated growth is responsible for the radial striations in calculi but it is not always obvious or visible to the eye.

By X-ray diffraction numerous crystalline compounds were found in urolithiasis which were classified into the following five groups to simplify discussions on composition: uric acids, urates, oxalates, phosphates (calcium and magnesium) and others (includes cystine, xanthine and any unidentified material).

In table 1 there are presented the main metallic components of various types of urolithiasis, the mineralogical name of compounds as well as the corresponding chemical formula.

Table 1. Classification of uroconcrements according to the main metallic component

Metal	Mineralogical name	Compound	Formula
Ca	Calcit, vaterit, aragonit	Calcium carbonate	CaCO_3
	Monohydroxycalcite	Calcium carbonate monohydrate	$\text{CaCO}_3 \cdot \text{H}_2\text{O}$
	Whewellite	Calcium oxalate mono- hydrate	$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$
	Whedellite	Calcium oxalate dihydrate	$\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
	Hydroxyapatite	Calcium phosphate	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
	Carbonate-apatite (dahllit)	Calcium phosphate carbonate	$\text{Ca}_{10}(\text{PO}_4\text{CO}_3)_6(\text{OHCO}_3)_2$
	Brushite	Calcium hydrogen phosphate dihydrate	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$
	Monetite	Calcium hydrogen phosphate	CaHPO_4
	Whitlockite	Tricalcium phosphate	$\text{Ca}_3(\text{PO}_4)_2$
	--	Octacalcium phosphate pentahydrate	$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$
	--	Calcium hydrogenurate	$\text{CaC}_5\text{H}_3\text{N}_4\text{O}_3$
Mg	Struvite	Magnesium ammonium phosphate hexahydrate	$\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$
	Newberryite	Magnesium hydrogen phosphate trihydrate	$\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$
	Bobierite	Trimagnesium hydrogen phosphate octahydrate	$\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
	--	Trimagnesium phosphate pentahydrate	$\text{Mg}_3(\text{PO}_4)_2 \cdot 5\text{H}_2\text{O}$
	--	Trimagnesium phosphate 22-hydrate	$\text{Mg}_3(\text{PO}_4)_2 \cdot 22\text{H}_2\text{O}$
	Hannayite	Trimagnesium phosphate diammonium octahydrate	$\text{Mg}_3(\text{NH}_4)_2\text{H}_4(\text{PO}_4)_4 \cdot 8\text{H}_2\text{O}$
	Dittmarite	Trimagnesium phosphate diammonium monohydrate	$\text{Mg}(\text{NH}_4)_2\text{H}_4(\text{PO}_4)_4 \cdot \text{H}_2\text{O}$
Na	--	Sodium dihydrogenurate monohydrate	$\text{NaC}_5\text{N}_4\text{O}_3 \cdot \text{H}_2\text{O}$
K	--	Potassium hydrogenurate	$\text{KC}_5\text{H}_3\text{N}_4\text{O}_3$
Zn	Hopeite	Zinc phosphate tetrahydrate (hexahydrate)	$\text{Zn}_3(\text{PO}_4)_2 \cdot 4\text{H}_2\text{O}$
Fe	Humboldtite	Iron oxalate dihydrate	$\text{FeC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$

Calcium was found in whewellite, weddellite, brushite, monetite, whitlockite crystals. Magnesium is the main metal ions in struvite, newberryite, bobbierite, hannayite, dittmarite crystals. Sodium is a component of sodiumdihydrogenurate, monohydrate, potassium of potassiumhydrogenurate. More rare are the hopeite crystals with zinc and humboldtite crystals with iron. There are no main component metals in cystine, xanthine and uric acid, uric acid dihydrate kidney stones.

2.2. Epitaxy in uroconcrements

Orientated crystal growth is an important factor in the deposition of most constituents, and epitaxy, or the orientated growth of one compound on another, may play an important role in the formation of stones of mixed composition.

Epitaxy is a crystal-growth phenomenon. It involves the orientated growth or deposition of one crystal, the deposit, on other, the substrate, with a near geometric fit between the respective networks that are in contact (Sutor, 1984).

The condition for epitaxy to occur may be expressed in the following way: the sides of the network on the surface of the substrate are designed ps and qs and those of the deposit, pd and qd . These are translation vectors which are calculated from the unit-cell dimensions of the material given for stone constituents.

GENERAL CONCLUSIONS

Studies concerning crystallographic aspects of urolithiasis allow a wider approach of the bioinorganic mechanism of concrements formation. In this framework the investigation of the crystals in urine may have a prognostic character or diagnostic guide line in the case of spontaneous elimination of uroconcrements fragment. This applicative aspect is of interest for the urological clinic.

Also, the crystallographic studies recognize the structure and texture of simple (e.g. oxalic, phosphatic) and/or mixed (e.g. oxalates-phosphates; urate-oxalates) composition of uroconcrements.

Extracorporeal shock wave lithotripsy (ESWL) having as result the elimination of uroconcrement fragments is considered an other applicative domain in which the study of microcrystals is of great importance. Results obtained by such studies could offer a helpful guide-line for the metaphylaxy of urolithiasis.

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STUDIES ON THE TOXICITY OF CADMIUM AND BENZO(A)PYRENE

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ABSTRACT

Polycyclic aromatic hydrocarbons and heavy metals are among the most ubiquitous environmental pollutants. Lower or higher levels of them are found in water, air, soil and also in food. Benzo(a)pyrene - usually noted by the acronym B(a)P - is a polycyclic aromatic hydrocarbon is the most often found cancerogen in our food. The compounds of the heavy metal cadmium (Cd) are widespread in soil, water, air and food.

The aim of the study was the assessment of possible toxic effects of benzo(a)pyrene and cadmium iodine on the experimental animals (Wistar strain rats organism).

Our results revealed that: low doses of benzo(a)pyrene and cadmium iodine introduced in the animal organisms caused kidney damage; cadmium alone caused changes in hepatic indices, on spermatogenesis, and on hematological indices; benzo(a)pyrene introduced alone did not produce toxicity.

Key words: Polycyclic aromatic hydrocarbons, heavy metals

INTRODUCTION

Polycyclic aromatic hydrocarbons and heavy metals are among the most ubiquitous environmental pollutants. Lower or higher levels of them are found in water, air, soil and also in food.

Benzo(a)pyrene (BP) – a polycyclic aromatic hydrocarbon is the most often found cancerogen in our food. According to the data of International Agency of Cancer Research, benzo(a)pyrene belongs to the group 2A of cancerogens, i.e. , to substances which are cancerogenic to animals and most likely- to man (Uleckiene, 1993). In Lithuania the level of benzo(a)pyrene in foodstuffs fluctuates from 0,05 mg/kg in bread to 1,00 mg/kg in unrefined sunflower -seed oil. In contaminated areas the level of BP in food may be as high as several tens of mg/kg (e.g. 50,0 in red beet) (Dagiene and Milukaite, 1992).

The compounds of a heavy metal cadmium (Cd) are widespread in soil, water, air and food. The level of cadmium in biosphere, especially in those areas where industry is developed, especially non-ferous metal industry and where the traffic is heavy, is constantly increasing (Zakrzewski, 1991). The principal sources of cadmium pollution in Lithuania are the following: fuel (coal and oil) combustion products, combustion products of rubbish and various wastes, industrial (industry of cement and phosphates fertilizers), emissions, emissions in the working environment – at the works of welding and soldering. From these pollution sources cadmium reaches the soil and ground waters. Through this chain cadmium gets into food. By the data of Republican Nutrition Centre, in some foodstuffs the level of cadmium exceed the

MAC (in pork liver, sea fish, semolisia, carrots and potatoes) (Kranauskas and Tomkiene, 1996). It is found that the dietary uptake of cadmium is the highest (90–96%) among nonsmokers. According to the data of World Health Organization, in many European countries the average daily intake of cadmium with drinking water and food per man (not smoking) is $1\mu\text{g}$ and $10\text{--}25\mu\text{g}$, accordingly. In Lithuania the MAC for drinking water is 0.003 mg/l and for raw stuffs and foodstuffs-ranges from 1.0 mg/kg in the leaves or 0.3 mg/kg in cattle and poultry subproducts to 0.01 mg/kg in beef, butter, curd products, pork. It means that the daily dose of cadmium per man is equal to 0.001 mg/kg .

The aim of study : assessment of possible toxic effect of benzo(a)pyrene and cadmium iodide on the animal organism.

MATERIALS AND METHODS

Experiment were carried out in accordance with Methodical Recommendations 407 of OECD Guidelines for Testing of Chemicals. For tests there were used 80 Wistar line male rats of 6–7 weeks of age, with $100\text{--}120\text{ g}$ weight at the baseline level.

In the short-term test the following doses were tried: 0.0000015 mg/kg of benzo(a)pyrene, 0.001 mg/kg of cadmium. The experimental animals were fed with the substances under study for 28 days (5 times per week). There were 5 groups : group 1 received cadmium iodide, group 2 – benzo(a)pyrene, group 3 Benzo(a)pyrene and cadmium iodide, group 4 – tap water, group 5 – rape-seed oil. Each experimental group consisted of 10 animals. The level of cadmium in the standard feed was 0.025 mg/kg , in drinking water no cadmium was found. During the trial there were performed clinical observations of animals (behaviour, skin, eyes, intake of food and water, gain in body mass). In the case of diseases out of humanity, the animals were decapitated. At the end of the trial, the state of the organism was evaluated according to the indices of the nervous system, kidneys, liver and hematologic indices. The gonadotoxic effects on male rats was assessed according to the indices of spermatogenesis.

The data obtained were prossed statistically using Student's t – test. The results were considered significant when $p < 0,05$.

RESULTS AND DISCUSSIONS

The short term results of the repeated oral toxicity test showed that the toxic effect on different indices of the functional state was not uniform while being exposed to separate substances or their combinations. Under exposure to these substances the indices reflecting the most of all the function of liver and kidneys were changing (Table 1).

Table 1: Biochemical indices of rats under exposure separate substances (benzo(a)pyrene – 0.0000015 mg/kg and cadmium – 0.001mg/kg) or their combinations

Indices	Control		Benzo(a)-pyrene	Cadmium salt	Benzo(a)pyrene + cadmium salt
	H ₂ O	oil			
Serum glutamic-pyruvic transaminase U/L	139.5±6.3	179.9±10.9	198.0±10.0	113.6±4.6 p< 0.002	152.5±8.3
Serum glutamic-Oxalo-acetic Transaminase U/L	94.2±8.07	179.14±3.8	167.13±4.59	107.7±10.7	139.2±8.1 p< 0.001
Alkaline phosphatase U/L	48.6±4.2	69.8±7.2	28.22±3.7	75.99±5.3 p<0.001	58.3±5.0
Serum creatinine µmol/l	104.7±9.1	83.4±9.9	74.7±11.4	58.2±11.0 p<0.002	262.2±24.8 p<0.001
Urine creatinine mmol/l	7.3±1.3	5.1±0.5	7.8±0.8	5.1±0.8	2.3±0.5 p<0.002
Serum urea mmol/l	13.3±0.5	7.4±0.6	5.9±0.4	9.1±0.3 p<0.001	8.2±0.4
Urine urea mmol/l	286.9±40.2	731.8±81.1	327.1±63.3 p<0,002	260.9±27.3	417.9±75.1 p <0.05
Urine hipuric acid mg/ml	7.5±1.1	25.4±2.2	26.1±0.5	19.4±2.5 p<0.001	11.5±0.6 p< 0.001
Urine excreted daily ml	3.1±0.4	3.8±0.9	5.8±1.1	7.6±1.5 p<0.01	4.2±1.2
Urine specific weight	0.9917±0.005	1.0069±0.005	1.0057±0.005	0.9982±0.003	1.0048±0.003

For the assessment of liver pathology, blood enzymes are very important, some changes may be caused even by low doses of chemicals. In blood of the animals who received cadmium a significant increase in alkaline phosphatase was found (56%), it shows that the enzymic function of the liver has been affected. Statistically significant reduction in the serum urea level shows its disturbed synthesis in the liver. There was observed a statistically significant increase in the relative weight of the spleen.

The decreased amount of hipuric acid (55%) in the urine of the animals who received doses of benzo(a)pyrene and cadmium may be evaluated as a disorder in the protective liver function when liver cells are affected and they are not capable to render harmless the introduced sodium benzoate into the organism and to synthesize hipuric acid. In the same group of animals there were found significant changes in the urine and serum. Increased serum level of creatinine (214%) showed that the impaired activity of the renal function characteristic of acute and chronic disease of Kidneys. Meanwhile the decreased amount of creatinine and urea in the urine showed disturbance in the renal secretion function.

Analysis of the hematologic indices revealed blood alterations among the animals who received cadmium salt. Reduction was found in blood hemoglobin concentration (g/l) and in the count of erythrocytes ($10^{12}/l$): 144.0 ± 1.0 and 4.3 ± 0.3 (control) and 129.0 ± 2.5 and 3.8 ± 0.1 (experimental group), $p < 0.001$. It implied the signs of anemia.

The indices of the nervous system were not changed with the introduction of separate substances as well as their combinations.

Among the indices of spermatogenesis, the number of spermatozooids in the suspension of the testicle epidymis has decreased significantly from 41.2 ± 3.9 (control) to 16.0 ± 1.9 (experimental group), $p < 0.0001$, although their viability has increased ($p < 0.02$). For the animals who received benzo(a)pyrene with calcium salt the relative weight of spermatid vesicles has fallen down ($p < 0.001$) and the relative mass of the testicle epidymis has grown. ($p < 0.001$).

All the statistically significant changes obtained did not exceed (>2.5) the physiological standard limits.

CONCLUSIONS

On this basis of the study results the following conclusion can be made: at the level of low doses the introduction of benzo(a)pyrene and cadmium iodide into the organism of animals have caused damage to the function of kidneys. Cadmium iodide alone has caused changes in the hepatic indices, those of spermatogenesis and in the some hematologic indices. The statistically significant changes obtained did not exceed the physiological standards limits. At the same time no toxicity signs were produced by benzo(a)pyrene.

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AGRICULTURAL USE OF IRRADIATED SEWAGE SLUDGE

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ABSTRACT

The designers considered that the cheapest solution for sludge disposal was its application on agricultural land. Two field experiments with irradiated and non-irradiated sewage sludge were organised on a Haplic Phaeozem, at Teleorman Agricultural Research Station, using oats and maize as test crops. No significant modifications of chemical characteristics of soil occurred after application of low doses of sludge (up to 400% of recommended N rate, i.e., about 20 tonnes/ha) within 2 years period. The percentage of nitrogen from fertilisers (Ndff) which is used by oat crops was reduced (4.1-4.9%). An important percentage (11.1-27.7%) of nitrogen came from sewage sludge (Ndfss). The total heavy metal contents of soil were not subjected to significant increases under the influence of sludge applied. Plants heavy metal contents did not increase too much, so that their quality as animal fodder did not diminish. Even for low values of nitrogen recovery, sludge fertilisation is favourable, being a method to efficiently use an otherwise waste material, its fertilising potential and to alleviate environmental pollution.

Key words: sewage sludge, ¹⁵N, utilisation degree of nitrogen, heavy metals

INTRODUCTION

In Roumania there are large quantities of sewage sludge resulted from municipal waste water treatment plants that are not used and just cover important areas, polluting the environment. The designers considered that the cheapest solution for sludge disposal was its application on agricultural land. In this way, the shift of important areas of land from agricultural use was avoided, and the organic matter and nutrients in sludge were efficiently used.

To be applied on agricultural lands, sewage sludge should meet the following demands:

- not to be a source of contamination with pathogenic agents; methods used in Romania for sludge sterilisation are: mesophilic anaerobic digestion and lime stabilisation of liquid sludge; in other countries, many other methods are used, e.g.: pasteurisation, incineration, composting, pyrolysis, wet oxidation, thermophilic aerobic digestion, irradiation, etc. (Baraldi, 1997);
- not to contain pollutants over the maximum allowable limits (Hall et al., 1992; International Atomic Energy Agency - IAEA, 1994);
- to be applied according to the application technologies (Environmental Protection Agency - EPA, 1983,1984).

An adequate monitoring system of environmental factors quality should be implemented within the areas where sludge is applied (Davies, 1983). Research

previously carried out by International Atomic Energy Agency (IAEA) demonstrated the effectiveness of ionising radiation in the inactivation of the pathogenic micro-organisms and protozoa parasites (IAEA, 1994;1997).

MATERIALS AND METHODS

Two field experiments with irradiated and non-irradiated sewage sludge, on a Haplic Phaeozem, at Teleorman Agricultural Research Station, Drăgănești Vlașca, using oats and maize as test crops, were organised. The experiments were set up in the field according to a complete randomised block design with 10 treatments (T_1 : 100 kgN/ha from 1% a.e. ^{15}N labelled fertiliser; T_2 : 20 kgN/ha from 10% a.e. ^{15}N labelled fertiliser; $T_3 - T_6$: non-irradiated and respectively $T_7 - T_{10}$: irradiated sewage sludge equivalent to 100, 200, 300, 400 kgN/ha + 20 kgN/ha from 10% a.e. ^{15}N labelled fertiliser) in 4 replicates.

Ammonium sulphate with 10% ^{15}N abundance and simple ammonium sulphate with 21% nitrogen were used as N sources for isotopic dilution.

The sewage sludge used in the experiments was taken from the waste water treatment plant of Pitești. The irradiation of the sludge was done in the High Activity Gamma Irradiation Station (SIGMA) from the Institute of Nuclear Research-Pitești. SIGMA uses as sources ^{60}Co , produced by "IZOTOP" from the former USSR. The sludge was irradiated being packed in airtight polyethylene bags. All samples were irradiated under the same conditions. The radiation dose absorbed by sludge samples was 4.95 KGy.

The sewage sludge had the following average chemical characteristics: 2.39% total nitrogen, 336 mg/kg $\text{NH}_4\text{-N}$, 70% moisture content, pH 6.80, 0.34% P, 0.35% K, 0.48% Na, 1.19% Ca, 0.35% Mg, 141 mg/kg Cu, 979 mg/kg Zn, 177 mg/kg Pb, 14 mg/kg Co, 41 mg/kg Ni, 321 mg/kg Mn, 462 mg/kg Cr, 11 mg/kg Cd. By comparing the heavy metal contents with the maximum admissible limits for agricultural use of sewage sludge in Roumania (10 mg/kg Cd, 50 mg/kg Co, 500 mg/kg Cr, 1200 mg/kg Mn, 100 mg/kg Ni, 300 mg/kg Pb and 2000 mg/kg Zn) it can be seen that this sludge could be used as organic fertiliser.

RESULTS AND DISCUSSIONS

No significant changes occurred in soil pH, in organic matter, total nitrogen, mobile phosphorus, mobile potassium, total and soluble heavy metal contents of soil after application of up to 400 kg/ha equivalent N from sludge (20 t/ha x 2 years).

Fertilisation with higher doses of sludge significantly influenced the yield of oats grains and straws (Table 1). The highest yield level was obtained at maximum dose of sludge treatment. Application of two consecutive annual doses of 20 tonnes sludge per hectare assured an yield level approximately equal to that given by 100 kgN/ha mineral fertilisation.

Data presented in Table 1 emphasise the effect that sewage sludge fertilisation has on the quantity of nitrogen taken up by oat grains. The fraction of nitrogen derived from fertilisers (Ndff) took low values (4.2-5.3%) at sludge treatments; an important percentage (7.7-25.7%) of nitrogen came from sewage sludge. Fraction of nitrogen derived from 100 kgN/ha mineral fertiliser was much higher (27.7%). With oat straws, again, low values for Ndff were obtained. The fraction of nitrogen derived from sewage sludge (Ndfss) varied between 11.4 and 28%.

Table 1: Effects of sewage sludge application on the yield and on the amounts of nitrogen taken up by oat plants

Characteristic		Mean values										HSD 5% Tukey
		T ₁	T ₂ *	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
Grain yield	t/ha	2.30 bcd	1.95 abc	1.72 a**	1.79 a	2.38 d	2.34 cd	1.84 a	1.91 ab	1.80 a	2.00 abcd	0.39
Total N	%	1.24 a	1.38 a	1.25 a	1.47 a	1.51 a	1.57 a	1.37 a	1.54 a	1.37 a	1.53 a	0.49
¹⁵ N abundance	%	0.64 a	0.92 b	0.87 b	0.83 b	0.86 b	0.80 ab	0.84 b	0.85 b	0.81 ab	0.77 ab	0.17
Ndff	%	27.7	5.7	5.3	4.9	5.2	4.5	4.9	5.0	4.6	4.2	
Ndfss	%			7.7	14.8	9.7	21.0	14.4	11.5	19.5	25.7	
Straw yield	t/ha	6.00 ab	5.46 a	6.50 bcd	7.20 d	6.92 cd	7.10 d	6.19 abc	6.15 abc	6.70 Bcd	7.25 d	0.83
Total N	%	1.37 a	1.31 a	1.38 a	1.39 a	1.45 a	1.61 a	1.39 a	1.49 a	1.53 a	1.54 a	0.30
¹⁵ N abundance	%	0.68 a	0.90 c	0.84 bc	0.81 abc	0.82 bc	0.78 bc	0.82 bc	0.81 abc	0.79 Abc	0.75 ab	0.13
Ndff	%	31.0	5.5	4.9	4.6	4.7	4.3	4.7	4.6	4.4	4.0	
Ndfss	%			11.4	16.9	14.6	22.3	15.6	16.7	20.8	28.0	
Ndfs	%	69.8	94.4	84.0	78.6	81.4	73.1	79.9	79.3	74.8	68.3	
Soil N yield	kg/ha	77.3	92.9	93.4	99.3	110.9	110.5	88.8	96.7	95.1	97.2	
Ndff	%	30.2	5.6	4.9	4.6	4.8	4.4	4.7	4.6	4.5	4.1	
Fertilizer N yield	kg/ha	33.4	5.5	5.5	5.9	6.6	6.6	5.3	5.7	5.7	5.8	
Ndfss	%			11.1	16.8	13.8	22.5	15.4	16.1	20.7	27.7	
Sewage sludge N yield	kg/ha			12.3	21.2	18.7	34.0	17.1	19.6	26.3	39.4	
Recovery of sewage sludge N	%			12.3	10.6	6.2	8.5	17.1	9.8	8.7	9.9	

* reference for treatments T₃-T₁₀;

** means within a row followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

The K, Ca, Mg, Co, Mn and Ni contents in oats grains and straws were not significantly changed. Table 2 illustrates the effect of municipal sewage sludge application on Cu, Zn, Pb and Cd concentrations in soil and in oat plants.

The low doses of sludge applied, the short period of time for experimentation and also the low Cu content of sludge are causes for the fact that no statistically significant changes in total and soluble Cu contents in soil occurred. Fertilisation with sewage sludge led to a tendency of accumulation of higher quantities of copper in oat grains and straws, but the level reached is low and within normal limits.

No significant accumulations of zinc in soil and oat grains as a result of fertilisation with sludge were evidenced. A tendency of increasing the content of zinc in oat straws occurred, but the level remained within normal limits.

Neither the total Pb content in soil was significantly changed by sewage sludge addition. However, a slight tendency of increasing the soluble Pb content in soil at higher

doses of sludge, and especially at irradiated sludge treatments occurred. Increasing of soil soluble Pb content led to increasing the Pb content in oat straws within treatments with higher doses of irradiated sludge. Increases of Pb contents were also evidenced in oat grains, the highest values corresponding to maximum dose of sludge applied. The level of Pb content in oat straws and grains do not raise problems concerning the quality of yield.

The Cd content in soil and in oat plants was not significantly changed under the influence of fertilisation with sewage sludge.

Table 2: Effects of sewage sludge application on concentrations of copper, zinc, lead and cadmium in soil and in oat plant

Characteristic			Mean values										HSD 5% Tukey
			T ₁	T ₂ *	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
Cu in plant	grain	mg/kg	4.9 ab	4.6 ab	4.3 a	5.9 bc	4.9 ab	5.7 bc	6.3 c	7.0 cd	7.9 de	8.6 e**	1.3
	straw	mg/kg	7.8 ab	7.5 ab	7.4 ab	9.0 bc	7.8 ab	11.3 c	5.8 a	5.8 a	8.5 b	8.9 bc	2.4
Cu in soil	soluble	mg/kg	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.3 a	0.1
	total	mg/kg	26 a	25 a	25 a	27 a	28 a	27 a	27 a	27 a	28 a	27 a	3
Zn in plant	grain	mg/kg	43 a	50 a	47 a	50 a	48 a	50 a	46 a	52 a	50 a	52 a	11
	straw	mg/kg	31 a	27 a	28 a	40 ab	37 ab	41 ab	37 ab	40 ab	43 ab	54 b	17
Zn in soil	soluble	mg/kg	0.2 a	0.2 a	0.3 a	0.3 a	0.3 a	0.2 a	0.3 a	0.2 a	0.3 a	0.4 a	0.2
	total	mg/kg	61 a	61 a	62 a	68 a	72 a	69 a	68 a	64 a	69 a	67 a	14
Pb in plant	grain	mg/kg	0.8 a	0.9 ab	0.8 a	0.8 a	1.0 ab	1.5 ab	1.3 ab	1.3 ab	1.5 ab	1.6 b	0.7
	straw	mg/kg	1.8 a	1.4 a	1.3 a	2.4 a	2.3 a	1.4 a	3.1 a	3.2 a	4.0 ab	6.0 b	2.7
Pb in soil	soluble	µg/kg	3.2 a	2.9 a	2.5 a	2.5 a	3.1 a	4.9 ab	7.4 bc	7.3 bc	7.2 bc	7.8 c	2.8
	total	mg/kg	60 a	58 a	57 a	59 a	58 a	59 a	57 a	58 a	63 a	67 a	20
Cd in plant	grain	mg/kg	0.2 a	0.1 a	0.2 a	0.2 a	0.2 a	0.3 a	0.2 a	0.3 a	0.3 a	0.2 a	0.2
	straw	mg/kg	0.2 a	0.2 a	0.2 a	0.3 a	0.3 a	0.2 a	0.2 a	0.2 a	0.2 a	0.3 a	0.1
Cd in soil	soluble	µg/kg	2.6 a	2.6 a	2.6 a	3.4 a	4.1 a	4.4 a	3.4 a	3.2 a	3.6 a	3.8 a	1.8
	total	mg/kg	1.4 a	1.5 a	1.4 a	1.6 a	1.3 a	1.2 a	1.2 a	1.1 a	1.2 a	1.3 a	0.5

* reference for treatments T₃-T₁₀; ** means within a row followed by the same letter did not differ at the 0.05 significance level using Tukey's honestly significant difference procedure.

The fertilisation with municipal sewage sludge led to significant grain maize yield increases (Table 3). No significant differences between the effects of fertilisation with irradiated and respective non-irradiated sludge were observed.

The nitrogen content of maize plant showed no significant differences depending on the dose of sludge applied.

Table 3: Effects of sewage sludge application on the yield and on the amounts of nitrogen taken up by grains and leaves of maize plants

Characteristic		Mean values										HSD 5% Tukey
		T ₁	T ₂ *	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	
Grain yield	t/ha	12.7	10.4	10.8	11.9	12.0°	12.2	11.1	11.6	12.4	12.0	1.6
		c**	a	ab	abc	abc	bc	abc	abc	bc	abc	
Total N	%	1.17	1.31	1.28	1.26	1.44	1.36	1.18	1.31	1.32	1.29	0.39
		a	a	a	a	a	a	a	a	a	a	
¹⁵ N abundance	%	0.06	0.13	0.12	0.11	0.12	0.11	0.13	0.12	0.12	0.11	0.04
		a	b	b	b	b	b	b	b	b	b	
Ndfs	%	94.0	98.7	95.6	87.1	88.6	82.5	97.9	90.2	91.0	87.9	
Soil N yield	kg/ha	139.7	134.4	132.1	130.6	153.1	136.9	128.3	137.1	149.0	136.1	
Ndff	%	6.0	1.3	1.3	1.2	1.2	1.1	1.3	1.2	1.2	1.2	
Fertilizer N yield	kg/ha	8.9	1.8	1.8	1.8	2.1	1.8	1.7	1.8	1.9	1.8	
Ndfss	%			3.1	11.7	10.2	16.4	0.8	8.6	7.8	10.9	
Sewage sludge N yield	kg/ha			4.3	17.5	17.6	27.2	1.0	13.1	12.8	16.9	
Recovery of sewage sludge N	%			4.3	8.8	5.9	6.8	1.0	6.6	4.3	4.2	
Leaves yield	t/ha	3.4	3.1	3.5	3.1	3.1	3.5	3.3	3.2	3.2	3.2	0.6
		a	a	a	a	a	a	a	a	a	a	
Total N	%	1.18	1.23	1.19	1.29	1.25	1.25	1.24	1.54	1.48	1.54	0.38
		a	a	a	a	a	a	a	a	a	a	
¹⁵ N abundance	%	0.11	0.20	0.19	0.15	0.14	0.11	0.19	0.16	0.16	0.14	0.09
		a	a	a	a	a	a	a	a	a	a	
Ndfs	%	89.2	97.9	89.3	73.4	68.6	53.7	91.6	76.7	76.2	65.2	
Soil N yield	kg/ha	35.8	37.3	37.2	29.4	26.6	23.5	37.5	37.8	36.1	32.1	
Ndff	%	10.8	2.1	1.9	1.6	1.5	1.2	2.0	1.7	1.7	1.4	
Fertilizer N yield	kg/ha	4.3	0.8	0.8	0.6	0.6	0.5	0.8	0.8	0.8	0.7	
Ndfss	%			8.8	25.0	29.9	45.1	6.4	21.6	22.1	33.4	
Sewage sludge N yield	kg/ha			3.7	10.0	11.6	19.8	2.6	10.7	10.5	16.5	
Recovery of sewage sludge N	%			3.7	5.0	3.9	5.0	2.6	5.4	3.5	4.1	

* reference for treatments T₃-T₁₀;

** means within a row followed by the same letter did not differ at the 0.05 significance level using Tukey' honestly significant difference procedure.

The utilisation degree of nitrogen from the fertilisers (Ndff) applied at maize plants was low and slightly decreased with the dose of sludge applied.

The fraction of nitrogen derived from sewage sludge (Ndfss) in maize grains and leaves increased with dose of non-irradiated and irradiated sewage sludge. The contribution of soil, mineral fertiliser and sewage sludge to the amounts of nitrogen taken up by maize grains and leaves are also presented in Table 3.

No significant accumulations of phosphorus in soil and maize leaves, husks, cobs and grains as a result of fertilisation with sludge were evidenced. A slight tendency of decreasing the content of phosphorus in maize stem occurred.

Data provided by Table 4 point out that municipal sewage sludge fertilisation did not cause accumulations of total copper in soil. A slight tendency of increasing the content of Cu in maize leaves, husks and grains occurred. Municipal sewage sludge application did not lead to the increase of zinc content in soil and in various organs of maize plants; tendency of increasing the soluble zinc content in soil with increasing irradiated and non-irradiated sewage sludge doses can be observed (Table 4).

Table 4: Effects of sewage sludge application on concentrations of copper, zinc, lead and cadmium in soil and in maize plant.

Characteristic			Mean values										HSD 5%
			T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	Tukey
Cu In plant	Grain	mg/kg	2.8 a**	2.9 ab	2.9 ab	3.0 ab	3.2 ab	3.1 ab	3.4 ab	4.1 ab	4.1 ab	4.2 b	1.3
	Cob	mg/kg	4.0 a	3.7 a	4.3 a	4.3 a	4.8 a	5.1 a	4.7 a	5.0 a	5.4 a	5.4 a	1.9
	Husks	mg/kg	1.6 a	1.7 ab	1.9 ab	2.0 ab	2.1 ab	2.2 ab	1.9 ab	2.0 ab	2.2 ab	2.4 b	0.7
	Leave	mg/kg	14 abc	12 a	13 ab	15 abc	15 abc	16 abc	14 abc	16 abc	19 bc	20 c	6
	Stem	mg/kg	2.3 a	2.2 a	2.3 a	2.3 a	2.4 a	2.5 a	1.9 a	2.2 a	2.2 a	2.3 a	0.8
Cu in soil	Solubl	µg/kg	4.5 a	4.6 a	4.5 a	4.7 a	4.5 a	4.4 a	2.9 a	3.2 a	3.9 a	3.8 a	1.9
	Total	mg/kg	21 a	22 a	24 a	23 a	24 a	25 a	24 a	23 a	24 a	25 a	5
Zn in plant	Grain	mg/kg	33 a	33 a	35 a	33 a	35 a	36 a	34 a	36 a	33 a	36 a	14
	cob	mg/kg	28 a	26 a	28 a	29 a	32 a	33 a	29 a	29 a	31 a	32 a	8
	husks	mg/kg	25 a	26 a	26 a	27 a	28 a	30 a	24 a	27 a	30 a	31 a	8
	leave	mg/kg	44 a	43 a	46 a	50 a	54 a	64 a	52 a	65 a	66 a	69 a	33
	stem	mg/kg	43 a	42 a	41 a	43 a	44 a	39 a	45 a	46 a	48 a	55 a	26
Zn in soil	solubl	µg/kg	40 a	39 a	68 ab	142 abc	135 abc	253 c	81 ab	117 abc	148 abc	201 bc	157
	total	mg/kg	56 a	54 a	55 a	56 a	59 a	61 a	61 a	61 a	63 a	65 a	14
Pb in plant	grains	mg/kg	2.2 a	2.2 a	2.3 a	2.3 a	2.5 a	3.2 a	3.1 a	3.5 a	3.6 a	4.0 a	1.9
	cob	mg/kg	2.9 a	2.8 a	3.0 a	3.1 a	3.3 a	3.4 a	3.0 a	3.1 a	3.3 a	3.4 a	1.4
	husks	mg/kg	1.5 a	1.4 a	1.5 a	1.6 a	1.8 a	1.9 a	1.3 a	1.5 a	1.6 a	1.6 a	1.0
	leave	mg/kg	1.1 a	1.0 a	1.1 a	1.0 a	1.3 a	1.4 a	1.1 a	1.2 a	1.2 a	1.4 a	0.7
	stem	µg/kg	513 a	50 a	512 a	568 a	607 a	630 a	501 a	542 a	569 a	603 a	242
Pb	solubl	µg/kg	6.7	6.4	5.6	6.2	5.8	6.2	6.0	5.9	5.3	5.6	2.3

CONCLUSIONS

1. No significant modifications of chemical characteristics of soil occurred after application of low doses of sludge (up to 400% of recommended N rate, i.e., about 20 tonnes/ha) within 2 years period.

2. Fertilisation with higher doses of sewage sludge significantly influenced the yield of oat grains and straws. The highest yield level was obtained at maximum dose of sewage sludge treatment. Application of two consecutive annual doses of 20 tonnes sludge /ha assured an yield level approximately equal to that given by 100 kgN/ha mineral fertilisation.

3. The percentage of nitrogen from fertilisers which is used by oat crops was reduced(4.1-4.9%), irrespective of irradiated or non-irradiated sludge application. However, an important percentage (11.1-27.7%) of nitrogen came from sewage sludge. Fraction of nitrogen derived from sludge is higher at irradiated than at non-irradiated sludge treatments.

4. Under the influence of treatments applied, the heavy metal (Cu, Zn, Pb, Cd) contents of oat plants suffered no such changes as to raising problems in connection with yield quality.

5. The fertilisation with municipal sewage sludge led to significant grain maize yield increases.

6. No significant accumulations of phosphorus in soil and maize leaves, husks, cob and grains as a result of fertilisation with sludge were evidenced.

7. For maize grains and leaves, the fraction of nitrogen derived from fertilisers (N_{dff}) took similar low values, for all either irradiated or non-irradiated sludge treatments. The values of nitrogen derived from sewage sludge (N_{dss}) increased with dose of non-irradiated and irradiated sewage sludge a subjected to significant increases under the influence of sludge applied. Soil content in soluble zinc had a tendency of increase in irradiated and non-irradiated sludge treatments.

8. No significant accumulations of heavy metals in maize plants were observed. A tendency of increasing the content of Cu in maize leaves, husks and grains occurred.

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INFLUENCE OF LITHIUM ON THE LIPIDIC COMPOSITION OF RAT BRAIN OPIOID RECEPTOR MEMBRANES AFTER PRE- AND POSTNATAL STRESS. ROLE OF MAGNESIUM

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ABSTRACT

Lithium, given to pregnant "control rats", increases the cholesterol, the phosphatidyl-ethanolamine and the phosphatidyl-serine levels and decreases the phosphatidyl-choline level. It seems that lithium has a "protective effect" on the lipidic composition of the brain opioid receptor membranes only against prenatal stress and only when it is given during pregnancy. Lithium, whatever the time of delivering to the mother does not avoid the effects of the postnatal stress. Lithium-magnesium interactions may be involved.

Keywords: pre- and postnatal stress - opioid receptor membranes - lithium - magnesium - lipids - fatty acids - rats.

INTRODUCTION

Lithium has been used to treat a multitude of somatopsychic disorders over the years. Its influence on the responses to stress has not been described in clinic. However, alterations in opioid receptors after chronic treatment with lithium have been suggested (Steengard-Pedersen and Schou, 1983).

Brain endogenous opioids have been implicated in the processes of both pre- and postnatal stresses but also in stress responses in general (Insel et al., 1990; Zagon and McLaughlin, 1989; Millan, 1981). These effects are mediated by membrane receptors, the sensitivity to lipids of which has been well documented (Medzihradsky, 1989). It is tempting to suggest that stress occurring at a time of high neuronal plasticity, will modulate either the lipidic environment or the lipidic composition of the receptor itself and so modify the function of the receptor.

Lithium effects may take place through alterations in the composition and/or the dynamic properties of the neuronal membrane (Lopez-Corcuera et al., 1988). Animal experiments suggest that Lithium may act by interfering with the metabolism of membrane lipids (Braham and Riddell, 1995). It is therefore possible that lithium counteracts the stress effects at the level of the lipidic composition of the receptor.

On the other hand, interactions lithium-magnesium have been described. In rats, lithium treatment causes a significant increase in the level of serum and brain magnesium (McEachron et al., 1982). Lithium presents chemical similarities to magnesium (Smith, 1977). It may compete with magnesium by removing it from its binding sites (Lipton, 1978). This competitive interaction has been shown in many systems (Wang et al., 1974).

Stress modifies magnesium serum concentration in rats (Cordova et al., 1990) and pigs (Szilágyi et al., 1997). Moreover, magnesium deficiency affects lipid

metabolism and membrane lipid physical properties (Rayssiguier et al., 1994.; Kisters et al., 1995).

It is therefore possible that, in case of stress, the lithium effects on the lipidic composition of the membranes may be modulated by subsequent modifications of the magnesium level.

Therefore brain opioid receptor membranes of rats submitted to pre- and postnatal stress were isolated. Lipid and fatty acids composition of these membranes was determined. The influence of lithium and the role of magnesium was studied.

MATERIALS AND METHODS

Experiments were performed on Wistar rats.

Placed with a male, statistically females are pregnant after 5 days. On the morning of gestation day 1, day 7, day 15, the females were exposed to a regimen of heat and restraint stress comprised of placing the female into a plexiglass restraint tube over which will be poised two 100 W flood lights. This stress procedure produced an ambient temperature within the restraint tube of approximately 38°C. Four 30 mn. stress sessions were conducted during 1 day.

Female "Lithium rats" received 1 mg/ml LiCl in their drinking water, during 3 weeks before pregnancy (1) or only during the pregnancy (2). They were then either exposed to the same regimen of heat and restraint stress as previously described, or left undisturbed for the duration of the pregnancy.

After birth, offsprings were raised by their biological mothers until the 25th day of postnatal life. After 1 month either they were sacrificed (prenatally stressed offsprings "PS-O" or prenatally stressed offsprings from mother receiving lithium before "PS-Li-O"1, or during pregnancy "PS-Li-O"2) or they were exposed to the same restraint stress and then sacrificed (pre- and postnatally stressed offsprings "P and PoS-O" or "P and PoS-Li-O"1, 2).

1st month old offsprings of non-stressed mothers were exposed to the restraint stress and sacrificed (postnatally stressed offsprings "PoS-O" or "PoS-Li-O"1, 2).

The results were compared to those obtained from offsprings of non-stressed mothers under lithium treatment ("Li rats"1, 2) or not under lithium treatment ("control rats").

Brain opioid receptor membranes were then isolated (Tocque et al., 1984). Rats were decapitated and the brains washed with bidistilled water until complete elimination of the blood. Brains were homogenized in 0.05M Tris-HCl, pH 7.4 at 4°C in the ratio 1:10 (w/v). Homogenates were centrifuged for 5' at 800 g. The supernatant was centrifuged for 30' at 40,000 g. The pellet was washed twice with the above mentioned buffer in the ratio 1:40 (w/v).

Proteins were determined according to Lowry et al., 1951.

The lipids of the membrane fraction were then extracted with chloroform-methanol 2:1 (v/v). Extraction was performed by 6x30" sonications with 30" between each sonication.

Lipids were separated by multiple two-dimensional thin layer chromatography (Pollet et al., 1978). Quantitative determination was performed by spectrophotometrical analysis (Pollet et al., 1978).

Fatty acid analysis was performed by GLC after methylation of the lipid extract (capillary column SE 30, temperature programming 10°C/mn 180°C-280°C) (Viani et al., 1991).

Serum lithium and magnesium were measured by Atomic Absorption Spectrophotometry in blood obtained after cardiac puncture with a plastic syringe containing sodium citrate 3.8% (w/v) in a ratio of 1 part to 9 parts of blood (Chiang et al., 1991).

The results expressed are the mean values $\pm$ SD.

RESULTS AND DISCUSSIONS

- After treatment with lithium salts by ad libitum intake in the drinking water, the plasma levels of Li^+ were 0.8 ± 0.12 mEq/l (mean of 15 animals);.

- Influence of lithium on the lipid composition of the brain opioid receptor membranes of:

1- Unstressed 1 month old rats from non stressed mothers under lithium treatment or from non stressed mothers without lithium treatment (Table 1)

Table 1. Influence of lithium on the lipid composition of the brain.

$\mu\text{mol/mg protein}$	control rats	"Lithium rats"(1)	"Lithium rats"(2)
Cholesterol	0.430 ± 0.100	0.430 ± 0.100	0.500 ± 0.100
Galactolipids (hexoses)	0.125 ± 0.050	0.120 ± 0.050	0.125 ± 0.050
% Cerebrosides	44.000 ± 1.000	42.000 ± 1.000	43.000 ± 1.000
Sulfatides	36.000 ± 1.000	38.000 ± 1.000	35.000 ± 1.000
Gangliosides	20.000 ± 1.000	20.000 ± 1.000	22.000 ± 1.000
Gangliosides (neuraminic acid)	0.020 ± 0.005	0.020 ± 0.005	0.020 ± 0.005
Phospholipids	0.710 ± 0.100	0.720 ± 0.100	0.830 ± 0.100
% PC	43.000 ± 1.000	43.000 ± 1.000	36 ± 1
PE	36.000 ± 1.000	36.000 ± 1.000	41 ± 1
PS	11.000 ± 0.700	11.000 ± 0.700	14 ± 0.7
PI	3.000 ± 0.400	3.000 ± 0.400	3 ± 0.4
Sph	5.000 ± 0.400	5.000 ± 0.400	4 ± 0.4
PA	1.000 ± 0.200	1 ± 0.2	1 ± 0.2
Cholesterol/Phospholipids	0.605	0.600	0.602

When lithium is given during the pregnancy (2), a slight increase of cholesterol and phospho-lipids can be observed in the lipidic composition of the brain opioid receptor membranes of offsprings of lithium treated non-stressed mothers. There also may be an increase in PE and PS and a decrease in PC.

PC: Phosphatidyl-choline, PE: Phosphatidyl-ethanolamine, PS: Phosphatidyl-serine, PI: Phosphatidyl-inositol, Sph: Sphingomyelin, PA: Phosphatidic acids.

2- After prenatal stress, post natal stress following or not prenatal stress (Tables 2, 3 and 4)

Table 2. Influence of lithium on the lipid composition of the brain after prenatal stress.

μmol/mg protein	"PS-O"	"PS-Li-O" (1)	"PS-Li-O" (2)
cholesterol	0.600±0.100	0.600±0.100	0.480±0.100
galactolipids (hexoses)	0.120±0.050	0.125±0.05	0.120±0.050
% Cerebrosides	43.000±1.000	44.000±1.000	43.000±1.000
Sulfatides	36.000±1.000	35.000±1.000	37.000±1.000
gangliosides	21.000±1.000	21.000±1.000	20.000±1.000
gangliosides (neuraminic acid)	0.020±0.005	0.020±0.005	0.020±0.005
phospholipids	0.680±0.100	0.710±0.100	0.800±0.100
% PC	45.000±1.000	45.000±1.000	35.000±1.000
PE	37.000±1.000	36.000±1.000	42.000±1.000
PS	8.000±0.700	8.000±0.700	14.000±0.700
PI	2.000±0.400	2.000±0.400	3.000±0.400
Sph	5.000±0.400	5.000±0.400	5.000±0.400
PA	1.000±0.200	1.000±0.200	1.000±0.200

Table 3. Influence of lithium on the lipid composition of the brain after post natal stress.

μmol/mg protein	P and PoS-O"	"P and PoS- Li-O" (1)	"P and PoS- Li-O" (2)
cholesterol	0.600±0.100	0.610±0.100	0.600±0.100
galactolipids (hexoses)	0.125±0.050	0.125±0.050	0.125±0.050
% Cerebrosides	44.000±1.000	42.000±1.000	43.000±1.000
Sulfatides	37.000±1.000	38.000±1.000	37.000±1.000
gangliosides	19.000±1.000	20.000±1.000	20.000±1.000
gangliosides (neuraminic acid)	0.020±0.005	0.020±0.005	0.020±0.005
phospholipids	0.720±0.100	0.700±0.100	0.720±0.100
% PC	45.000±1.000	44.000±1.000	44.000±1.000
PE	37.000±1.000	37.000±1.000	37.000±1.000
PS	9.000±0.700	8.000±0.700	9.000±0.700
PI	1.500±0.400	2.000±0.400	2.000±0.400
Sph	5.000±0.400	5.000±0.400	5.000±0.400
PA	1.000±0.200	1.000±0.200	1.000±0.200

$\mu\text{mol/mg protein}$	"PoS-O"	"PoS-Li-O" (1)	"PoS-Li-O" (2)
cholesterol	0.610 ± 0.1	0.600 ± 0.1	0.610 ± 0.1
galactolipids (hexoses)	0.130 ± 0.05	0.130 ± 0.05	0.130 ± 0.05
% Cerebrosides	45 ± 1	44 ± 1	46 ± 1
Sulfatides	34 ± 1	34 ± 1	33 ± 1
gangliosides	21 ± 1	22 ± 1	21 ± 1
gangliosides (neuraminic acid)	0.02 ± 0.005	0.02 ± 0.005	0.02 ± 0.005
phospholipids	0.700 ± 0.1	0.710 ± 0.1	0.720 ± 0.1
% PC	44 ± 1	44 ± 1	44 ± 1
PE	37 ± 1	37 ± 1	37 ± 1
PS	8.5 ± 0.7	8 ± 0.7	9 ± 0.7
PI	2 ± 0.4	2 ± 0.4	2 ± 0.4
Sph	5 ± 0.4	5 ± 0.4	5 ± 0.4
PA	1 ± 0.5	1 ± 0.2	1 ± 0.2

Compared to control rats (table 1), pre-and postnatal stresses (tables 2, 3 and 4) increase the cholesterol level but do not change the total phospholipids, the galactolipids and the gangliosides levels in the rat brain opioid receptor membranes. However, it seems that both stresses slightly decrease the PS and PI levels.

In case of prenatal stress, lithium seems to have a "protective effect" on the lipidic composition of the brain opioid receptor membranes only when it is given during pregnancy (2) (lipidic composition similar to the "Lithium rats 2" - table 1).

Lithium does not "protect" the membranes in case of postnatal stress following or not prenatal stress (lipidic composition similar to that observed in case of pre- or post natal stress without lithium - tables 3 and 4).

Table 5. Serum magnesium levels in prenatal stress (mM/l)

Control rats	Lithium rats 1	Lithium rats 2	"PS-O"	"P and PoS-O"	"PoS-O"	The serum magnesium level increases only when lithium is given
0.9 ± 0.18	1.0 ± 0.2	1.5 ± 0.2	1.0 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	

to control rats during pregnancy (2) or only when postnatal stress occurs after or not prenatal stress (Table 5).

Table 6. Serum magnesium levels in post natal stress (mM/l)

"PS-Li-O" (1)	"PS-Li-O" (2)	"P and PoS-Li-O" (1)	"P and PoS-Li-O" (2)	"PoS-Li-O" (1)	"PoS-Li-O" (2)
1.0 ± 0.2	0.9 ± 0.18	0.9 ± 0.18	1.6 ± 0.2	0.8 ± 0.18	1.7 ± 0.2

Under lithium treatment, the magnesium level is increased in the offsprings only when lithium is given during pregnancy (2) and only when postnatal stress occurs (table 6).

The effects of lithium on the brain opioid receptor membranes are observed with a lithium plasma level equivalent to the therapeutic level usually found in human (0,8 mEq/l). There are no signs of intoxication.

- Lithium given to pregnant “control rats” during pregnancy increases the cholesterol, PE and PS levels and decreases PC level in the brain opioid receptor membranes of offsprings. The cholesterol/phospholipids ratio, however, does not significantly change after lithium treatment (Table 1).

These results suggest that the membrane cholesterol content plays no important role in the alteration by lithium of the properties of the brain opioid receptor membranes. The increase in PE and PS content while PC content decreases (the more unsaturated phospholipid classes, the less unsaturated phospholipids) may however play a role. Similar results have been obtained in rat synaptic membranes after chronic lithium treatment (Lopez-Corcuera et al., 1988).

The fact that these results are observed only when lithium is given during pregnancy suggest that lithium may only act during the development of the foetus.

- It seems that lithium have a “protective effect” on the lipidic composition of the brain opioid receptor membranes only against prenatal stress and only when it is given during the pregnancy. After one month the lipidic composition of the offsprings is similar to that observed when lithium is given to unstressed mother during pregnancy.

We have looked at the lipidic composition of such offsprings after one year and compared it to 1 year old control rats (Table 7).

Table 7. Lipidic composition of the offsprings

μmol/mg protein	1 year old control rats	1 year old “Lithium rats” (2)
cholesterol	0.600±0.1	0.610±0.1
phospholipids	0.710±0.1	0.720±0.1
PC	44±1	42±1
PE	36±1	38±1
PS	10±0.7	11±0.7
PI	3± 0.4	3± 0.4
Sph	5±0.4	5±0.4
PA	1±0.2	1±0.2
Cholesterol/ phospholipids	0.845	0.847

This lipid composition is similar to that observed in the control rats. It means that the effects of the postnatal stress on offsprings of stressed mothers treated with lithium during pregnancy disappeared with the time.

The fact that the “protective effect” of lithium against prenatal stress occurs only when lithium is given to the mother during pregnancy confirms that lithium may only act during the development of the fetus.

Lithium, whatever the time of delivering to the mother, does not avoid the effects of the postnatal stress. These results suggest that both stresses involved different mechanisms.

There is extensive evidence for the correlation of membrane fluidity and physiological activity including membrane function (Kates and Manson, 1984; Aloia et al., 1988). Stress occurring at a time of high neuronal plasticity will, through changes in membrane lipid composition, modify membrane fluidity and therefore modify the function of the receptors. We have shown that whatever the stress, pre- or postnatal, the effects on the brain opioid receptor membranes lipid composition are the same. However the action of lithium is different depending of the nature of the stress.

This result suggests that either

- different opioid receptors are involved in the prenatal stress and the postnatal stress

- or the postnatal stress may not involved opioid receptors.

- or other factors such as the interactions between lithium and magnesium have to be considered.

Prenatal stresses does not modify the serum magnesium level of the offsprings from mother not receiving lithium (table 5). The serum magnesium level of the offsprings is increased only when lithium is administered during the pregnancy of their mother and only during postnatal stress (table 6). It is tempting to suggest that when postnatal stress occurs, the increased serum magnesium concentration will allow higher amount of magnesium to be present in the brain. Therefore, magnesium, displacing lithium from its site will prevent the "protective effect" of the lithium observed when only prenatal stress occurs

Further experiments are necessary to clarify these results.

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COMPLEX COMBINATIONS OF TRANSITIONAL METALS - POSSIBLE CHEMICAL MODELS OF NITROGENASE

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ABSTRACT

The biological and abiological reduction of dinitrogen supposes the existence of some MoFe_3S_4 type clusters linked by cisteinic S in the protein inside, $[\text{Fe}_3\text{O}_4(\text{H}_2\text{O})_{12}]^{2+/3+}$ respectively, in an iron hydroxide (model) or of some complex dinitrogen combination beside a powerful reducer. The dinitrogen reduction takes place in stages, by diazenase and hydrazenase for ammonia.

Key words: nitrogen fixation

INTRODUCTION

Although for a long time various objections had been raised against dinitrogen coordination during the last decades a new class of coordinative compounds of transitional metals with dinitrogen has been developed and diversified, many of them being particularly stable.

This new class coordinative compounds has a particular importance both from a theoretical and practical point of view as it could contribute to elucidating the mechanism of biological conversion of dinitrogen into ammonia and at the same time it could open new elaboration ways of some unconventional ways of ammonia synthesis under mild conditions of temperature and pressure.

The interest manifested by the specialists towards this reduction way of dinitrogen fixation has stimulated various researches and the publication of a large volume of specialized papers and monographies.

The area of the preoccupations connected to the formation of dinitrogen compounds and dinitrogen reactions, under mild conditions supposes the existence of two methods, and namely :

- the biological method of genetic modification which should enable the genes passing from the nitrogen fixation bacteria to other organisms;
- the developing of some catalytic systems which should enable the dinitrogen fixation, as far as possible, under usual conditions of temperature and pressure.

BIOLOGICAL FIXATION OF DINITROGEN

On the basis of some recent researches of biological fixation of atmospheric nitrogen and its conversion into ammonia, one has established that at the basis of the process there is a complex enzymatic system, known under the denomination of nitrogenase.

It is in the presence of this catalytic system that the breaking of the triple link $\text{N}\equiv\text{N}$ and NH_3 forming by hydrogen atoms linking, become possible. Structurally, the nitrogenase is a complex enzyme with molybdenum and iron content, consisting actually of two proteins:

- molybdoferredoxine (Figure 1), a bigger protein (molecular weight 180,000 - 300,000), in the structure of which there are 1-3 molybdenum atoms, 17-36 iron atoms and 14-28 free sulphur atoms, out of which 50% are engaged in the forming of some MoFe_3S_4 clusters. Of all the elements it is only the vanadium which can replace the molybdenum so that the protein activity be not lost whereas the presence of some elements such as Cd, Cu, Zn, Ni inhibits the process.

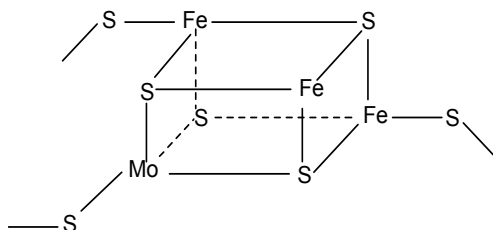


Fig. 1: Structure of molibdoferredoxine

- azoferredoxine (Figure 2), a smaller protein (molecular weight 40,000 - 60,000), in the structure of which there are 2-4 iron atoms and 1-4 sulphur atoms which make up one Fe_4S_4 cluster.

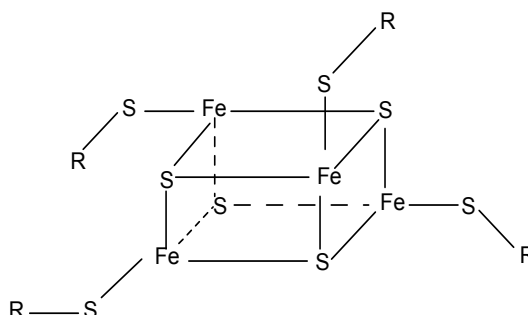


Fig. 2: Structure of azoferredoxine

The mechanism of biological fixation of dinitrogen has been elaborated by Schrauzer, Shilov and others. At present, one has accredited the idea that the dinitrogen reduction takes place on molybdoferredoxine, the process being

conditioned by the existence in the system of the monomagnesian salt of ATP, on the basis of a mechanism which includes diazene and hydrazine as intermediaries.

Actually, the dinitrogen reduction takes place in stages, through some species of the type N_2H_x ($x = 1-4$). In this process, the azoferredoxine has the role of electron conveyor between the reducing agent and the molybdoferredoxine which stocks them in the clusters of the type Fe_4S_4 . Here from they pass through the active centre to the formed ammonia is replaced by the dinitrogen and the process starts again.

CHEMICAL MODELS FOR THE REDUCTIVE FIXATION OF DINITROGEN

The first studies concerning the reductive fixation of dinitrogen have been carried out in aprotic media by means of the coordinative compounds of the transitional metals and they were due to Volpin, who developed a rich activity in this field. Mention should also be made of the papers of Shilov, Tamelen, Yamamoto and others.

The characteristic feature of these reactions is that they are produced in systems formed of a transitional metal complex unsaturated from a coordinative point of view, capable of complexing and activating the dinitrogen and a reducing agent which is strong enough to function as an electron source as compared to the activated dinitrogen. The reactions take place in organic solvents under normal conditions of temperature and pressure (Table 1).

Table 1 : Systems capable of the reductive fixation of dinitrogen in aprotic media and the yields in ammonia

Crt.no.	System	Reactives ratio	Solvent	Yield in NH_3 (moles NH_3 /g Ti)
1.	$[(\pi-C_5H_5)_2TiCl_2] + EtMgBr$	1:9	Ether	0.67
2.	$[(\pi-C_5H_5)_2TiCl_2] + Li$	Li excess	THF	0.75
3.	$[(\pi-C_5H_5)_2TiCl_2] + Li/Hg$	1:10	THF	0.77
4.	$[(\pi-C_5H_5)_2TiCl_2] + C_{10}H_8Li$	1:6	THF	0.96
5.	$[(\pi-C_5H_5)_2TiCl_2] + C_{10}H_8Na$	1:4.3	THF	0.90

No matter used system and the nature of the reducing agent, all the dinitrogen fixation reactions have at their basis a common mechanism with that presented in Figure 3, and which is developed in more stages.

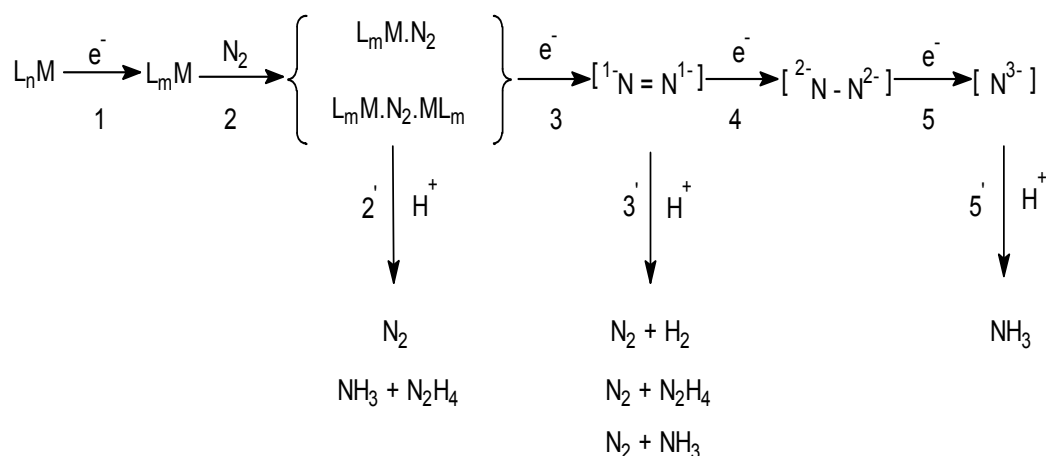


Fig. 3: Mechanism of reductive fixation reactions of dinitrogen in aprotic media.

Comparing the chemical systems capable of the dinitrogen fixation in aprotic media with the enzymatic ones, Volpin and Shur have ascertained that they have the following common features:

- the transitional metal represented by molybdenum or vanadium in the enzymatic reactions and by titanium, vanadium, molybdenum or others in the chemical ones, is comprised in the active centre;
- in both of the cases the dinitrogen is reduced to ammonia.

POSSIBLE MECHANISMS OF DINITROGEN REDUCTION ON SYSTEMS WITH Fe

The $Fe(OH)_2$ activity in the reductive fixation of dinitrogen is controversial. The system is, probably, the simplest of all the chemical systems capable of dinitrogen reduction. Schrauzer has published an article on the systems which release hydrogen of the $Fe(OH)_2$ aqueous suspension type and which react with many reducing substrates, including dinitrogen. The dihydrogen forming leads to elementary Fe forming, Fe_3O_4 respectively, by $Fe(OH)_2$ disproportioning :



For N_2H_4 and NH_3 forming, various mechanisms have been proposed. The elementary iron generated by $Fe(OH)_2$ disproportioning, is dispersed in such a way, as to be able to react with more reducing substrates. The dihydrogen releasing is inhibited by C_2H_2 , O_2 , CO , N_2 . The inhibition observed for the dihydrogen releasing from $Fe(OH)_2$ by unsaturated hydrocarbons is attributed to the interaction with elementary iron, generated in the reaction conditions. The C_2H_2 reduction mechanism, by Fe_n type clusters generated from $Fe(OH)_2$, is presented in Figure 4.

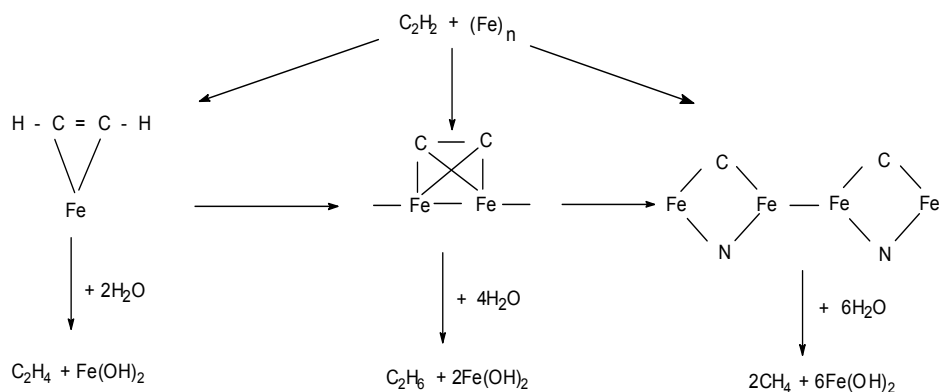


Fig. 4: Mechanism of C_2H_2 reduction by Fe_n clusters

The fact that dinitrogen inhibits, in a similar way, the dihydrogen releasing in iron suspension, suggests that this substrate is also reduced. This fact has been confirmed by experiences with N_2 enriched in $^{30}N_2$ as substrate and by direct colorimetric determinations of N_2H_4 and NH_3 . The reduction mechanisms, of dinitrogen by the Fe_n type clusters generated from $Fe(OH)_2$, are presented in Figure 5.

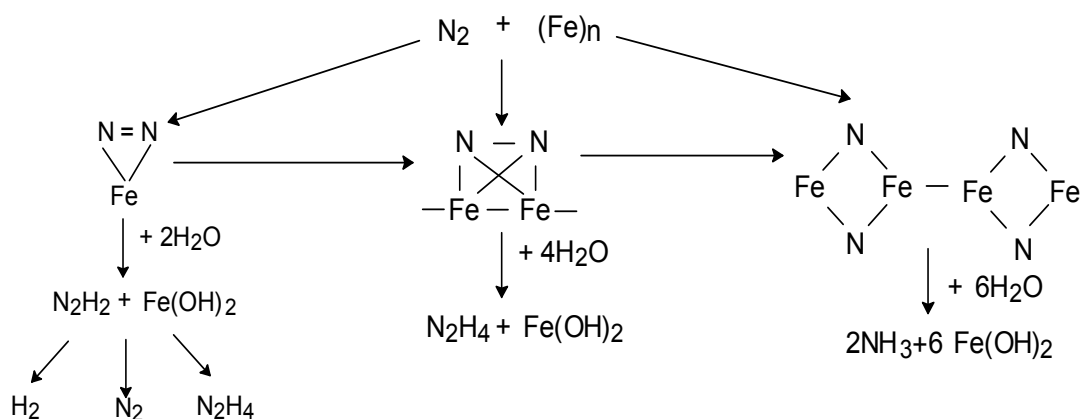


Fig. 5: Reduction mechanism of dinitrogen by Fe_n type clusters.

A number of experimental data concerning dinitrogen reduction in systems with $Fe(OH)_2$ are given in Table 2 and Figure 6.

Table 2 : Results concerning dinitrogen reduction in systems with $\text{Fe}(\text{OH})_2$
(room temperature and atmospheric pressure)

Crt. no.	pH value	Reaction duration(min .)	Concentration Fe (%) $\times 10^{-2}$	Solution colour	NH_3 moles/l $\times 10^{-5}$	N_2H_4 moles/l $\times 10^{-5}$
1.	12.5	20	1	Grey	3.97	3.2
2.	13.0	20	2	Grey	7.10	4.4
3.	13.0	20	2.2	Grey	7.90	4.8
4.	13.2	20	4	Grey	9.53	5.8
5.	13.2	20	10	Grey	5.55	2.7
6.	12.8	20	20	Grey	5.56	2.8

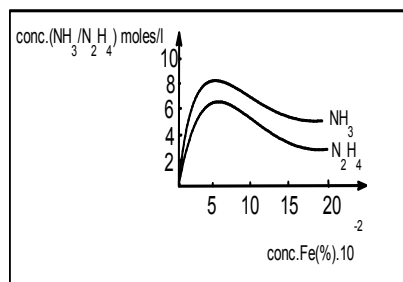


Fig. 6: NH_3 and N_2H_4 yield dependence on Fe concentration in the reaction mixture ($t^\circ = 20^\circ\text{C}$, $p = 1 \text{ atm.}$).

By similitude with a system in the specialized literature we could draw, for the possible structure of the precipitates and co-precipitates the following conclusions. It is very likely that a Fe^{2+} and OH^- ions organization respectively, exists in the form of cuboidal clusters in order that the stereochemical and energetical conditions for dinitrogen reduction be created. Identically as in the effects have been ascertained, the same supposition may be made for the Fe/O clusters (Figure 7).

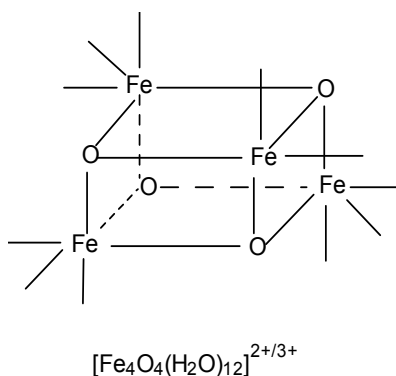
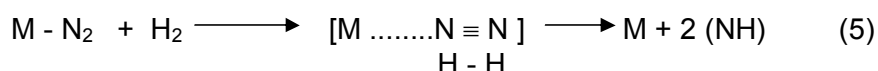
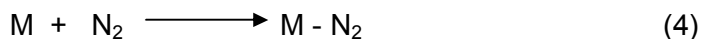


Fig. 7: Proposed model for the clusters of the type $[\text{Fe}_4\text{O}_4(\text{H}_2\text{O})_{12}]^{2+/3+}$.

CONCLUSIONS

1. The effected studies for elucidating the mechanism which is to be found at the basis of the biological and abiological conversion of dinitrogen have led to the conclusion that two stages seem to be important in this process, and namely: absorption and activation of dinitrogen and reduction of activated dinitrogen. For the systems of complex combination type, the two stages may be represented by the N_2 molecule coordination and subsequent splitting of the $N \equiv N$ molecule through the molecular hydrogen:



2. The possible mechanisms of dinitrogen reduction by the Fe_n type clusters generated from $Fe(OH)_2$ are presented in Figure 5 and they, as the experimental data existent at present, indicate an insufficient activity of N_2 molecule which still remains inert enough to Fe.

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DYNAMIC CHARACTERISTICS OF BIOLOGICAL PROCESSES IN RELATION WITH METALLIC IONS

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ABSTRACT

In living organisms transformations take place of a continue, interrelated, reversible or non-reversible manner. These transformations constitute the basis of the so-called dynamic characteristics of biological processes: metabolism, chronobiochemistry, homeostasis, homeorhesis. These processes will be discussed in relationship with metallic cationic bioelectrolytes. Metabolism – is defined as the complex assembly of biochemical interactions that are the basis of physico-chemical transformations of the compounds with exogenous and endogenous origin from the living matter. It is a dynamic characteristic of biological processes, which stays at the origin of biotransformations in the living organism. The metabolic processes reveal two aspects: the specificity and the universality. Bioelectrolytes from the group of the metallic cations undergo no metabolic transformations in ana- or catabolic processes but, being present, take part in the processes of biotransformation. Chronobiochemistry - is defined by the variation of the quantum of metabolites due to biochemical and physiological modifications that are statistically validated, which present a wavy, reproducible character in some frequency domains. In the case of metals one can observe important physiological and physiopathological differences during the circadian (K, Na, Ca), selenar (Fe) and circannual rhythms (Ca, Mg). Homeostasis – represents the capacity of the biologic system to maintain in constant limits an own status when environmental conditions vary continuously. In the case of an organism this signifies the maintenance in some limits of a “biologically normal” status. Metal homeostasis e.g. Ca, Mg, Na, K, Fe a.o. is relevant for the biomedical status of living matter. Their dyshomeostasis may lead to nutritional and endocrine diseases. Homeorhesis – is the capacity of an organism to achieve a “distable equilibrium” under the variations that appear in the internal environment. A characteristic example is given by the complex mother-conceptus. In this case too, the metallic bioelements play an important role during the processes of morphogenesis and intrauterine development of conceptuses (embryos and fetuses).

Key words: dynamic biological processes – metallic ions.

INTRODUCTION

Complex biochemical interactions take place in the living organism during which continuous exchanges of substance, energy and information with the outside environment take place.

After the sources of substance and energy it is considered that the organisms can be: photosynthesisant – which have as base of their activity the solar radiation and chemosynthesisant – which use the chemical energy stored in some chemical compounds of organic or inorganic nature.

The biotransformations produced in living organisms of continuos, inter -related, reversible or non-reversible manner, are the bases of the so-called dynamic characteristics of biological processes. Among these there are described: 1) metabolism; 2) chronobiochemistry; 3) homeostasis; 4) homeorhesis (Gârban, 1993 b).

As a result of the transformations that take place in living organisms, the own bioconstituents are formed, assuring on this way the perpetual morphological renewal. With regard to the structure, the bioconstituents belong to diverse classes of compounds: carbohydrates, lipids, protids, minerals. As to minerals, they can be of cationic nature, e.g.: Ca^{2+} , Mg^{2+} , Na^+ , K^+ , Fe^{2+} , Zn^{2+} etc. and anionic nature, e.g.: phosphates, carbonates etc. (Eichhorn, 1973; Anke et al., 1990-1998; Anonymous, 1997).

1. OVERVIEW ON THE DYNAMIC CHARACTERISTICS

The dynamic characteristic are presented in an integrative way – regarding the organism in its complexity and continuously related to cationic minerals of metallic nature (i.e. metallic ions). Evidently, in this case there will be presented various aspects regarding the biometals – metals usually present in living organisms (especially limited to the human organism).

Biom mineral compounds have many important roles in the organism: contribute in the processes of morphogenesis, being part of different tissues, mostly the sustaining ones; intervene as metabolic effectors (activators or inhibitors) in enzymatic reactions; maintain in physiological limits of the osmotic pressure of liquid medium in the living organism, denominated by Cl. Bernard (cited by Rhoades and Pflanzner, 1992) as “milieu interieure”; maintain of the normal oncotic (colloid-osmotic) pressure; former of buffer systems to regulate the acid-basic equilibrium of the organism; achieving of bioelectric systems of the transmembrane transport; bioconstituents of some chemical compounds (e.g. Co in vitamin B₁₂, Fe in heminenzymes, Zn in carbon anhydrase etc.).

It is reminded the fact that in the human organism there were also decelated metals with toxicogene potential as a consequence of environmental contamination (water, food, air).

Further on there will be presented diverse aspects regarding the dynamic characteristics of biological processes in relation with the metal ions.

Investigation of the dynamic characteristics of biochemical processes is extremely important for molecular biology, biochemistry, physiology, pharmacology a.o. In order to understand some general information regarding this subject is resumed.

1.1. METABOLISM

Morphological, physiological and biochemical peculiarities of living matter are achieved during some transformations specific for the various biochemical pathways which are defining for the metabolism.

In its assembly metabolism is a complex of physico-chemical transformations of chemical compounds (exogenous and endogenous) from the living organism.

As a biologic process, the metabolism is characterised by two phases: a) *catabolism* – the totality of the processes of physiological biodegradation of the macromolecules of exogen (food) and endogen (metabolites of the internal environment) origin; b) *anabolism* – the totality of physiological biosynthesis processes during which the biomolecules of the own organism are formed. An essential characteristic of the metabolism consists in the development of the biodegradation and biosynthesis reactions in steps, leading to intermediary products, which constitute the intermediary metabolism. The succession of interactions specific for the intermediary metabolism are characterized by the so-called metabolic pathways. These pathways can be catabolic, anabolic (differ by the number of enzymatic steps), anphybolic (identity of the number of steps in catabolism and anabolism). In the case of physiologic modifications there are used the terms catabolism or biodegradation, respectively anabolism or biosynthesis.

Intermediary products of a normal metabolism are called metabolites. Metabolic turnover of various biomolecules - characterized by the half time of the metabolites - shows differences related to the nature of the component and the morphofunctional peculiarities of the tissue. Table 1 shows the turnover of some components from various tissues of rats (Lehninger, 1980).

Table 1: Turnover of some tissular bioconstituents in rats

Tissue	Metabolite	Metabolic turnover (hours)
Liver	Total proteins	5 - 6
	Triacylglycerols	1 - 2
	Phosphoglycerolipids	1 - 2
	Cholesterol	5 - 7
	Glycogen	0.5 - 1.0
Brain	Triacylglycerols	10 - 15
	Phosphoglycerolipids	about 200
	Cholesterol	over 100
Muscle	Total proteins	30
	Glycogen	0.5 - 1.0

Experimental data reveal the existence of a metabolic turnover with a reduced half time in liver than in brain or in muscle in the case of identical components. The tissues in which appear composition modifications have a rapid turnover.

Metabolic processes can be characterized by some peculiarities such as: a) *specificity* – defined by chemical and energetical efficiency and development in mild temperature, pressure and pH conditions - specific for the internal environment; b) *universality* – characterised by biotransformation mechanisms similar in microorganisms, plants, animals and man.

1.2. CHRONOBIOCHEMISTRY

Biochemical interactions specific for metabolic processes develop in time and in space so that chronobiochemistry and topobiochemistry can correlate the biologic systems. For the same biologic process there are periodical alternations having the characteristics of a rhythm. Biochemical modifications of the quantum of metabolites are genetically conditioned. Chronobiochemistry approaches the problem of biological rhythms (biorhythms) and their implications in the integration of biological systems (Feuer and Iglesia, 1985; Gârban, 1993 b).

The classification of biorhythms is made into three classes, established after the type of periodicity: 1) ultradians – lasting for seconds, minutes or hours; 2) circadians or nictemerals – lasting about 24 hours (21-28 hours); 3) infradians – lasting days, e.g.: hebdomadars (7 days), circamensuales (1 month), circannuals (1 year). Conditioning has a two-fold origin: intrinsic, determined by hereditary factors (e.g. activity of heart, estral cycle) and extrinsic determined by environmental factors (e.g. thermoregulation, diuresis, etc.). It is also influenced by the metabolic processes, i.e. biochemical catabolic and anabolic interactions.

Circadian rhythms are the most frequent ones in man, animals and even plants. Bioperiodicity appears as an ubiquitary phenomenon, regarding all the aspects of life (biochemical and biophysical interactions, physiological processes). One can observe a morphophysiological bioperiodicity regarding: biochemical interactions - e.g. biochemical mediators; biophysical variations - e.g. membrane potential; physiological processes - e.g. cardiac chronotropism; morphological modifications - e.g. the heart in the alternance (systole-diastole). The study of chronobiochemical modifications needs an interdisciplinary approach.

Diverse chronobiological aspects are well known, e.g. heart rhythm, reproduction processes in animals and plants. A cyclic, regular variation - specific for biological rhythms - can be assimilated to a sinusoidal function, which offers a mathematical approach. One can reveal the existence of some characteristic para-

meters for each biochemical, biophysical or physiological rhythm (fig.1). Among these an essential importance have the parameters which define the chronobiochemical characteristics: a) *the period* – the duration of a complete cycle of variation; b) *the acrophase* – corresponds to a maxim value (pic) of the concentration of a metabolite taken into study; c) *the amplitude* - estimated by the diagrammatic redrawing of the chronobiochemical variations of the studied metabolite (Reinberg, 1973; Gârban, 1984).

The effects of chronobio-chemical modifications are not limited only at circadian variations, they are also extended at selenar and circannual level. The concentration of electrolytes in biologic fluids shows also circannual variations.

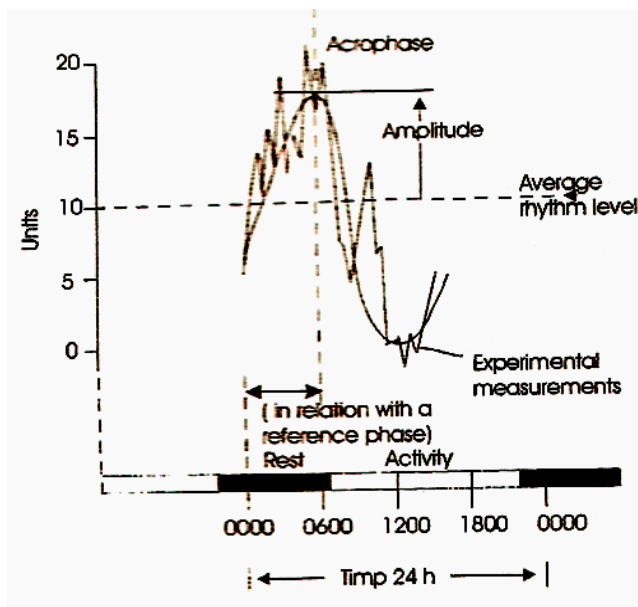


Fig. 1.: Chronobiochemistry of a circadian rhythm-characterization of parameters

Bioperiodicity is a peculiar phenomena of the organisms. The chronobiochemical autonomy of the biological rhythms is realised in the dualism endogenous (genetic) component – exogenous (areal, meteorologic factors etc.) component. There also exists the possibility of “synchronization” realised by the interrelation genotype-phenotype-environment and the manipulation of environmental stimuli. Under chronobiochemical point of view the synchronization is correlated with modifications in the material metabolism, e.g.: chromoproteins (hemoglobin in animals, chlorophyll in plants), nucleoproteins; lipids (glycero-

phospholipids, sterides); carbohydrates (glycogen - in animals; cellulose - in plants).

Studying the problems of chronobiology made possible the establishment that biorhythms are the consequence of the processes that exist at cellular, tissular, organs level or even at populational level. The extent of chronobiochemical studies to man and animals led to the elucidation of numerous problems and interdisciplinary connections, determining the appearance of new domains such as: chronophysiology (e.g. electrocardiogram-ECG; electroencephalogram-EEG, electromyogram-EMG), chronopathology (e.g. urolithiasis in summer), chronopharmacology (e.g. cytostatic chemotherapy), chronotoxicology (e.g. storage of metals with toxicogen potential).

One can observe some similitudes of the periodicity of movements in macrouniverse(e.g. planetary system), microuniverse (e.g. atomic nucleus) and in biosphere studied with the help of biochemistry or biophysics.

1.3. HOMEOSTASIS

The term “homeostasis” (gr. homeios - the same, stasis - state) was introduced in physiology and biochemistry in 1929 by the american physiologist W. Cannon (cited by Pora, 1981).

As a dynamic characteristic, homeostasis presents two interrelated characteristics: a) *homeostatic regulation* (or autoregulation) – the series of processes which lead to augmentation, depression or annulment of some variations in the functioning of a system; b) *homeostatic equilibrium* – an alternation of states with equilibrium tendencies.

In animal and vegetal organisms the introduced nutrients (biomolecules) determine fluctuations of the concentration in the internal environment. However, finally, the composition of the internal environment is maintained - due to the homeostatic mechanisms - relatively constant. Among the elements with a good homeostasis is included the sequence of the DNA nucleotides which form the chromosomal DNA.

Homeostasis is achieved at all levels of supramolecular organisation. In the living world, homeostasis has two distinct functions: stabilization, to assure the biological equilibrium, and optimization by which the living organisms realize successive equilibrium (“adaptation phenomena”). It is a characteristic present at all levels, from the subcellular one to that of biocenosis (Zetkin et al., 1968; Feuer and Iglesia, 1985).

In correlation with homeostasis, in pathological states one can discuss the concept of heterostasis, that is followed by physiopathological and morphopathological modifications with unfavorable prediction.

1.4. HOMEORHESIS

This concept imposed in biochemistry by fundamental scientific research and also by applicative research.

Homeorhesis is defined, principally, as the capacity of an organism to maintain a “distant equilibrium” in variable conditions occurred in the internal medium. The existence of the distant equilibrium is marked, for example, by a continuous exchange of matter, energy and information in the complex mother – conceptus (embryo or fetus). The phases of the distant equilibrium – homopoiesis and heteropoiesis - are interactive and interrelated. By homeorhesis, a systematic dynamic equilibrium is realised, called in the system theory as “autoisodiasostic system” (Dioguardi, 1989 - cited by Gârban, 1993 b). The study of metabolites in homeostasis led even to the idea of the existence of a “gestational anabolism” (Brätter et al., 1992). In the diphasic equilibrium the status of metabolites has also a nutritional conditioning (Gârban, 1993 a). The status of the metabolites depends on the exogenous intake of nutritive principles.

The mode of achieving the distant equilibrium is evaluated by the relation intake-flux-retention-elimination in the case of the resulted metabolites on various biochemical pathways.

During the gestation period a supplementary retention appears, without accumulations in the conceptuses. In a larger context, homeorhesis can be defined as a constant retention of metabolites for fundamental physiological activities.

2. BIOMINERAL COMPOUNDS AND THE DYNAMIC CHARACTERISTICS

Dynamic characteristic are evidenced, *largo sensu*, as the interactions occurring in the organism which involve the organic bioconstituents (protids, lipids, carbohydrates) and inorganic bioconstituents (anionic / cationic biominerals and water).

Metabolism. Metallic bioelements, like other bioconstituents, realise a “metabolic pool”. In this context one can speak about the relationship intake-flux-retention-elimination. Their intake has exogenous (biomineral nutrients) and endogenous (bioinorganic metabolites, e.g.: metalloproteids; salts of gluconic acids; salts of fatty acids etc.) sources. Their flux is orientated toward the “metabolic pool” which - conceptually - may be imagined as the total content of metallic elements in the organism. From this level the metals are distributed in order to be stored (e.g. Cu in ceruloplasmin; Fe in hemine etc.) and eliminated by digestive, renal and cutaneous (in a lesser amount) route. In fig.2 a model of biochemical and physiological mechanisms involved in hydro-electrolytic metabolism is depicted.

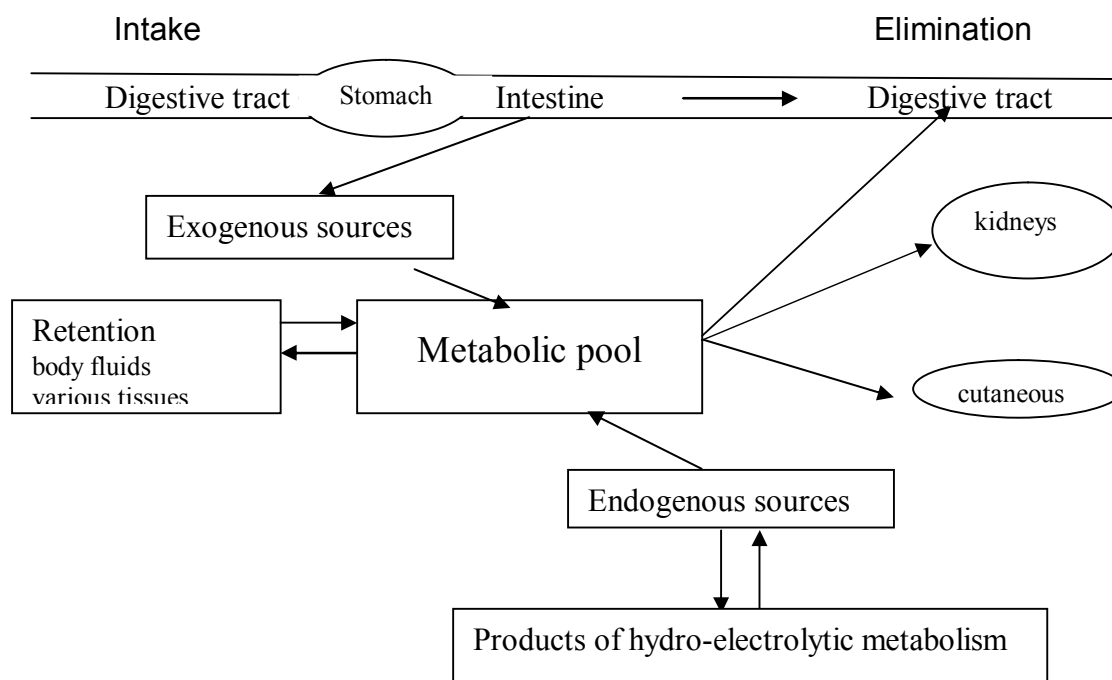


Fig.2. : Metabolic model of metallic bioelectrolytes in the relationship intake-flux-retention-elimination.

Using the conceptual model presented (see fig.2), the interconditioning metabolism-homeostasis could be understood easier. Thus, the “metabolic pool” will assure, according to the described relationship, the homeostasis of metallic electrolytes. The quantum of these varies in relatively low limits. Dyshomeostasis appears in limit situations, as the expression of disturbance in the hydro-electrolytic metabolism.

Chronobiochemistry. Observations on the metabolism must be completed with chronobiochemical peculiarities that involve the whole material metabolism (i.e. carbohydrate, lipid, protid and hydro-electrolyte). The biomedical interest for chronobiochemistry is important especially for its peculiar aspects with implication in chronophysiology, chronopharmacology, chronotoxicology etc.

Chronobiochemical studies made by Reinberg (1973) regarding some parameters specific for the material metabolism, e.g. blood, plasma, serum and urinary cationic (metal ions) and anionic (carbonates, phosphates etc.) metabolites revealed the modifications that appear in the alternation day / night, revealing maximum values (acrophase) during 24 hours - Table 2.

Table 2. Circadienne chronobiochemical variations of some bioelectrolytes in human

Specification	Type of variability	Acrophase (maximum values)
Blood	Ca ²⁺	_____) _____ (
	Na ⁺	_____ (_____)
	K ⁺ erythrocyte	_____) _____ (
	HCO ³⁻	_____ (_____)
Plasma or serum	Ca ²⁺	_____) _____ (
	Na ⁺	(_____)
Urine	Ca ²⁺	_____) _____ (
	Mg ²⁺	_____ (_____)
	Na ⁺	_____) _____ (
	K ⁺	_____ (_____)
	Na/K	_____) _____ (
	PO ₄ ³⁻	_____ (_____)
Periodicity		← day 24 hours night →

Homeostasis. Changes in the hydro-electrolytic metabolism, with characteristic chronobiochemical peculiarities, assure the biochemical homeostasis of metals. General data concerning the homeostatic status of some metals in blood serum are presented in table 3 (Altman and Dittmer, 1974; Anonymous, 1997).

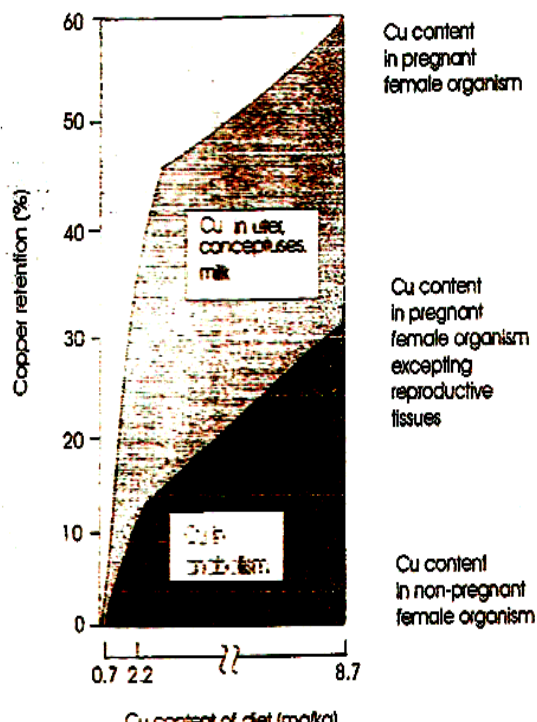
Table 3. Concentration of metallic bioelements in human serum

Specification	Range of values	Specification	Range of values
Na	310.0 - 356.0 mg/dL	Cu	11.0 - 20.0 µg/dL
K	16.0 - 20.0 mg/dL	Mn	0.5 - 1.3 µg/dL
Ca	9.0 - 9.3 mg/dL	Mo	0.2 - 1.2 µg/dL
Mg	1.0 - 3.0 mg/dL	Ni	0.1 - 1.3 µg/dL
Fe	50.0 - 150.0 µg/dL	Co	0.1 - 0.4 µg/dL
Zn	11.0 - 24.0 µg/dL	Cr	> 0.5 µg/dL

An essential characteristic of metallic bioelectrolytes as compared with other metabolites, especially with organic origin (such as carbohydrates, lipids, protids), is that they are not involved in biodegradative and/or biosynthetic processes in the organism. In conclusion one can say that in case of metals the physiological relationship intake-flux-retention-elimination is of great importance in their biochemical homeostasis status.

Homeorhesis. The problem of homeorhesis control, that express the so-called "distable equilibrium", implies the use of some accurate methods in analytical

biochemistry. In this context, in the case of metallic bioelements, their retention in materno-fetal complex is controlled. Thus, it was evidenced the existence of a specific "gestational anabolism" (Brätter et al., 1992).



Literature data show the problem of homeorhesis control, evaluating - for example - the constant concentrations of trace elements in milk related to an administered diet (Brätter et al., 1992; Kirchgessner, 1993).

Experimentally, in laboratory animals (female rats), the retention of copper related to gestational anabolism was studied (fig.3). It was observed that in gestational period appears a supplementary retention without storage in the offspring(s) organism. The "post partum" supplementary retention could be directed to milk production.

The intake of bioconstituents is achieved by exogenous (nutrients intake) and endogenous sources (substances resulted from intermediary metabolism). The flux of bioconstituents in the whole organism provides their participation to the distable equilibrium. Their constant

retention – used for growth, development, resorption, milk production a.o. – expresses in facto the homeorhesis in humans and, in generally, in mammals.

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MUSCULAR ACTIVITY STATUS RELATED TO METAL IONS: PARTICULARISATION FOR CALCIUM AND MAGNESIUM

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ABSTRACT

Minerals play an important role in the muscular activity both by the presence of cations, i.e. metallic ions of macroelements: Ca^{2+} , Mg^{2+} , Na^+ , K^+ and of some trace elements: Fe^{2+} , Zn^{2+} , Cu^{2+} and of anions, such as phosphate (HPO_3^{2-}), sulfate (SO_4^{2-}) and chlorides (Cl^-). Metallic ions of macroelements are involved in the membrane potential in the relationship of Na^+ / K^+ and even of $\text{Ca}^{2+} / \text{Mg}^{2+}$ and in muscular contraction (especially Ca^{2+} and Mg^{2+}). Trace elements participate as constituents of some protein molecules involved in physical effort, e.g. Fe^{2+} in hemoglobin and myoglobin; Zn^{2+} and Cu^{2+} in various proteins with enzymatic role. The anions have also remarkable roles knowing that ATP molecules are involved in energogenesis; sulfate is a constituent of the glycosaminoglycans such as chondroitin sulfate, keratan sulfate a.o. The latest ones are molecules present in tendond and aponevrosis - with role in the insertion and resistance of muscular tissue. Chloride, apparently less important, intervenes in the colloid-osmotic equilibrium of which dynamic is important in physical exercise. The aim of this work is to present assembly aspects on the biomineral compounds role in the muscular physiology (especially of Ca^{2+} and Mg^{2+}), revealing some peculiar aspects related to physical exercise and referring to ATP in relation with bioenergetical processes.

Key words: muscular activity ; alkaline-earth metal ions (Ca^{2+} , Mg^{2+})

INTRODUCTION

All the movements of higher animals including humans are accomplished through the contraction of muscles. Thus, the muscles are considered as biological motors that convert chemical energy into mechanical energy and translate the signals from the central nervous system into movements. The initiation and coordination of movement depend on the nervous system; the extra fuel and oxygen requirements of the muscles are supplied by appropriate adjustment of the cardiovascular and respiratory systems (Rhoades and Pflanzer, 1992).

It is known that the skeletal muscles are the most obvious muscles of the human body. These are large muscles used among other things for locomotion and for maintaining body posture. They usually attach to the skeleton and move the jointed bones with respect to one another.

MUSCULAR ACTIVITY: PHYSIOLOGICAL AND BIOCHEMICAL PECULIARITIES

Skeletal muscles are arranged in so-called antagonistic pairs. When a muscle moves a joint in one direction (e.g. flexion) another muscle moves the joint in the opposite direction, toward a more extended (i.e. extension) position.

The physiological relationship flexion-extension may be evaluated also at cellular level, e.g. contraction of sarcomeres and at molecular level, e.g. conformational modifications of fibrillar and globular proteins and the ions intervention as cell bioconstituents or as biochemical effectors (enzymatic ones).

The contractile elements of muscle consist of many different proteins, among these the most important are: myosin, actin monomer (G-actin), tropomyosin, troponin (with the subunits Troponin-C, Troponin-I, Troponin-T). alpha-actinin, beta-actinin, C-protein, M-protein (Rhoades and Pflanzner, 1992; Gârban, 1996).

Mostly the protein called myosin makes up thick filaments. A thick filament is composed about 300 to 400 myosin molecules. The head portion of myosin molecule has the biochemical and enzymatic properties that participate in the actual contractile function. The C-protein is involved in the assembly of about 400 molecules of myosin. The M-protein is involved in holding the tail sections of myosin.

The thin filaments are made up by the globular protein called G-actin. When it aggregates, the fibrous actin, i.e. F-actin appears. G-actin contains a specific binding site for ATP and a high affinity binding site for divalent metal ions. The Mg^{2+} ion is most likely the physiologically important cation, but Ca^{2+} also binds tightly and competes with Mg^{2+} for the same tight binding side. It is the G-actin-ATP- Mg^{2+} complex that aggregates to form the F-actin polymer (Bogert et al., 1984).

MUSCULAR ACTIVITY - ENERGETICAL ASPECTS

Contraction of skeletal muscle is initiated by a nerve impulse being transmitted across the neuromuscular junction through the intervention of the neurotransmitter acetylcholine. When a nerve impulse reaches the neuromuscular junction of skeletal muscle, Ca^{2+} is released into the sarcomere. The Ca^{2+} ion binds Troponin-C subunit of troponin, causing a conformation change that results in the exposure of the myosin binding sites on the actin molecule. Myosin binds to actin and undergoes a conformation change that allows the actin-myosin complex to bind ATP. Thus, the binding of ATP facilitates the dissociation of myosin.

In skeletal muscle contraction, the activation of the contractile proteins is triggered by the rise of intracellular ionized concentration, due to the movement of calcium from the storage site in the sarcoplasmic reticulum to the myoplasm. During muscle contraction, Ca^{2+} is released from the sarcoplasmic reticulum and binds to troponin in the thin, actin-containing muscle filaments.

In all types of physical exercise, the final common physiological event in the human body is the contraction of the skeletal muscle. The basic biochemical alterations at the muscle cell level that convert chemical energy into mechanical energy are accompanied by increases in the integrated activity of many organ systems, including the central nervous system (CNS), which initiates coordinated skeletal muscle activity. The respiratory system and circulatory system increase their activity during exercise to provide increased oxygen and nutrients to active muscle cells and to remove CO_2 , other metabolites and heat. Contracting muscle cells may increase heat production to 20 times than resting levels; thus, exercise needs extra demands on the temperature regulating system. The extent of the physiological adjustments in organ systems in response to exercise is often a direct measure of the strain or stress of exercise on the body.

Exercise is often classified as either dynamic exercise or static exercise. Dynamic exercise is performed any time muscle length changes. In a physical sense resistance are overcome along a certain distance. In this type of exercise, which includes such activities as bicycling, running, stair climbing or mountain climbing, exercise is quantified in physical units such as watts. In positive dynamic exercise, such as ascending a mountain, the muscles act as a "motor". In negative dynamic exercises such as descending a mountain the muscles act as "brakes".

The elevated metabolic demand is the common feature of all types of exercise and in most instances this is more important for physiological adjustments than any other factors (Murase et al., 1981).

Source of primary energy for skeletal muscle contraction and for other cellular work, are high energy phosphate compounds such as adenosin triphosphate (ATP), krectine phosphate a.o. These are produced by both anaerobic and aerobic metabolic processes. Three important metabolic systems that supply energy fo muscle contraction are: 1) the phosphatogen system; 2) the glycogen-lactic acid system; 3) the aerobic system (the citric acid cycle). General data on these are presented below:

1) Phosphatogen system. The energy supply from this system is used for short bouts of maximal exercise. The amount of energy present as ATP in the muscle is enough to maintain maximal exercise for only 5 to 6 seconds. The phosphatogens ATP and phosphocreatine can maintain maximal muscle contraction for only 10 to 15 seconds.

2) Glycogen-lactic acid system. In muscle this system can also supply energy for contraction. The stored muscle glycogen can be split into glucose (glycogenolysis) and then used for energy. Glycolysis occures in the absence of the oxygen and is therefore anaerobic. This system can produce ATP molecules more rapidly than the oxidative mechanisms in the mithochondria and is used as a rapid source of energy for muscle contraction.

3) Aerobic energy system. The aerobic system involves the use of oxygen within the mitochondria for the metabolism of glucides, lipids and proteins in the citric acid cycle and electron transport chain to produce ATP molecules for every molecule of glucose. Only 20% of the energy released is in the form of the mechanical energy of contraction; 80% is released as heat energy. The heat release during exercise place additional demands on the cardiovascular system and sweat response which play major roles in temperature regulation and the transfer of heat from body to the environment.

With repeated contraction there is a progressive loss of force producing capacity, termed muscle fatigue. The fatigue that is defined is originated from: continuos high frequency stimulation, repeated tetanic stimulation, long duration rhythmic activity, continuated convalescent period etc.

The failure of Ca^{2+} sequestration by sarcoplasmic reticulum plays a major role in muscle fatigue. The reduced Ca^{2+} concentration in sarcoplasmic reticulum may be due to an excess being bounded to troponin either to a reduction in the amount of ATP necessary to drive Ca^{2+} back.

Adaptation to physical exercise depends on the type and the severity of the work what is performed and the state of training of the individual. Muscular work may be thought of in two categories: dynamic work in which muscles contract through some distance against a resisting force (as running or cycling) and static work in which the muscles contract to support a load or exert a force without movement.

THE EFFECTS OF CALCIUM AND MAGNESIUM IONS

In the organism of a human adult (with an average weight of 70 kg) one can find 1.1-1.5 kg calcium. From the whole calcium content 98% is in the bones and the other part in cells and biological fluids. Calcium in serum consists of three components: ionized, complexed and protein bound. Approximately 45% calcium is associated with proteins (mainly albumin 80% and globulins 20%), 50% is in free state (unbound, ionized, ionic etc.) and 5% is bound to substances not associated with proteins.

Calcium metabolism is influenced by two parathyroid hormones (parathormone and calcitonine) and calciferols (vitamins D).

The complexed and protein-bound components act as a reservoir to maintain the equilibrium of the ionized fraction. The bound components may also have important role in the configuration and function of the associated proteins. The mineral phase not only imparts rigidity of bones and teeth, but also, in bone is a reservoir for extracellular ions (Devlin, 1992; Garban, 1996).

The ionized Ca is involved in important mechanisms such as: neuronal excitability, membrane permeability, hormonal liberation, enzymes activity, bone mineralization, skeletal muscle contraction etc. (fig.1).

Absorption and elimination of calcium vary in rather large limits. It had been shown that the required daily intake of calcium by the organism is: for adults 800-900 mg/day; for pregnant females 1500 mg/day; for adolescents 1200 mg/day; for children 1000 mg/day. Usually the Ca, P and Mg for organism are taken from food.

International organizations such as W.H.O. and F.A. O. established that the normal calcium require for organism is 400-500mg, with an adequate vitamin intake.

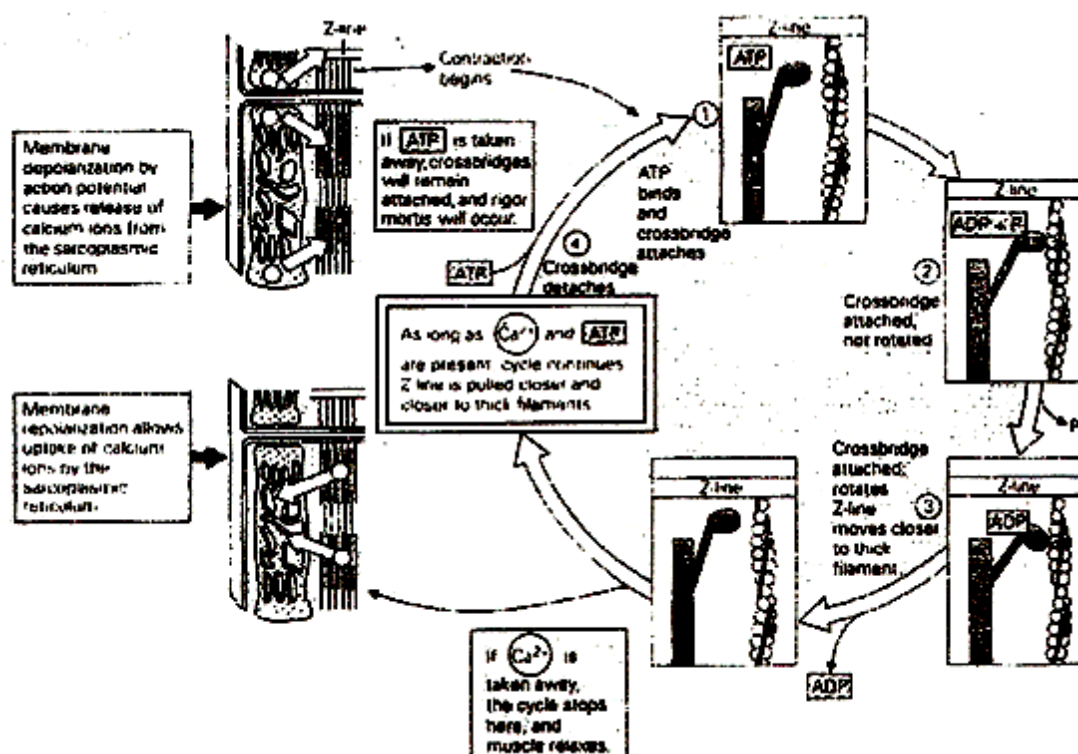


Fig. 1. Calcium and ATP participation in the muscular contraction (after Rhoades and Pflanzner, 1992)

Among the nutrients, milk and dairy products bring the highest calcium quantity and have the best Ca/P ratio, permitting a good absorption. It was demonstrated that the Ca/P ratio of food plays an important role in the bioavailability of these minerals realizing a very good absorption when it is between 0.5-2.0; if the ratio is lower than 0.5 at the intestinal level an insoluble salt (tricalcic phosphate) will be formed and eliminated by feces. The consecutive hypocalcemia will lead to the appearance of some clinical manifestations called spasmophilia.

Besides an optimal Ca/P ratio in food which influences the calcium absorption there are numerous other factors which reduce the absorption of minerals at intestinal level. So, the concentration of various substances at intestinal level determines the quantity of the absorbed minerals. among these substances we will mention the followings:

a) Oxalic acid - present in various nutrients such as spinach, orach tea, cocoa a.o. in increased concentration forms insoluble complexes with calcium and magnesium. If the oxalic acid concentration in a nutrient is twofold higher than the calcium concentration the absorption of calcium decreases. So it is not recommended the association of milk with cocoa or chocolate in patients with a maximum require for calcium.

b) Phytic acid - being in high quantity in beans, green peas, bred and other food stops the absorption of Co and Zn.

c) Vitamin D - is very important for the intestinal absorption of calcium. It is not yet known exactly the quantity of Vitamin D required for the adult organism. In geographic areas where the sunshine period is reduced the daily quantity is 400 i.u. and even 800 i.u. during lactation. The main source of vitamin D is the cutaneous ultraviolet radiation. The studied patients with spasmophilia had short exposures or no exposing to sunshine not even in summer. So, one can conclude that the population of our country presents a deficiency in vitamin D. Hepatic and renal diseases reduce or activate fallacious vitamin D, too decreasing the quantity of its active products.

d) Lactase deficiency - frequently the patients with spasmophilia can not support milk. It is because the deficiency of lactase enzyme, leading to the appearance of dyspeptic symptoms.

e) Giardiasis - is also an important etiopathogenic factor that may perturb the intestinal absorption of calcium. This disease was revealed in 40% of the patients with spasmodophilia.

Magnesium is an other important biomineral constituent of the organism. Its quantity in the adult human body (average weight 70 kg) is 25-30 mg. About 60% is founded in bones and 40% in cells, extracellular space and biological fluids (only 1% is present in serum: 45% is ionized and 55% represents complexed magnesium and protein bound magnesium).

Magnesium is an activator for about 300 enzymes mainly involved in energy transfer reactions (cofactor of transphosphorylation reactions during energy transfer in muscle contraction - transformation of chemical energy into physical energy). A maximal capacity in these transfer reactions is essential for competitive athletes. In addition, this ion is necessary for biosynthesis of proteins and maintenance of membrane integrity.

The necessary Mg supply for man for optimal maintenance of biochemical pathways and function of organs is known.

Environmental factors such as acid rains increase the Mg loss in soils and thus reduce availability of Mg to plants and animals and consequently to humans. Under normal conditions humans take up about 300 mg Mg or less daily, an insufficient amount to assure a normalized Mg balance.

Many factors can affect magnesium homeostasis, e.g.: *physiological factors* – sex, age, pregnancy, lactation; *genetical factors* - regulation of red cell levels, different kind of transport systems, HLA BW 35; *nutritional factors* - decreased ingestion, malabsorption, calcium, phosphate, phytates and fat intakes; *water supply* (soft and hard waters); *alcoholism*; *endocrine regulation*; *stress*; *physical activity*; *circadian rhythms* (Laires and Monteiro, 1987; Gârban and Daranyi, 1998).

Calcium and magnesium deficiency could be: *primary*, caused only by low intake and *secondary*, due to iatrogenic and non-iatrogenic causes (Table 1). In primary carency we must consider two forms: *deficiency*, the most usual, treated by oral administration and *depletion* not treated by oral administration.

Table 1. Secondary causes of hypocalcemia and hypomagnesiemia

General causes	Specification concerning biochemical and physiological modifications
iatrogenic	Decreased ingestion or malabsorption Hypocaloric and hyperproteic diets, Kempner diet, amagnesian perfusions, extensive bowel resection, intestinal bypass
	Nervous and metabolic dysfunction Stress (as surgery), neurotrophics, psyhanaleptics, hypercalcemic drugs, thyroid extracts, oral contraceptives, digoxin
	Excessive elimination Gastrointestinal-laxative abuse, gastrointestinal aspiration, kidney-loop diuretics, aminoglycosides, renal transplantation
non-iatrogenic	Decreased ingestion Great malnutrition, chronic alcoholism, pancreatitis, diffuse bowel disease (regional enteritis, ulcerative colitis)
	Alterations in homeostasis (with increase or decrease) Stress, endocrinopathies
	Excessive elimination Gastrointestinal – vomiting, diarrhea, steatorrhea, sprue; renal nephropathies, hyperaldosteronism, uremia; skin-burns, excessive sweating by strenuous physical activity

In developed countries Mg intake is often marginal and prolonged intensive exercise is a factor which exposes subjects to Mg deficit through metabolic depletion linked to exercise itself, which can only aggravate the consequences of frequent marginal deficiency. Mg deficit enhances susceptibility to free radical mediated injury and could increase the tissue damage that results

from strenuous physical exercise. Competitive athletes are especially inclined to loose Mg in sweat and urine (Noble, 1986).

According to the clinical routine of sports medicine only a relatively small attention was given to Mg if compared to K or Ca, however it is known that physical exercise is a factor leading to changes in Mg metabolism which depend on many factors, such as type, duration and intensity of exercise. When metabolism is accelerated ad during exercise, the requirement for Mg increases, which is justifiable by its key role in the use of energy rich compounds, in the maintenance of the membrane properties and in the muscle contraction.

An optimal function of metabolism is only ensured when the diet of the competitive athlete is well balanced. Wide significance is particularly attached to the maintenance of biochemical water and electrolyte homeostasis.

Among the limiting factors in obtaining maximal physical output is energy metabolism, the conversion of the acquired energy into motion, e.g. muscle contraction, coordinative abilities and finally physic components.

Several studies that included swimmers, runners, fencers, soccer players etc. have shown reduced plasma Mg levels in these persons. Mg deficiency was due to increased sweat and urine excretion of Mg and it was possible to reduce it by a corresponding Mg supplementation.

Open, blind or double blind tests showed that the Mg supplementation in physical exercise had positive and significant effects. Biochemical parameters, such as oxygen uptake, lactate formation, integrity of membrane function, blood coagulation clearly indicated an improvement in cellular energy metabolism, regeneration and adaptation of the organism to the physical stress situation.

Bioavailability of calcium and magnesium is an other important problem related to the absorption, metabolism and retention of these metals by the human organism. The concept of bioavailability defines the relationship established between the biologically active substances (Ca^{2+} and Mg^{2+} presented in this paper) and organism.

In case of a usual nutrition the bioavailability is estimated in relation with: 1) intrinsic variables (physiological): a) absorptive mechanisms; b) metabolic interactions; 2) extrinsic variables (nutritional): a) solubility (in our case that of Mg and Ca brought in organism by foods); b) synergistic effects; c) antagonistic effects.

In the context of bioavailability the problem of the biologically active substances with nutritional importance and consumed as food supplements, e.g. micronutrients from the group of vitamins and/or from the group of biominerals (part of them containing Ca and Mg) may be also approached. Food supplements consumption may assure a better homeostatic status during more intensive and sometimes of long duration muscular activity.

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THE STUDY OF THE POTENTIAL TOXIC METAL CONTENT IN ANIMAL FEED AND PORK, BEEF AND MOUFFON IN TIMIȘ AND CARAȘ COUNTY

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ABSTRACT

This paper represents a study of the content of some trace elements (Cu, Zn) and some potential toxic metals (Pb, Cd) of animal feed and also pork, beef and mouffon meat from Timiș and Caraș – Severin counties. The study was performed for 3 years: 1996; 1997; 1998 and for analyses of Cu, Zn, Pb and Cd content in the products we used the atomic absorption spectroscopy method (AAS).

We can conclude, in our study that the analyses for preliminary animal food products from this counties have not high content of Cu and Zn, and generally, we didn't find Pb and Cd residues exceeding the admission values.

Key words: potential toxic metals, atomic absorption spectroscopy method.

INTRODUCTION

At this end of century we assist at an abnormal industrial and agricultural pollution of the environment. In this category, the contamination with high doses of trace elements and potential toxic metals of soils, water sources, air, and of course of food products – both vegetal and animal type – can be included (Popa and Stănescu, 1981; Anke et al., 1993; Lăcătușu et al., 1998). The process of contamination has to be hardly studied and supervised because there is the possibility of some major disturbances at environment level. This perturbation of the balance can generate great alterations of the human organism status.

The maximum values of analysed trace elements and potential toxic metals, in Roumania, for meat products are: Cu 3 ppm; Zn 50 ppm; Pb 0.5 ppm; and Cd 0.1 ppm (based on The Health Ministry Order 611 / 3 April 1995).

MATERIALS AND METHODS

In this paper we present the study's results of the quantum of two biometals (Cu, Zn) and two metals with potential toxic (Pb, Cd) in processed meat form registered entertimes of Timiș and Caraș – Severin counties. We also present the quantum of potential toxic metals form feed animal in State Agricultural Enterprise form the same counties.

Totally, from 1996 to 1998, there were analysed 2578 meat samples (muscle tissues, liver and kidney), and 279 animal feed samples. For the quantitative analyses

we used flame atomic absorption spectroscopy and a GFU – 202 aparate, with double fascicle, monocanal, that had a lamp for fond correction (Seracu, 1986).

The determinations were made at different wavelengths: for Cu – 324.7 nm; for Zn – 213.9 nm; for Pb – 217.0 nm; and for Cd – 228.8 nm. The method sensibility it was evaluated at 0.09 ppm for cooper; 0.02 ppm for zinc; 0.20 ppm for lead; and 0.01 ppm for cadmium.

RESULTS AND DISCUSSIONS

The evaluated data for three years (1996, 1997, 1998) of determinations are presented in table 1. These results show the number of determined samples from animal meet and organs tissues.

Table 1. The quantum of some trace elements and some heavy metals in animal meet and organs tissues.

Year	County	Number of determined samples				Number of over-doses samples
		Cu (<3 ppm)	Zn (<50 ppm)	Pb (<0.5 ppm)	Cd (<0.1 ppm)	
1996	Timis	582	582	582	582	2 samples beef kidney tissues with 1.5ppm Cd
	Caras–Severin	236	236	236	236	-
1997	Timis	719	719	719	719	-
	Caras–Severin	320	320	320	320	-
1998	Timis	582	582	582	582	-
	Caras–Severin	139	139	139	139	-

According to these results, the beef organs – kidney presented two samples of over doses of cadmium caused by contamination.

Data of the determined samples for animal feed, evaluated on three years (1996, 1997, and 1998) are given in table 2.

There were tested 276 samples of animal feed between 1996 and 1998 in Timis and Caras–Severin county. None of these samples showed any contamination of animal feed with heavy metals with toxic potential (Pb and Cd) or over maximum admitted doses of trace elements (Cu and Zn).

Cooper and zinc are essential microelements for human organism. Cooper has multiple biologic roles and is considered indispensable for life. In organism cooper is bound to albumin, achieving equilibrium of cooper concentration in blood. The main organ of storage in human organism is the liver, but high quantum of cooper can be found in hearth, kidney, brain, and hair too (Rader and Spauling, 1978; Gârban et al., 1995; Vincu et al., 1998). The tested samples did not show an excess of cooper over the maximum admissible limit of 3ppm.

Zinc plays an activator or inhibitor role of many metabolism reactions, because there are about 30 “zinc-dependent” enzymes. The presence of Cd inhibits the Zn transfer through the intestinal wall, but also Zn is a protector against Cd intoxication.

Table 2. The quantum of some trace elements and some heavy metals in animal feed.

Year	County	Number of determined samples				Number of over doses samples
		Cu (<3 ppm)	Zn (<50 ppm)	Pb (<0.5 ppm)	Cd (<0.1 ppm)	
1996	Timis	114	114	114	114	-
	Caras-Severin	3	3	3	3	-
1997	Timis	92	92	92	92	-
	Caras-Severin	-	-	-	-	-
1998	Timis	70	70	70	70	-
	Caras-Severin	-	-	-	-	-

Same of cooper, the main organs of zinc storage are the active metabolic tissues: liver, hearth, pancreas, but high quantum of zinc can be found in bones, hair and skin too (Rader and Spauling, 1978).

Lead – according to literature data – is considered as an industrial pollutant and in the present study the quantum of analysed samples had not exceeded the maximum admissible value: 0.5 ppm. In low doses the lead influences the endocrine system (decreases the tiroidic activation) and other biochemical mechanisms. Thus, some metals from enzyme structures can be affected and finally the enzymatic complex is inactivated. Lead can bind to the thiol, orthophosphate and carbonyl rests contained in macromolecules ligands and biologic membranes. Therefore it is considered that an increase of the protein content, the intake of vitamin C and PP, of some minerals such as calcium, iron and phosphates decreases lead toxicity.

Cadmium, same with lead, is a major contaminant of the food products. In the human and animal organism, cadmium can be found as a metalloprotein, metallo-tioneine in liver and kidney. According to the results of these study two samples (beef kidney and mouton kidney) of all analysed samples presented high concentration of cadmium: 0.15 ppm compare to the maximum admitted level of 0.1ppm.

Metals with toxicogene potential present importance for biochemistry, nutrition, biochemical pathology, toxicology etc. In some conditions these metals may become contaminants of the human environment (water, soil, food, air) and therefore, in order to prevent accidents by intoxication, their distribution in the nature is systematically investigated (Dumitrescu et al., 1979; Kliment, 1996; Le Blanc and Bain, 1997).

In food industry there is a trend to use equipment based on special alloy that protect contamination of food products (Drăgănescu, 1998).

Usually the concentration of Pb and Cd is determined but the investigations could be extended also to Hg, Sn a.o. Beside the concentration of the potential toxicogene metals it is useful to determine also the concentration of some biometals (Cu, Zn, Fe, Mn etc.) which in excess could become harmful. In the last years important investigations upon the aluminium distribution in the environment were also performed. Knowing that in the precedent decades in the food industry there were used equipment based on aluminium the determination of Al content in food products is of great importance. In case of canned food the Sn concentration was determined.

To control the concentration of the potentially toxicogenic metals in food products is of great importance in some geographical areas and a computer assisted monitoring could be helpful (Fântână, 1998).

Generally one can mention that there are special laboratories for metals determination in food products and water. Experimental studies on laboratory animals as well as epidemiological research are performed in order to find out the distribution of metals in environment and especially in some industrial areas.

CONCLUSIONS

The animal feed used in Timis and Caras-Severin county, tested over three years of study, did not exceed the maximum admissible level for Cu, Zn, Pb and Cd – and this confirmed that the soil from Banat are not contaminated yet with these pollutants.

1. Meat samples collected from different Commercial Societies are not contaminated with lead residues.

2. Cadmium contamination is presented only in two cases from 582 analysed samples in 1996 in Timis county.

3. The quantum of Cu and Zn did not exceed the maximum level for meat and organ samples or for animal feed samples in 1996 – 1998 years.

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OBTAINMENT OF SOME STABLE SALTS OF TIO- AND OXOTIOMOLYBDATES

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ABSTRACT

Using procaine (Proc) as a stabilising agent, thermal stable salts in watery solution or in acetonitril were obtained, salts of the tio- and oxotiomolybdenic anions, of Proc₂MoO₂S₂ or Proc₂MoS₄ type, which can be used as additives or antidots against the molybdenum deficiency in animals or plants.

Key words: tio-, oxotiomolybdates

INTRODUCTION

Molybdenum is one of the essential trace elements of the animal organism, implicitly of the human organism. It is the constitutive element of three important enzymes: xanthine oxidase, aldehyde oxidase and sulphit oxidase, which play an important role in the detoxification of the organism as well as in producing another bioconstituents. Reducing the activity of sulphit oxidase can be dangerous for the organism by affecting the nervous system pre- and post natal.

Deficiency as well as toxicity of molybdenum in animals are due to the interaction Mo-Cu (Diemann and Müller, 1973; Gergen et al., 1988). Molybdenum deficiency in animals was only experimental observed, molybdenum concentrations in fodders being enough high in order to assure an optimal nutrition of the animals. Reduced concentrations of molybdenum in plants (<0.1ppm) and a great contribution of copper (10-20ppm) can favour the copper toxicity (Mason et al., 1989).

Molybdenum toxicity in animals, known as molybdenose was relative frequently diagnosed in different regions of the globe, animals being, in comparison with plants, little tolerant to molybdenum excess in food. The effects of fodder consumption with high molybdenum concentrations are indirectly, molybdenum excess interfering with the absorption and the utilisation of copper. The young animals are most affected by molybdenose and mostly then when are fed with fresh grass. The potentially toxic Mo concentrations in plants are about 10ppm (dry matter) but these can appear even at lower concentrations related to the quantity of consumed fodder. Due to the interaction Mo-Cu, the level of copper in fodders influences the critical level of Mo, this one being the higher the copper concentration is higher. Thus, at copper concentrations of 4-10ppm on pasture, the toxic level of Mo was 10ppm, when copper concentrations were reduced, Mo toxicity was shown at 3.10ppm Mo (Mason, 1990; Price et al., 1987). Treatments with Mo salts in soil or plants can prevent the deficiency of this microelement and remove the nocive effects of nitrates at sensitive plants.

Ammonium tiomolybdate or ammonium dioxoditiomolybdate used as antidots present the disadvantage of their instability in time, in solid state and mostly in watery solution, in normal conditions. The instability in keeping those salts and in handling the watery solutions at boiling temperature needed for sterilizing injectable solutions, determined us to make researches in order to obtain salts stable in time, in normal conditions, which permit the obtainment of some stable watery solutions at the boiling temperature necessary for their sterilisation.

RESULTS AND DISCUSSIONS

In order to choose the right substances we considered that quaternary ammonium, phosphonium, tetraphenyltetrazolium, alkaloids salts can give more stable substances with tio- and oxotiomolybdenic anions (Rana and Kumar, 1982) but these substances present neuroleptical toxicity for animals (Thornton and Webb, 1980).

Procaine (p-aminobenzoate of 2-diethylaminoethyl), a complex basic ester with pharmaceutical properties, known also by its biostimulating properties (Zota, 1985), was identified to react very easy as chlorhydrate, in watery solution, with the watery solutions of ammonium tio- or oxotiomolybdates.

There are obtained with very good efficiency (75-90%) red or yellow precipitates which can recrystallize from hot water resulting red-orange crystals (for the tiomolybdate) or yellow-orange crystals (for dioxoditiomolybdate), extremely stable in time, in normal conditions. Physico-chemical characterisation of $\text{Proc}_2\text{MoS}_4$, $\text{Proc}_2\text{MoO}_2\text{S}_2$ (Proc=procaine) compared with $(\text{NH}_4)_2\text{MoS}_4$ and $(\text{NH}_4)_2\text{MoO}_2\text{S}_2$ is given in Table 1 and Table 2.

Table 1: Chemical composition and characterisation of the obtained substances

Compound	Form, colour	Melting (decomp.) temperature (°C)	Mo% calc/obtained	S% calc/obtained	N% calc/obtained
$(\text{NH}_4)_2\text{MoS}_4$	crystals dark red	under 80 (decomp.)	36.92/36.50	49.23/50.00	10.77/11.01
$\text{Proc}_2\text{MoS}_4$	crystals red-orange	112-114	13.79/13.65	18.39/19.05	8.05/8.15
$(\text{NH}_4)_2\text{MoO}_2\text{S}_2$	microcryst. yellow-brown	under 80 (decomp.)	42.10/42.27	28.07/27.51	12.28/13.02
$\text{Proc}_2\text{MoO}_2\text{S}_2$	crystals yellow-range	94-98	14.46/14.13	9.61/10.12	8.41/8.20

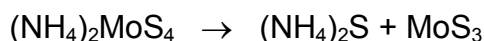
Table 2: Vibration frequencies characteristic for the Mo-S and Mo-O bonds in the obtained tio- and oxotiomolybdates (cm^{-1})

Compound	$\nu^s_{\text{Mo-S}}$	$\nu^{as}_{\text{Mo-S}}$	$\nu^s_{\text{Mo-O}}$	$\nu^{as}_{\text{Mo-O}}$
$(\text{NH}_4)_2\text{MoS}_4$	460	480		
$\text{Proc}_2\text{MoS}_4$	450-467	485-490		
$(\text{NH}_4)_2\text{MoO}_2\text{S}_2$	450	473	820	787
$\text{Proc}_2\text{MoO}_2\text{S}_2$	447-450	466	840-845	815-820

Comparing the obtained IR spectra before and after water crystallization of $\text{Proc}_2\text{MoS}_4$ and $\text{Proc}_2\text{MoO}_2\text{S}_2$ results that the two substances do not present observable transformations by IR spectroscopy. Vibration frequencies characteristic for Mo-O and Mo-S bonds remain in the same domaine.

$\nu_{\text{Mo-S}}$ for $\text{Proc}_2\text{MoS}_4$	recrystallized	$450\text{-}485\text{ cm}^{-1}$
$\nu_{\text{Mo-S}}$ for $\text{Proc}_2\text{MoS}_4$	non recrystallized	$467\text{-}490\text{ cm}^{-1}$
$\nu_{\text{Mo-S}}$ for $\text{Proc}_2\text{MoO}_2\text{S}_2$	recrystallized	$447\text{-}460\text{ cm}^{-1}$
$\nu_{\text{Mo-S}}$ for $\text{Proc}_2\text{MoO}_2\text{S}_2$	non recrystallized	$450\text{-}460\text{ cm}^{-1}$
$\nu_{\text{Mo-O}}$ for $\text{Proc}_2\text{MoO}_2\text{S}_2$	recrystallized	$815\text{-}840\text{ cm}^{-1}$
$\nu_{\text{Mo-O}}$ for $\text{Proc}_2\text{MoO}_2\text{S}_2$	non recrystallized	$820\text{-}845\text{ cm}^{-1}$

Ammonium salts of tio- and oxotiomolybdates can not recrystallize because at reheating the watery solutions they decompose:



and $(\text{NH}_4)_2\text{MoO}_2\text{S}_2$ suffers complex reactions of condensation and oxido-reduction leading to the formation of the complex anion $[\text{Mo}_2\text{O}_2\text{S}_2(\text{S}_2)_2]^{2-}$.

The solutions of $\text{Proc}_2\text{MoS}_4$ and $\text{Proc}_2\text{MoO}_2\text{S}_2$ in acetonitril, heated for 2 hours at boiling temperature (80°C) do not suffer transformations observed in the visible electronic spectrum, as it can be seen in figure 1.

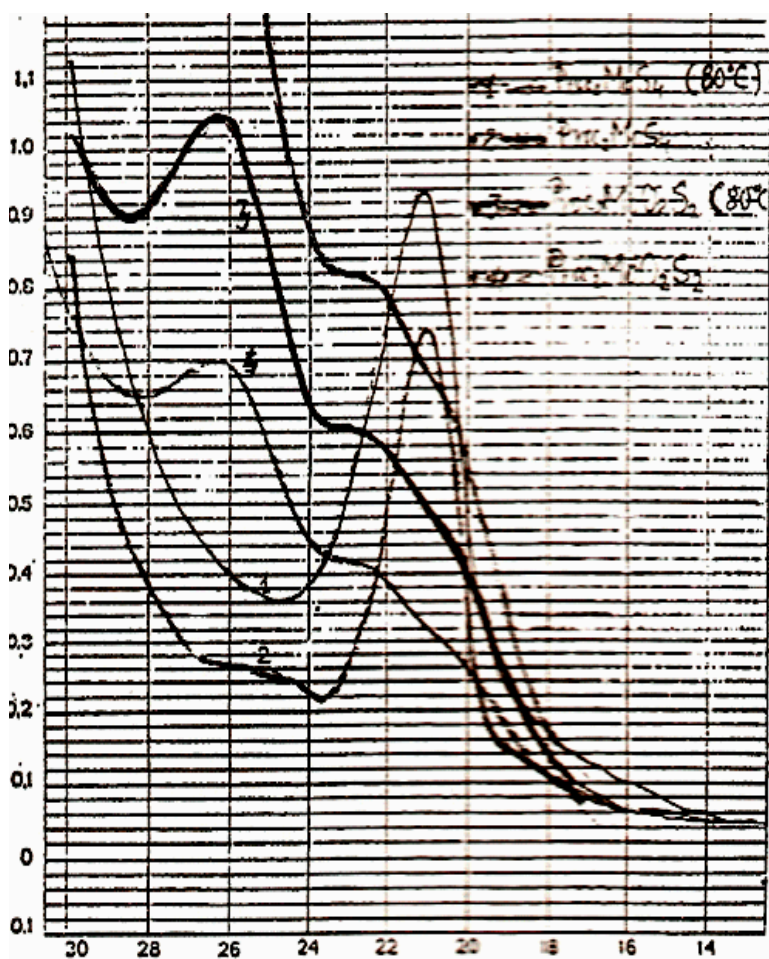


Fig. 1: Visible electronic spectra of $\text{Proc}_2\text{MoS}_4$ and $\text{Proc}_2\text{MoO}_2\text{S}_2$ solutions in acetonitril, before and after heating

The results obtained with the two substances show their special stability, either in solid state or in watery solutions or in acetonitril solutions, where they are partially soluble.

CONCLUSIONS

Using procaine as a cation to stabilize the tio - and oxotiomolybdenic anions there were obtained thermal stable salts of these anions in watery solution as well as in acetonitril. The obtained substances being not toxic can be used to remove molybdenum deficiencies in the animal and vegetal reign.

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PRESENCE OF TOXIC ELEMENTS, MICRO- AND MACRO-ELEMENTS AND TOTAL BETA ACTIVITY IN HONEY

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ABSTRACT

A total of 17 samples of honey, originating from different localities in Vojvodina (Yugoslavia), were included in this study. The contents of Hg, Pb, Cd, Zn, As, Cu and Fe, were determined in 9 samples. These elements were selected for examination because their maximal tolerable contents (MTC) in food are defined by national law regulations. In two examined samples, the content of Zn (sample no. 9: 14,12 mg/kg) and Fe (sample no 4: 20,40 mg/kg) were found to be above the MTC. Besides elements regulated by the law, several other micro- and macro-elements also significant from the nutritional point of view were also determined: Ca, P, Mn, Co, Mg, K and Na. In the analyzed samples, relatively high contents of Ca, P, Na, K, Fe and Zn were found. In 7 samples of honey the total beta activity (TA) was determined, ranging from 7,05 Bq/kg to 19,63 Bq/kg. The content of stable potassium detected in the examined samples indicates that the contribution of ^{40}K (a natural radionuclide) in TA, varies from 88,2% to 96,0%, suggesting that the major amount of TA in honey originates specifically from ^{40}K activity.

Key words: toxic elements, total beta activity, honey

INTRODUCTION

The rapid development of industry and technology world-wide unavoidably results in the increase of global pollution of the environment, which has direct or indirect impact on food contamination. At the end of the 20th century, we are witnessing an enormous number of contaminants of different origin, among which heavy metals and radionuclides certainly play an important role. With the aim of improving environmental protection and preserving human health, continuous surveillance of the level of contamination of the biosphere, among other measures, is of particular significance. Due to the characteristics of honey especially the relatively vast area which bees cover in order to collect the basic components for its production: nectar and pollen, honey is certainly one possible indicator of contamination in a certain region. On the other hand, honey has become a popular component in human nutrition in the past few years, thus data characterizing its quality and food safety are of major importance.

MATERIALS AND METHODS

Samples of honey were collected in the second half of 1997 and first half of 1998, from different localities in the northern part of FR Yugoslavia (province of Vojvodina). A total of 9 samples of honey were collected for the determination of

toxic micro- and macroelements and 7 samples of honey were analyzed for their total beta activity ($T_{\beta}A$).

Sample preparation. Samples were prepared by the method of "wet burning" (briefly summarized the samples were heated simultaneously with sequential addition of $Mg(NO_3)_2$, concentrated nitrate acid and hydrogen peroxide), applied in the analysis of toxic elements (except Hg) and macro- and micro-elements. Following exposure to $450^{\circ}C$ the resulting ash were dissolved in chloride acid and diluted with demineralized water to an appropriate volume (25 cm^3). Sample preparation for mercury detection was carried out by mineralization with sulfate acid and potassium permanganate. Decolorization of the obtained solutions was carried out with hydroxylamine-hydrochloride, while the reduction of ionic mercury into its elementary form was performed by a solution of tin (II) chloride.

Detection. The detection of toxic elements (Pb, Cd, Hg) and several micro- and macro-elements (Mg, Mn, Co, Zn, Fe, Cu) was performed by the atomic absorption spectrophotometric method using the Varian SpektrAA-10 spectrometer. Mercury content was determined by applying the "cold vapor" method. The contents of Na, K and Ca were defined by flame emission analysis on the same apparatus while the concentration of phosphorous and arsenic determined spectrophotometrically.

The total beta activity level ($T_{\beta}A$) was defined in the mineral residue (ash) obtained after mineralization of the samples. $T_{\beta}A$ in samples was measured on an anticoincidental device (OMNI GUARD, Tracerlab, USA) intended for measuring low levels of beta - activity, featuring basic activity less than 1 imp/min.

RESULTS AND DISCUSSION

Comparing the detected amounts of toxic elements in the analyzed samples of honey (Table 1) with MTC (Table 2) set by the law, it can be concluded that two samples are not in accordance with the tolerable levels: elevated Zn content in sample no. 9 (14,12 mg/kg) and Fe content in sample no. 4 (20,40 mg/kg).

Table 1. Content of toxic elements in honey (mg/kg)

Sample		Pb	Cd	Zn	Cu	Fe	As	Hg
1.	Wildflower honey	0.197	< 0.02	1.89	0.300	9.14	< 0.10	< 0.005
2.	Flower honey	0.244	< 0.02	4.54	0.191	3.84	< 0.10	< 0.005
3.	Linden honey	0.259	< 0.02	1.97	0.062	14.56	< 0.10	< 0.005
4.	Black locust honey	0.216	< 0.02	2.38	0.189	20.40	< 0.10	< 0.005
5.	Wildflower honey	0.077	< 0.02	1.69	0.065	4.86	< 0.10	< 0.005
6.	Black locust honey	0.101	< 0.02	2.27	0.130	5.36	< 0.10	< 0.005
7.	Mixed honey	0.096	< 0.02	3.24	0.331	9.60	< 0.10	< 0.005
8.	Flower honey	0.166	< 0.02	3.42	0.075	8.61	< 0.10	< 0.005
9.	Mixed honey	0.350	< 0.02	14.12	0.756	0.83	< 0.10	< 0.005

Table 2. Maximal tolerable contents of toxic elements in honey, regulated by national legislative norms

Toxic Element	Pb	Cd	Zn	As	Cu	Fe
Maximal tolerable contents (mg/kg)	0.5	0.03	10	0.5	1	20

It is also worthy mentioning that concentrations of the most toxic elements (As, Cd, Hg) in all the examined samples were below the detection limit of methods applied in their detection, i.e. considerably lower than the MTC for certain elements.

Table 1 shows that the amount of lead in all samples of honey taken from all localities were measurable, ranging from 0,077-0,350 mg/kg, which is below the MTC (Table 2).

Table 3. Content of micro- and macro-elements in honey (mg/kg)

Sample	Na	K	Ca	Mn	Co	P
1. Wildflower honey	24.7	221.4	19.48	0.277	< 0.05	56.77
2. Flower honey	45.4	344.2	48.25	0.139	< 0.05	54.94
3. Linden honey	48.05	966.1	47.42	1.133	< 0.05	44.76
4. Black locust honey	19.5	256.8	26.61	0.352	< 0.05	51.06
5. Wildflower honey	64.4	345.5	7.73	0.104	< 0.05	48.25
6. Black locust honey	42.9	347.7	3.07	0.096	< 0.05	44.96
7. Mixed honey	39.8	208.4	2.88	0.214	< 0.05	101.5
8. Flower honey	37.1	570.1	40.59	0.816	< 0.05	40.38
9. Mixed honey	185.5	2197	141.3	1.936	< 0.05	169.5

Values obtained after determining the contents of other micro- and macro-elements (Table 3) indicate similar values of contents of certain elements in most samples of honey. The average composition of individual elements in Table 3 in the examined samples is as follows: Na 40,2 mg/kg, K 407,5 mg/kg, Ca 24,5 mg/kg, Mn 0,391 and P 55,3 mg/kg.

Sample no. 9, in which a multiple increase in the concentration of almost all the examined elements was seen, was excluded from the above mentioned average values. Due to the fact that sample no. 9 originates from close proximity to a cement factory with intensive production, it is very possible that this is the cause of the measured extreme values in almost all the analyzed elements.

On the basis of data presented in Table 3 it can be concluded that the presence of Co was not observed in any of the examined samples, although data found in the literature states the presence of Co in honey ranging from 0,50 to 1,40mg/kg.

Table 4. Level of total beta-activity ($T_{\beta}A$), content of total potassium, activity of ^{40}K ($A^{40}K$) and ^{40}K contribution ($C^{40}K$) in total beta activity in honey

Sample	$T_{\beta}A$ (Bq/kg)	K (g/kg)	$A^{40}K$ (Bq/kg)	$C^{40}K$ (%)
1. cvetni med	7.88 ± 0.42	0.262 ± 0.013	6.98 ± 0.35	88.2
2. Kelebija	11.80 ± 0.54	0.403 ± 0.020	10.68 ± 0.53	90.5
3. Lipov med	12.76 ± 0.63	0.462 ± 0.023	12.25 ± 0.61	96.0
4. Bagremov med	17.77 ± 0.87	0.633 ± 0.032	16.78 ± 0.84	94.4
5. Livadski med	7.05 ± 0.36	0.244 ± 0.012	6.47 ± 0.32	91.2
6. Livadski med	17.04 ± 0.88	0.599 ± 0.030	15.88 ± 0.79	93.2
7. mesani med	19.63 ± 0.94	0.685 ± 0.034	18.16 ± 0.91	92.5

The $T_{\beta}A$ content and ^{40}K activity are presented in Table 4. $T_{\beta}A$ content in the examined samples of honey varied from 7,05 Bq/kg (sample no. 5) to 19,63 Bq/kg (sample no. 7). ^{40}K activity, as a natural radionuclide, was measured to be approximately near the $T_{\beta}A$, more precisely the contribution of ^{40}K ranged from 88,2 % to 96,0% of $T_{\beta}A$.

On the basis of the measured $T_{\beta}A$ content and calculations of the $T_{\beta}A$ from the measured content of total potassium ($A^{40}K$), variances and Fisher's coefficient (F) were computed for both methods. Comparing the computed coefficient (F) with the Table containing values of the critical coefficient (F_{cr}), we can conclude that variances do not differ significantly among all the examined samples. Since standard deviations, in both series of measurements, do not differ significantly in all samples, we can calculate their common standard deviation and Student's coefficient (t_s). In samples no. 3 and 4 $t_s < t_{tab}$, which leads to a conclusion that there are no statistical differences (at the 5% level of significance) between the measured ($T_{\beta}A$) and computed ($A^{40}K$), consequently the measured beta activity in these samples originates from ^{40}K . In samples no. 1, 2, 5, 6 and 7 $t_s > t_{tab}$, indicating that the differences between the measured ($T_{\beta}A$) and calculated ($A^{40}K$) are statistically significant; thus, the remaining portion of the total beta activity originates from other beta emitters.

CONCLUSIONS

1. From the total number of examined samples of honey, only two samples exceeded the MTC set by national regulations (Zn in sample no. 9 and Fe in sample no. 4).
2. Contents of the most toxic elements: Hg, Cd and As were below the detectable limits of the applied methods, and considerably lower than MTC for the observed elements.
3. Contents of other elements significant in human nutrition (Zn, Cu, Fe, Mn, Ca, P, Na, K) varies slightly depending on the kind of honey and its origin, excluding sample no. 9 in which a multiple increase of almost all the determined elements was observed, which can be connected to the locality from which the examined samples were taken from.
4. The total beta activity of honey in samples no. 3 and 4 originate from ^{40}K as the most widely distributed natural radionuclide. In samples no. 1, 2, 5, 6 and 7 the remaining amount of $T_{\beta}A$ originates mainly from small amounts of other beta emitters.
5. Due to the low concentrations of detected toxic elements and radionuclides and contents of micro- and macro-elements, the obtained results indicate that honey from the examined localities is a healthy and ecology sound food component. However, with the aim of protecting the environment and preservation of human health, continuous surveillance of the content of toxic elements and radionuclides in honey, particularly in localities with increased risk of contamination, is necessary.

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SOURCES AND CONCENTRATIONS OF HEAVY METALS IN SOUTH-WEST ROMANIA

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ABSTRACT

The Banat soil between Carpathians, Tisa, Mureș and Danube have now a relative high content in heavy metals. Because of the lack of industrial pollutants, the causes which have favoured the accumulations of heavy metals can be put only on the fact of the high geochemical founds of the rocks and bed-materials from the mountain zone and the modality of their transport towards the west, towards the piedmont areas and the plain ones.

Key words: pollution, heavy metals, Banat, Mureș-Tisa-Danube euroregion

INTRODUCTION

The geogenous and anthropogenous abundance of heavy metals in Banat rocks and soils (between Carpathians-Tisa River-Mureș River and Danube) is conditioned by the paleogeographical evolution, by the type and seen of the direction of weathering that occurred especially during the Quaternary as well as by the direction and the characteristics of the pedogenetical processes.

The Banat soils do now have a high content of heavy metals that are almost always above the background domains. In the absence of predominant industrial polluters the causes that have favoured the accumulation of heavy metals can only be explained by the high geochemical background of the rocks and of the parental materials of the eastern mountain zone and by the manner of transport towards these areas and the modifications that occurred in the chemistry of the soils during the pedogenesis process.

The researches done took into consideration the knowledge about the geochemical endowment of Banat in order to appreciate the capacity of the soils of being able to reach or not extra loading of heavy metals. The identification of heavy metals on levels of content and their map materialisation is allowing the characterisation of the place, the appreciation of their origin (autochthonous, alochtonous or anthropogenous) as well as the establishing of some correlation's between the total forms and the mobile ones of each studied element.

MATERIALS AND METHODS

Towards estimating the geochemical background of the etno - historical Banat, within the surface of the Romanian Banat there have been taken about 400 soil samples from the horizon placed at different depths (from 0-150 cm) from over 100 places. The analysis methods relied in the weathering of the prevailed samples with a mixture of acids nitric, sulphuric and perchloric, in a 2 : 0,1 : 1 (I.C.P.A. - 1986) proportion and spectometrical atomically absorption dosation in variant of air-acetylene flame atomisation.

The manner in which the position cartographies were made presenting the area disposition of the researched oligoelements relied in the superposition of the following maps: the map of main geomorphologic units of Banat, the map of the intensely mineralised materials, the geological map, the map of the depth of the underground waters, the map of the rocks and of the parental materials, the map of the soil associations (Ianoş, 1997), the map of Banat along XVIII and XIX century, the map of the humus supplies (0-50 cm), the map of the soil acidity.

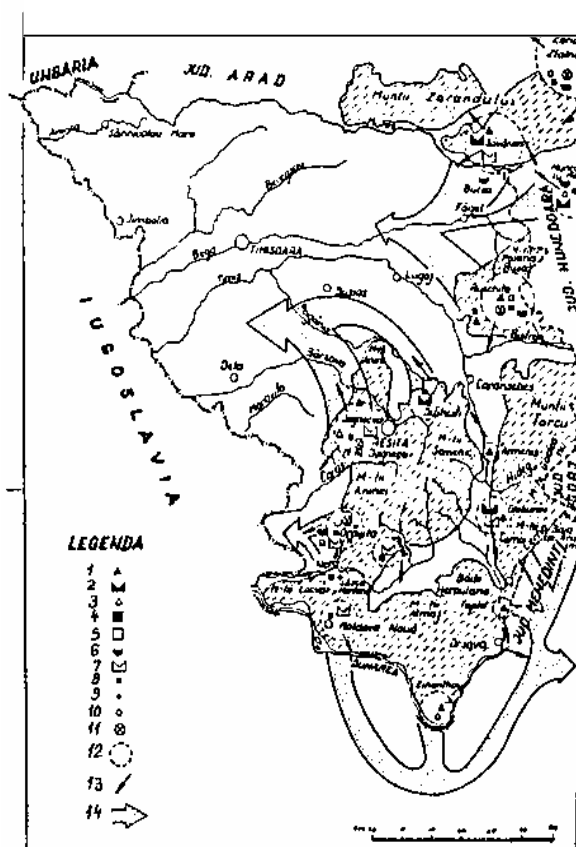


Fig. 1: Areal with anomalous mineralizations in the mountain Banat region

Legend: 1. Fe; 2. Mn; 3. Cr; 4. Cu; 5. Pb; 6. Zn; 7. Mo; 8. Ni; 9. Co; 10. Mg; 11. Complex polysulphurs; 12. Anomalous areas with mineralizations; 13. Direction of spreading of the eroded materials; 14. General direction of transport and sedimentation of the intense mineralized materials.

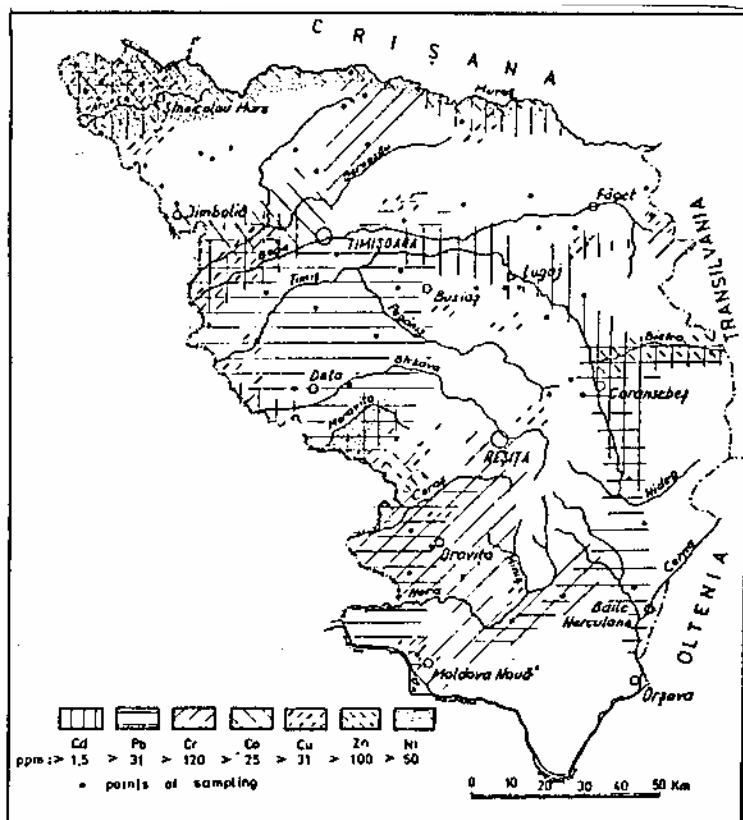
Functions of the particularities of occurrence of the researched metal microelements (Cd, Co, Cu, Cr, Fe, Mo, Mn, Ni, Pb, Zn) in the subjacent and parental rocks (Rankama, 1970), of the environment and the manner in which these elements are taken out of rocks by weathering, of the behaviourism of these rocks during the transportation as well as of the situation created during the sedimentation there have been delimited new areas with similar concentrations on content levels.

On the basis of the above presented considerations to which there can be added lithological, gliptogenetical or pedogenetical particularities in the zones where there were not gathered and analysed samples the conclusions that could be drawn were extrapolated to that the future analytical determinations to confirm or to deny the formulated suppositions.

RESULTS AND DISCUSSIONS

In its eastern part, Banat is limited by more or less homogenous mountain massifs. Made during the alpine orogenesis they have taken at the same time or after that an intense magmatic activity generated by the postlaramic tectonically movements after which on the fracture line there were pushed towards the surface the banatitic intrusions especially formed on granodioritic type of rocks (Oncescu, 1965; Folea, 1980). Parallel there have been taken place large processes of contact metamorphism and selective metasomatozoa and as a result there were

replaced especially by transformation of the limestone's the skarns (Gheorghită, 1975; Petrulian, 1979). Later, the endo- and exogene skarns were influenced by a hydrothermal metamorphism (Folea, 1980) with the making of polymetal sulphurs (pyrite, blend, calcopyrite, galena, aikinit, bismuthit, cerussite, crocoite, piromorphite, molybdenite, etc). A short inventory of the areas with intense mineralisation are indicating us a zone of positional accumulation in the nord-west part of Banat (Metaliferi Mountains) where there are predominating the polisulphurs of plumb, copper, zinc. Towards west and south west Savu (1970)



mentioned rich mineralisations of molybden and manganese or iron in the eastern part of Zarand Mountains. From both zones, Mureș River and its tributaries have taken denuded materials and put them towards west building the hills and the plains in the central and western part of Banat. There follows, on the south, the metalogenetical areas in the mountain massifs Poiana Rusca-Rușchița (lead, copper, zinc, molybdenum); Dognecea (iron, copper, chromium); Anina-Sasca (cobalt, nickel); Almăj-Moldova Nouă (copper).

During the geological time, but especially during the quaternary, when the frequent climatically oscillations have been favouring an intense physical weathering in stages and a pronounced chemical weathering in the interstages, from the rocks of the mountain massifs there was released a part of the constituent chemical elements.

The positive epirogenetical movements of the Carpathian chain and the drainage of the Panonic lacustrine domain by Danube piercing through the iron gates have been accelerating the denudation processes, those of transport of the

materials resulted as well as an accelerated sedimentation in the piemountainous zones and then on the plain zones in the east-west direction. On the basis of these

considerations we have to underline the fact that not only the superficial lithological layers in the plain or piedmountain zones are loaded with heavy metals but also thick packs of rocks placed on deep depths. This fact is explaining some overloading with arsenic of the depth waters (60-200 m), there were registered in the northern part of Mureş River that are also continuing in Hungary.

A last stage in the process of rocks and soil loading with heavy metals is conditioned by the filling with alochtonous materials of the vast areas of subsidence in the eastern Banat, zones occupied by the end of the XVIII-th Century by large swampy domain where there existed a varied and rich hydrophile fitocenosis that was decomposed and there resulted rich amounts of sulphur (H_2S). Because the waters of the main rivers that were eroding Banat used to carry in suspension fine particles of eroded material or ions of the heavy metals in solution and as the majority heavy metals have a special affinity for the sulphur, at floods these elements were blocked in the fine textured materials loaded with sulphur compounds fact that lead to high occurrences of chemical elements in the clayed rocks especially the smectical ones.

The tectonic and climatic stability at the beginning of the Holocene marked a dwindling of the erosion processes, of the sedimentation, as well as an amplification of the solification processes function of the time lift from the beginning of the solification process of the granulometrical making of the parental rocks and of the soil, of the amount of humified organically matter, of the pH and rH of the solutions that are circulating in the soil, heavy metals placed here together with the solification materials have been suffering concentration modifications by lateral or vertical movements ascendant or descendent.

On the basys of the analytical data there can be inferred that in the Banat soils the metal microelements that were researched are especially to be found in the structure of the primary and secondary minerals as well as kept by different physico-chemical mechanisms by the organically colloid's and the mineral ones. The total supplies and the degree of mobility is conditioned by nature of the solification rock and by the processes of pedogenesis (eluvial-iluvial, bioaccumulation, oxido-reducing). Generally, in reduced and moderate weathering conditions, the influence of the rock is determinant and in the conditions of advanced weathering the main role goes to the pedogenetical processes.

After the alteration of the rocks, the great part of the metallic elements are passed in the solution under the form of the compounds (sulphate, chlorate) which are the then differentially transported and resedimented. The values of concentration in metallic element met all over the south-western Romania soils are in general high some of them over passing the medium limits.

Generally regarded and correlated with the data obtained by Lăcătuşu (1992) for Romania or by Adriano (1986), Alloway (1990) for other zones of the world, the microelements determined in the Banat soils are in a concentration (mean values) of 1.2 to 3 times higher, the maximum values being characteristic especially for the lead, cadmium, cobalt and chromium. We can notice from table 1 that only the molybden is inside medium limits from all the 9 metallic elements inquired in our

country. The rest of the elements are doubling or tripling the limits of medium constant or come up to 4-6 times higher for maximum limits.

Table 1: The geogene abundance in heavy metals of the soils from south-western part of Romania, reported to the values of the Clarks and to the medium content from the soil

Element	The Clark	The medium content in soils in		Maximum content in the SV part of Romania soils
		Romania	SV part of Romania	
Mn	1000	500	600-800	800-1200
Cu	47	20	20-30	30-60
Ni	58	20	30-40	40-60
Cr	83	30	80-100	100-130
Zn	83	50	50-70	70-90
Co	18	5	20-25	25-35
Pb	16	15	20-30	30-50
Mo	1	2	1-2	2-5
Cd	0.13	0.3	0.5-1.0	1.0-1.5

The highest contents of metallic elements (total forms) on concentrational typology of soils and areas have been met in the vertisols of the western part of the Torontal Plain (the Cheglevici-Valcani zone) and the Timiș Plain (the Checea-Iohanesfeld zone). It was also good to sign the great concentration of Oravița.

Reducing contents of heavy metals have been signalled on few areas in the podzolized soils, here the pedogenetical process has interconnected the geogene accumulation and withdrew great amounts of epipedogenetical elements, leading them to deep horizons of soil.

The soluble forms of microelements are generally determined by total forms of accumulation, but the pedogenetical process and especially the soil reaction has a decisive role in the establishment of their movement. Beside zinc and manganese, the other mobile form elements inquired have been identified in large accumulation in the same areas of clay soils in the western part of Banat. The mobile manganese is signalled in great amounts in the soils with humidity excess from the eastern piemountainous areas, but the zinc conditioned by the acid reactions is very much found in the non-bazified soils without a levigation process.

CONCLUSIONS

The presence of the heavy metals elements in the rocks of the mountainous area from the centre and the eastern part of Banat has determined levels of high concentration, especially in the plain soils.

Even if the Banatic soils have big amounts of heavy metals however, till there haven't been signalled polluted zones.

Function of the some conditions of realising transport and sedimentation most of the heavy metals presented in the Banat soils have a superior abundance in comparison with the neighboured areas or other parts of the world.

The microelements exist in the soils under mobile aspects and have a various area disposition, determined by the reaction of the solution, by the content of clay and intensive humus filed organically material.

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BIOCHEMICAL CONSIDERATIONS REGARDING WILSON'S DISEASE

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ABSTRACT

Wilson's disease or hepatolenticular degeneration, is a progressive disorder of early life which is frequently familial and is characterized pathologically by degeneration of certain regions of the brain, especially the corpus striatum, and cirrhosis of the liver, and clinically by increasing muscular rigidity and tremor.

It is believed that ceruloplasmin, which normally contains almost the whole of the copper in the blood is deficient. This leads to low serum copper level. The copper is carried loosely bound to albumin. Hence it is deposited in the tissues and excreted in urine. The disorder appears to be inherited as an autosomal recessive.

In this paper, emphasis will be placed on aspects of relatively low concentrations of thiols (i.e. decreased cysteine concentrations) in blood plasma of subjects affected by Wilson's disease, copper metabolism and fatty acids.

The complex biochemical pathways described in the human body are strictly controlled by an array of regulatory mechanisms. Many of these biochemical reactions involve energy transfer and release and can result in the production of free radicals, unstable and highly reactive molecules with unpaired electrons, which are important for normal cellular function and as a line of defense by macrophages, for the destruction of foreign material. Proceeding on the contention that deficiency of serum ceruloplasmin is one, if not the only, inherited biochemical defect in the majority of patients with Wilson's disease, Total Copper, Urinary Copper, Serum and Urinary Uric Acid, Plasma Thiols (as Free Cysteine) and Fatty Acids were estimated.

We studied four subjects (aged 19 to 25) and 20 neurologically normal controls. All Wilson's disease subjects had decreased serum ceruloplasmin and serum copper levels, elevated urine copper levels.

Key words: Wilson's disease, serum ceruloplasmin, copper, fatty acids a.o.

INTRODUCTION

Extracellular fluids contains low molecular weight antioxidants that are actively involved in the defense against reactive oxygen species. The antioxidant activity of these compounds is largely due to their ability to trap oxygen radicals. Our group studying from many years the implications of antioxidant status in autoimmune diseases (e.g. Multiple Sclerosis, AIDS), neuromuscular diseases (e.g. Duchenne Muscular Dystrophy) and liver diseases (e.g. Cirrhosis). These results suggest that elevation (even exogenous drugs) in the concentrations of total reduced thiols in plasma may improve its antioxidant activity under specific conditions. This may be particularly useful since other important antioxidant mechanisms such as urate, ascorbate and vitamin E appear to be inefficient. Our general aim is to introduce new medicinals among relevant probability to preserve the free thiol groups in active structure at a physiologically pH and with a very low toxicity. Our objective in projecting these drugs was to confer a greatest stability of the free thiolic groups not only in ampoules but to have a maximum concentrations of -SH groups in human

body, with a low toxically effects. In this paper we give a typical example of the alteration of antioxidant status of organism.

MATERIALS AND METHODS

The diagnosis of Wilson's disease was based on clinical history, neurologic examination and biochemical tests. Samples were obtained before breakfast. Samples of 5-6 ml of venous blood were added to heparinized test tubes and were immediately centrifuged at high speed. The supernatant plasma was removed with a Pasteur pipette, taking care not to aspirate any of buffy coat.

Aliquots to be used for quantitative cysteine analysis were deproteinized by adding 20 mg of solid sulfosalicylic acid per ml. of plasma shaking to mix, and centrifuging at high speed. Deproteinized plasma supernatants were stored at -70°C until analyzed. Very rapid deproteinization of plasma specimens is crucial for accurate measurement of cysteine. Delay in deproteinization results in loss cysteine through disulfide bonding to the sulfhydryl groups in plasma proteins (Perry and Hansen, 1969; Perry et al., 1975). Cysteine (-SH) was estimated according to the method of Ellman with 5,5'-dithio-bis-2 nitro-benzoic acid (DTNB) at pH 8,412 nm. Plasma aliquots were extracted with a system chloroform : n-octanol (2:1).

Fatty acid methyl esters were prepared in accord with Morrison method modified. and Smith (1964). The purified fatty acid methyl esters were dissolved 100 μl of iso-octane and 2.0 μl was injected into a Hewlett Packard 5840 a gas chromatograph, equipped with a dual flame ionization detector (FID). The chromatograph was fitted with a 30-m glass capillary column of 0.3 mm i.d., coated with SILAR 10C. The oven temperature was programmed from 100°C to 200°C , heating for 5 min. at the rate of $5^{\circ}\text{C}/\text{min}$, followed by $0.5^{\circ}\text{C}/\text{min}$. up to 40 min and finally $1^{\circ}\text{C}/\text{min}$ up to maximum temperature of 200°C , which was maintained for further 10 min. before cooling. The splitting ratio was 1:70, the column was operated with hydrogen as the carrier gas at a flow rate 2.5 ml/min. Injection and detector temperatures were 240°C . The peaks were identified by means of known standards (Preoteasa and Ionescu, 1995; Preoteasa et al., 1994; Ionescu and Mosoiu, 1990a; Ionescu and Mosoiu, 1990b).

Serum ceruloplasmin was estimated according to the method of Ravin (1956). Total copper serum was estimated by atomic absorption spectroscopy by a simple 10% v/v dilution of serum with 6% butanol. The use of the butanol solution as diluent significantly increases the sensitivity of copper. The fuel (acetylene), oxydant (air) and the counter flow jet position must be optimized for best precision. Wavelength 324.7 nm, aspiration rate 5 ml/min, Hallow cathode lamp: Cu, slit width 320 μm , lamp current 5 mA; AAS1 Carl Zeiss Jena.

RESULTS AND DISCUSSIONS

Data of the general laboratory investigations in healthy subjects and in patients are presented centralized in table 1.

Table 1. Biochemical findings in blood and urine of subjects

Specification	Cerulo-plasmin mg / dl	Total Cooper μg / dl	Uric acid mg / dl	Cysteine (-SH) μmol / l	Urinary cooper μg / 24 h	Urinary uric acid mg / 24 h
NORMAL N=20	32.20 $\pm$ 4.90	185.3 $\pm$ 14.10	3.75 $\pm$ 0.45	90.00 $\pm$ 20.54	12.54 $\pm$ 3.54	50.45 $\pm$ 6.30
PATIENS N =4	8.45 $\pm$ 7.32	99.44 $\pm$ 30.12	3.02 $\pm$ 0.25	68.34 $\pm$ 12.23	80.05 $\pm$ 32.23	43.33 $\pm$ 4.21
p	< 0.001	< 0.001	< 0.05	< 0.05	< 0.0001	< 0.05

Determinations on long chain fatty acids are given separately for healthy subjects (table 2) and the studied patients (table 3).

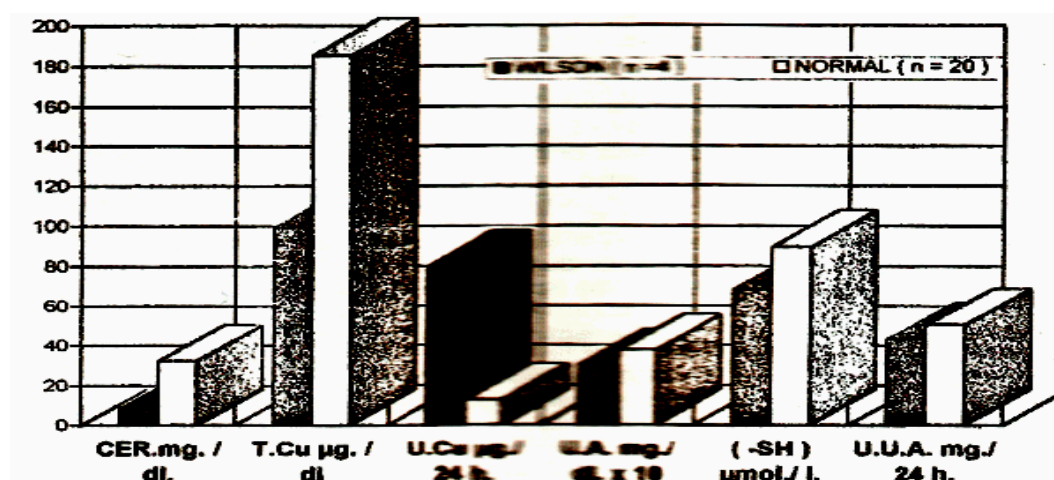
Table 2. Plasma saturated long chain fatty acids in normal persons (expressed as % of total fatty acids)

Age (years)	C: 20	C: 22	C: 24	C: 26	C: 22/ C: 20	C: 24/ C: 22	C: 26 C: 24	C: 26 C: 22	C: 26 C: 20
20	0.345	0.985	0.997	0.023	2.855	1.012	0.023	0.023	0.067
20	0.345	1.440	1.010	0.035	4.174	0.701	0.035	0.024	0.101
20	0.350	0.987	0.995	0.022	2.820	1.008	0.022	0.022	0.063
20	0.350	1.437	0.997	0.035	4.106	0.694	0.035	0.024	0.100
20	0.361	1.350	1.100	0.030	3.740	0.815	0.027	0.022	0.083
20	0.400	1.390	1.010	0.032	3.475	0.727	0.032	0.023	0.080
20	0.402	1.290	0.997	0.033	3.209	0.773	0.033	0.025	0.082
20	0.410	1.350	1.000	0.024	2.293	0.741	0.024	0.018	0.059
23	0.408	1.378	0.988	0.024	3.377	0.717	0.024	0.017	0.059
23	0.409	1.370	0.889	0.033	3.350	0.649	0.037	0.024	0.081
23	0.416	1.370	0.889	0.024	3.293	0.649	0.027	0.018	0.058
23	0.416	1.392	0.893	0.026	3.346	0.642	0.029	0.019	0.063
23	0.420	1.361	0.987	0.025	3.240	0.725	0.025	0.018	0.060
23	0.451	1.400	1.010	0.030	3.104	0.850	0.025	0.021	0.067
25	0.345	1.400	0.900	0.034	4.058	0.643	0.038	0.024	0.099
25	0.346	1.400	0.944	0.034	4.064	0.674	0.036	0.024	0.098
25	0.349	1.400	0.932	0.033	4.011	0.666	0.035	0.024	0.095
25	0.350	1.390	0.889	0.033	3.971	0.640	0.033	0.024	0.094
25	0.357	1.401	0.920	0.029	3.924	0.657	0.032	0.021	0.081
25	0.357	1.440	0.900	0.033	4.034	0.625	0.037	0.023	0.092
MEAN	0.379	1.347	0.962	0.030	3.521	0.730	0.030	0.022	0.079
+ SD	0.034	0.128	0.058	0.005	0.522	0.113	0.005	0.003	0.016

Table 3. Plasma saturated long chain fatty acids in subjects with Wilson's disease (expressed as % of total fatty acids)

Age (years)	C : 20	C : 22	C : 24	C : 26	C : 22 / C : 20	C : 24 / C : 22	C : 26 / C : 24	C : 26 / C : 22	C : 26 / C : 20
20	0.556	1.470	1.112	0.034	2.644	0.756	0.031	0.023	0.061
21	0.630	1.495	1.111	0.035	2.373	0.743	0.032	0.023	0.055
25	0.559	1.401	1.108	0.035	2.506	0.791	0.032	0.025	0.063
25	0.620	1.431	1.109	0.035	2.308	0.775	0.032	0.024	0.056
MEAN	0.581	1.449	1.110	0.035	2.458	0.766	0.032	0.024	0.059
+ SD	0.039	0.042	0.002	0.000	0.149	0.021	0.000	0.001	0.004
p	< 0.001	N.S.	< 0.001	< 0.05	< 0.001	N.S.	N.S.	N.S.	< 0.05

The general investigations (e.g.: ceruloplasmin, cooper, uric acid, cystine, etc.) were also represented by diagrams – fig. 1.

**Fig. 1:** Biochemical findings in blood and urine of subject

In figure 2 there are presented comparatively the data for long chain fatty acids in healthy subjects and in patients.

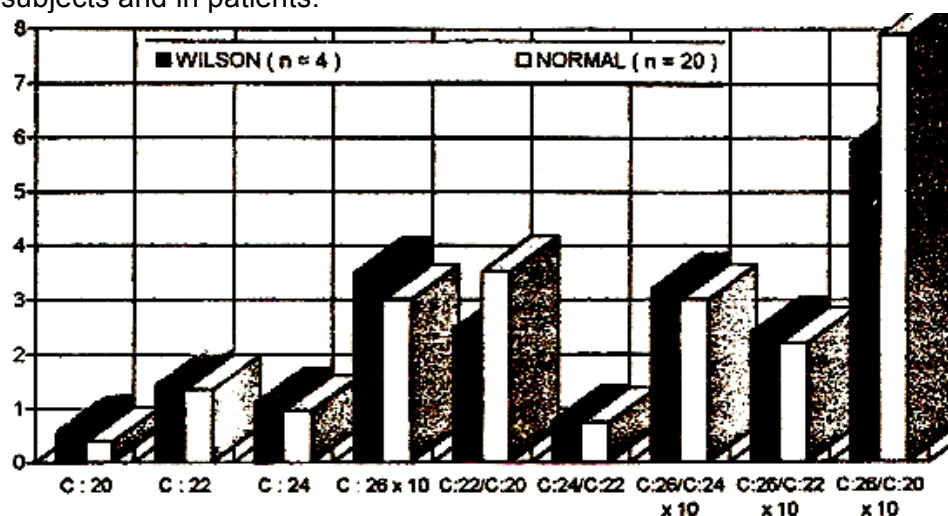


Fig. 2: Plasma saturated long chain fatty acids in subjects

CONCLUSIONS

1. Of all the biochemical observations pertaining to copper metabolism in the present investigation. The significantly lowered ceruloplasmin value for the group of patients as a whole was the most striking. There was not a single case with ceruloplasmin level higher than 15 mg/dl.
2. Our four patients showed a significantly lower plasma and urinary uric acid level compared with the normals.
3. We have measured concentrations of total cysteine in the fasting plasma of Wilson's and healthy subjects. We found a statistically significant decrease of cysteine
4. We found a statistically significant increase in plasma levels of saturated very long chain fatty acids in patients with Wilson's disease. The abnormality was most striking for the C:20, C:24, C:26 and less for C:22. The fatty acids ratios suggest that the abnormality involves mainly C:20 and C:26 saturated fatty acids with lesser involvement of C:22, C:24.
5. We do not exclude in this case an essential modification of antioxidant status for subjects with Wilson's disease.

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DISTRIBUTION PATTERNS OF METALS IN SEDIMENTS OF THE LOWER DANUBE SYSTEM

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ABSTRACT

As part of a research dealing with the biochemistry of metals in the Lower Danube System, the distribution in soil/sediment was characterized. No acute pollution was detected either in the lower Danube Floodplain or in the lakes and channels of the Danube Delta. In the aquatic ecosystem of the Danube Delta we found a contamination gradient of the superficial sediment related to the hydroconnectivity gradient from the Danube. The mechanism of the contamination seems to be related to sedimentation. No such clear patterns were found in the aquatic ecosystems of the Danube floodplain. The least contaminated sediments were found mainly in the aquatic ecosystems located in the center of the investigated alluvial island, suggesting a superposition of the effects of the two kin dof floods: over the natural levee and by channels. The concentrations of metals in the floodplain aquatic sediments were higher than those from the delta in the case of Pb, Zn, Cr, Zr and Ni, and smaller in the case of Cu. The differences between the different types of ecosystems of the floodplain were related mainly to soil/sediment structure and redox conditions.

Key words: metals, Lower Danube System, soil, sediment

INTRODUCTION

Heavy metals are stable pollutants of ecotoxicological importance (Ramade, 1992). Even if they reach acute concentrations in the Danube sediments only locally (Vadineanu and Cristofor, 1992; Equipe Cousteau, 1993), they may have negative effects on the populations and the integrating ecosystems by phenomena of bioaccumulation and bioconcentration (Wachs, 1998). It is well known that wetlands can be effective in the temporary retention of part of the fluxes crossing them (Finlayson, 1994), but to what extent this retention can affect their functioning it is still unclear. The answer depends on the characterization of the biogeochemistry of the metals in the envisaged ecological systems. A first step in this direction is to describe the distribution of metals in the ecosystem compartments, from which the main abiotic one is soil/sediment. Four work hypotheses were considered in this respect:

1. No acute levels of metals occur in the lower Danube System soil/sediments
2. There are significant differences in the contamination of the sediments from the Danube Delta compared to the lower Danube floodplain.
3. There is a gradient of the metals concentrations in sediments along the hydroconnectivity gradients stating from the Danube.
4. There are significant differences in the concentrations found in the soil/sediment of different types of ecosystems from the Danube floodplain.

The activity was developed in the frame of national and international research projects in 1992-94 in the Danube Delta (Mihăilescu et al., 1994) and in 1996-97 in the Danube floodplain (Iordache et al., 1998). The paper is based on the integrated analyses of the two pools of data.

MATERIALS AND METHODS

A transect of 800m length was established in the Fundu mare Island, which is a 20 km² island remained under natural flooding regimen. The transect consisted of five stations, starting from the shore (H1), crossing the natural levee (H2), an intermediary zone (H3), a marsh (H4) and ending (H5) near a lake (LC). Another transect in the same island started at the Danube, went along a channel (two stations, CC1 and CC2) and crossed five lakes connected by short channels (LC, LS, LI, LB and LF, each one with two or three stations). An early succesional reach was studied on a different island (Small Island of Brăila). In this case the transect consisted of seven stations: I1 (on a beach), I2, A1 (on a low levee), I3 (in an oxbow lake), I4, A2 (on the main levee), B (on a forested shore). A more detailed description is given elsewhere (Vădineanu, 1997, 1998). In the Danube Delta up to 20 stations were established in the main lakes and channels (Mihăilescu et al., 1994).

Soil/sediment was sampled monthly in the Danube floodplain in 3 to 9 replicates, depending on the ecosystem type and two times an year in the Danube Delta. The replicates from all the stations of an ecosystem were homogenized and analyzed for up to 20 elements (from which we present here 8: Cu, Cr, Fe, Mn, Ni, Pb, Zn and Zr) by mass spectrometry and X-ray fluorescence, with control samples analyzed for most of these elements (not for Zr) by graphite furnace atomic absorption spectrophotometry.

Control parameters such as surface and ground water level, soil granulometry, humidity, temperature, redox potential, loss on ignition, were determined by methods described elsewhere (Iordache et al., 1997b; Postolache et al., 1997). Information about the areas with high sedimentation rates in the Danube Delta was obtained from satellite images (Drost, 1991). Statistical analyses of the data was performed with the Statistica 4.5 package (StatSoft Inc., 1993).

RESULTS AND DISCUSSIONS

Hypothesis 1 and 2

Table 1a shows the average concentrations of metals in the studied types of ecosystems. Table 2 contains selected data from the literature concerning soil/sediment in wetland/aquatic systems at polluted/unpolluted sites. All the average concentrations are in the range reported in literature, excepting for Mn, which is slightly above the highest value and for Zr. However, in the case of Zr only one source was identified. In what concerns the ecotoxicologically important metals (Cr, Cu, Ni, Pb, Zn) they are all in the lower part of the reported range, with values higher than those found in unpolluted areas, but much lower than those from polluted areas. One can conclude that no acute pollution with metals occurs in the lower Danube system, which sustains earlier statements on this topic (Vădineanu and Cristofor, 1992).

Also in Table 1a one can see the results of the Mann-Whitney test applied for differences between the aquatic systems from Danube Delta and those from DANube floodplain. Only Cu had higher values of concentrations in the sediments of the Danube Delta. The sediments from the Danube floodplain were found to be richer in Zr, Zn, Ni, Cr and Pb. another interesting point is that Zn, Ni, Cr and Pb are a cluster of metals significantly and positively related (R values and p levels in given table 1b). Cu is negatively related with this cluster at lower R and higher p values. To explain this finding more detailed research is needed.

Table 1: a) Average concentrations of metals in the investigated ecosystem and the results of the statistical test; b) Regression matrix between the average metal concentrations in the studied aquatic ecosystems (significant at 0.05=*, 0.01=**, 0.001=***).

Table 3 The results of the Mann-Whitney U test for differences between the floodplain stations (significant at 0.05=*, 0.01=**, 0.001=***).

	Zr	Zn	Cu	Ni
H1=1, H2=2, H3=3 H4=4, H5=5	13* 23* 25*	12*** 13*** 14*** 15*** 24*** 25*** 35*** 45*	12** 24* 25** 35*	
I1=1, I3=2, B=3, I2=4 A1=5, I4=6, A2=7		12* 13*** 14*** 15*** 16** 17*** 25** 28* 27*** 32*** 34*** 35*** 45* 47*** 56* 57***	13** 14* 16* 17* 25* 32** 35* 36** 37* 56*	25* 27* 32*
	Fe	Mn	Cr	Pb
H1=1, H2=2, H3=3 H4=4, H5=5	12** 13*** 14*** 15*** 23*** 24** 25***	12*** 14* 23***		35*
I1=1, I3=2, B=3, I2=4 A1=5, I4=6, A2=7	12* 13*** 14*** 15*** 16** 17*** 25*** 27*** 32*** 3445** 47*** 56*** 58*** 59*** 67***	24*** 25*** 34*** 45*** 12* 13* 14* 15** 16** 17** 24* 25* 26* 27** 32** 34*		13* 15* 25* 32*

Table 4 Synthetic characterization of the studied ecosystems in the Danube floodplain.

Parameter / Station	H1	H2	H3	H4	H5	I1	I2	I3	I4	CC1,2	LC, LS, LI, LB, LF
Ecosystem type	1	2	3	4	4	1	2	5	2	0	7
Direct anthropogenic impact	0	1	0	0	0	0	1	0	1	1	1
Average water level (cm)	132	-208	26	63	82	48	-108	78	-221	200	150
Min water level (cm)	-100	-400	-150	-150	-80	-150	-300	-150	-400		
Max water level (cm)	420	80	210	280	320	298	163	343	48		
Water speed when flooded	1	3	3	3	3	1	2	2	3	2	3
Soil type	1	2	2	4	4	1	3	3	3	3	4
Soil humidity (% average)	28.8	30.2	44.8	43.2	48	27.5	29.5	38.2	29.1		
Soil pH (average)	7.43	7.51	7.19	7.19	7.21	7.35	7.59	7.33	7.8		
Min soil pH	7.02	6.84	6.53	6.45	6.65	6.28	6.03	5.98	6.22		
Redox potential in soil at 5 cm (mV, average)	126	380	108	54	-38	308	289	213	409		

Ecosystem type:

1 = shore, 2 = natural levee

3 = internal zone of intermediary
altitude 4 = depression

Anthropogenic impact:

0 = none

1 = low

Soil type:

1 = sandy and silty strips
alternating

2 = sand and silt in equal parts,
90%, 3 = silty, >70%

Water speed when flooded:

1 = high, 2 = low 3 = low or stagnant

Hypothesis 3

Figure 1 shows the relationship between a parameter describing the relative sediment contamination with metals in the Danube delta ecosystem (an average of standardized concentration by minimal values) and a parameter describing the position of the ecosystem in the landscape, basically depending on the distance from and the connection with Danube river, so describing a hydro-connectivity gradient (details for computation are given in Iordache et al., 1997a). One can see that there is a clear trend of decreasing contamination along the hydro-connectivity gradient.

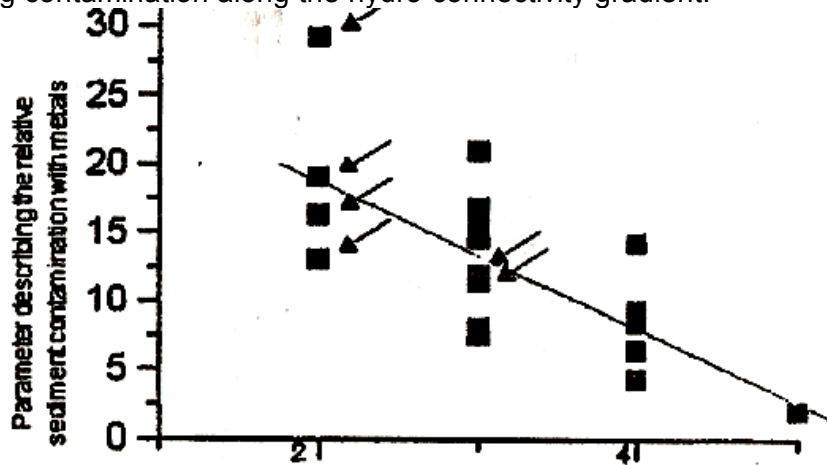


Fig. 1: Relationship between the position on the hydro-connectivity gradient and the relative contamination with metals for the aquatic systems of Danube delta

The arrows indicate those lakes which appear to have intensive sedimentation on remote sensing images. They are associated with high relative contamination, suggesting that sedimentation is an important mechanism controlling the metals concentration in sediments.

The same kind of representation performed for the aquatic ecosystems of Fundu Mare Island (Danube floodplain) did not suggest any simple trend. In figure 2 we present the situation for four selected metals (values on the plot standardized to SD to be comparable between metals, Mann-Whitney U test performed on raw values). Few differences between directly connected ecosystems are statistically significant, suggesting however that, at least in the case of Zn and Cu, higher concentrations are found in lakes located at the two ends of the gradient. The increasing Fe concentrations toward the middle of the islands is probably due to soil characteristics (high percent of clay, see table 4) whereas Cr shows no clear patterns of variation.

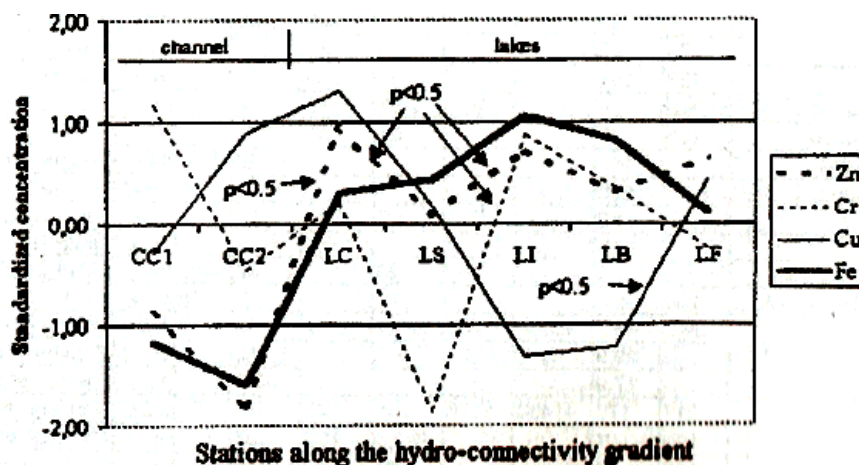


Fig. 2: The distribution of metals in the aquatic ecosystems of the Fundu Mare Island (standardized concentrations). Arrows indicate significant differences

In order to interpret these findings we have to take into consideration that in the floodplain the inundation takes place in two ways: by the channel during low floods, according with the hydro-connectivity gradient, and over the the natural levee at high flood, actually against the usual gradient. On the other hand, in both situations the relatively short length of the island compared to the Danube delta could lead to smaller differences in the sedimentation taking place in the aquatic systems.

Hypothesis 4

Table 3 shows the results of the Mann-Whitney U-test for differences in the yearly average concentrations found in the investigated transects in the floodplain (not included the aquatic ecosystems, already discussed above; they are comparable from the point of view of metals with marsh stations H4 and H5). There are very significant differences in the case of Zn, Fe and Mn. Cu and Pb shows also some heterogeneity on the distribution at landscape level, whereas Ni, Zr and especially Cr are much more homogenous distributed at this scale. Figure 3 presents a scatter diagram of Fe concentrations vs. Cu concentrations, indicating the different types of ecosystems to which the values correspond. Trends of variations of Mn, Zr and Cr are also indicated on the plot. One can remark that levee stations generally have high values of Cu, Mn, and average values of Fe and Zr. Shore and channel stations and the lakes are characterized by low values of Cu and Mn, but are different in what concerns Fe and Zr. This could be explained by reference to table 4. We suggest that Fe, Zr and Cr distribution is related to soil granulometry, whereas that of Cu and Mn depends more on redox conditions, which at least in the case of Fe, Zr and Mn is plausible (Alloway, 1995). Further, more specific research is needed to confirm these new hypothesis.

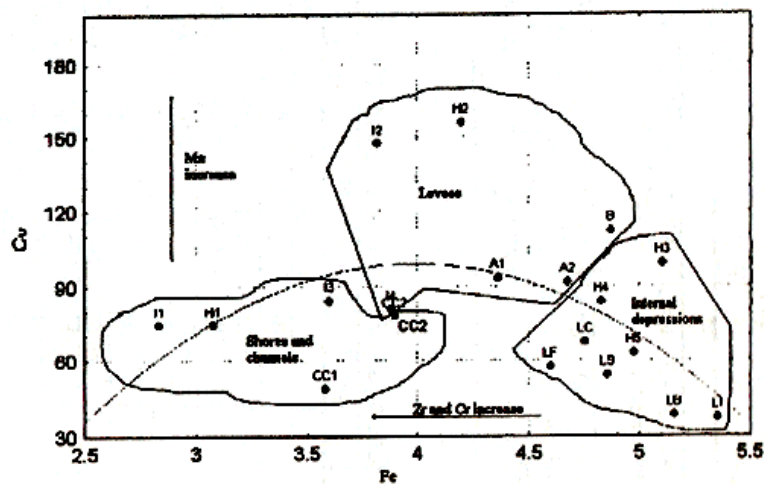


Fig. 3: Distribution of Fe and Cu in the floodplain ecosystems (% and ppm)

Table 3 The results of the Mann-Whitney U test for differences between the floodplain stations (significant at 0.05=*, 0.01=**, 0.001=***).

	Zr	Zn	Cu	Ni
H1=1, H2=2, H3=3 H4=4, H5=5	13° 23' 25"	12° 13' 14" 15" 24° 25' 35" 45"	12° 24' 25" 35"	
I1=1, I3=2, B=3, I2=4, A1=5, I4=6, A2=7		12° 13' 14" 15" 16" 17° 25' 28" 27° 32' 34" 35° 48' 47" 55' 57"	13° 14' 16' 17' 25" 32° 35' 36' 37' 56"	25° 27' 32"
	Fe	Mn	Cr	Pb
H1=1, H2=2, H3=3 H4=4, H5=5	12° 13' 14" 15° 23' 24" 25"	12° 14' 23"		35°
I1=1, I3=2, B=3, I2=4, A1=5, I4=6, A2=7	12° 13' 14" 15" 16" 17° 25' 27" 32° 34' 45' 47" 55° 57' 35" 36" 57" 67"	24° 25' 34" 45° 12' 13" 14" 15° 16' 17" 24" 25° 26' 27" 32" 34"		13° 15' 25' 32"

Table 4 Synthetic characterization of the studied ecosystems in the Danube floodplain.

Parameter / Station	H1	H2	H3	H4	H5	I1	I2	I3	I4	CC1,2	LC, LS, U, LB, LF
Ecosystem type	1	2	3	4	5	4	1	2	5	2	6
Direct anthropogenic impact	0	1	0	0	0	0	0	1	0	1	1
Average water level (cm)	132	-208	26	26	63	82	48	-108	78	-221	200
Min water level (cm)	-100	-400	-150	-150	-150	-80	-150	-300	-150	-400	150
Max water level (cm)	420	80	210	210	260	320	298	163	343	48	
Water speed when flooded	1	3	3	3	3	3	1	2	2	3	3
Soil type	1	2	2	2	4	4	1	3	3	3	4
Soil humidity (% , average)	28.8	30.2	44.8	43.2	48	48	27.5	29.5	36.2	29.1	
Soil pH (average)	7.43	7.51	7.19	7.19	7.21	7.21	7.35	7.59	7.33	7.8	
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Redox potential in soil at 5 cm (mV, average)	129	380	108	54	-38	-38	308	269	213	409	

Ecosystem type:

1 = shore, 2 = natural levee

3 = internal zone of intermediary
altitude, 4 = depression

Anthropogenic impact:

0 = none

1 = low

Soil type:

1 = sandy and silty strips

alternating

2 = sand and silt in equal parts,

90%, 3 = silty, >70%

Water speed when flooded:

1 = high, 2 = low 3 = low or stagnant

CONCLUSIONS

The main conclusions are:

1. No acute levels of pollution were detected in the Lower Danube floodplain or in the lakes and channels of the Danube Delta
2. On an average base the concentrations of metals in the floodplain aquatic sediments were higher than those from the delta in the case of Pb, Zn, Cr, Zr and Ni and smaller in the case of Cu.
3. In the aquatic ecosystems of the Danube Delta a contamination gradient of the superficial sediment was found, related to the hydro-connectivity gradient from the Danube. No such clear pattern was found in the aquatic ecosystems of the Danube floodplain
4. There are many significant differences between the different types of floodplain ecosystems in the case of Zn, Fe and Mn, fewer significant differences in the case of Cu, Pb, Ni and Zr and not significant differences in the case of Cr.

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SELENIUM LEVEL IN PATHOLOGICAL STATES OF POPULATION WITH LOW SELENIUM INTAKE

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ABSTRACT

Nowadays, there are plenty of evidences that a dietary deficiency of Selenium (Se), an important part of antioxidative defense system in organism, can predispose the living system to oxidative stress and contribute the increase in the number of patophysiological events including carcinogenesis, deteriorating the defense against maleffects of ionizing radiation and therapeutic drugs and facilitates aging. The global study of Se intake and blood content among Serbian population showed that we are a Se deficient area. The increased incidence of several kinds of cancer and cardiovascular disorders, correlate well with low Se content. Our investigation confirmed another data that patients with some cancers and cardiovascular diseases have lower Se blood level than healthy subjects from the same area. The common therapy in these cases, additionally reduced already low Se level enhancing the oxidative stress in the patient organism.

Key words: selenium blood content, selenium deficiency, malignant diseases, cardiovascular disorders, cancer therapy

INTRODUCTION

Oxygen is a required component of normal aerobic metabolism used to generate the energy (heat) necessary to drive the many reactions required to sustain life. To prevent damage to the cell from high levels of oxygen turnover and production of highly reactive forms free radicals (FR) of oxygen, safeguards are necessary. These safeguards exist as both enzymatic and nonenzymatic compounds. The antioxyenzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and enzymes of the hexose monophosphate shunt, require cofactors such as trace elements: selenium (Se), manganese (Mn), magnesium (Mg) and zinc (Zn), while the nonenzymatic systems rely on the presence of antioxidant molecules such as glutathione or vitamins E, A, C. FR damage to cells occurs when antioxidant defenses in the organism are overwhelmed induced the state of oxidative stress. A large number of scientific publications over the last three years have implicated oxidative stress in the pathology of more than 50 human diseases including AIDS, cancer, cardiovascular diseases, arthritis, multiple sclerosis, chronic fatigue syndrome, psoriasis and asthma (Halliwell 1987, Gutteridge et al., 1993, Esterbauer et al., 1993, Oliver 1995, Baker et al., 1988, Glabinski et al. 1993) and also in the normal aging process (Niva et al., 1989).

Furthermore, the therapies and drugs used in the treatment of these diseases, may themselves contribute to increased oxidative stress.

Trace elements play an important role in oxygen metabolism and formation of FR such as iron (Fe) and copper (Cu), being usually the main enhancers of its formation, whereas other trace elements, such as Zn, Mg and Se, protects against the harmful effects of these radicals. Alterations in status of some trace elements as the important part of antioxidant defense, may induce or contribute to the expression of physiological disorders, and at the same time, may be a consequence of the disease.

Selenium is one of the essential components of the endogenous antioxidative defense system. It is a constituent of enzyme glutathione-peroxidase that is the scavenger of hydroperoxides, phospholipid hydroxiperoxides, a part of selenoprotein-p and other selenoproteins, also a part of 5'-deiodinase important in thyroid metabolism, and some amino acids. The level and the ability of this essential trace element in the organism are dependent of the exogenous supplementation. On our Earth we may find areas with low level of Se in the ground and/or with low viability of Se for the food chain. Nowadays, we have plenty evidences that a deficiency of Se, as an important part of antioxidative defense, can enhance susceptibility of the living system to oxidative stress and disorders impaired with it (Combs and Combs 1986). Now, it has been established that the severe Se deficiency is the one of the major contributors for development of endemic cardiomyopathy - Keshan disease (The group of Environment and Endemic Disease from Chinese Academy of Science 1981), endemic osteoarthropathy, known as Kashin-Beck disease (The group of Environmental and Endemic Diseases, CAS. Kashin-Beck disease in China:1990) first described in China. Se deficiency has been linked with high risk for several kinds of cancer, cardiovascular failure, neuronal and muscular disorders (Virtamo et al., 1987, Tan and Huang 1991, Xia et al. 1992). In the last few years, it has been found that there is an increased susceptibility of Se-deficient individuals toward virus infection (like Cocksackie virus) and virally and chemically induced tumorigenesis (Beck et al., 1995, Baldwin and Parker 1986).

Ten years of geochemical investigation of waters and food in Serbia (Maksimovic, 1991, Maksimovic et al., 1992), showed that significant deficiency of Se exists in domestic food and majority of water samples. Analyses of soils and rocks also showed that most of the samples were low in Se content. Epidemiological investigations in Serbia confirmed previous observations that our population intake of Se is very low due to low Se content in the food chain. Consequently, it is found that Se level in blood of Serbian population is low. Data given for a few selected countries (Kumpulainen, 1989, Gissel-Nielsen, 1994, Bruggemann, 1996) indicate a similar correlation between the Se-intake and blood Se. On the other hand, cardiovascular diseases with 58.8% and malignant diseases with 14%, are the main cause of mortality among our population. The higher incidence of these diseases correlates well with Se deficiency areas (Maksimovic et al., 1992). The studies among the patients with cancer also showed a greater rate of mortality in the areas with higher deficiency of Se.

The aim of this study was to estimate the Se level in blood of patients of cardiovascular failure and several kinds of cancer that occurs most frequently in our population. It was also interesting to study the effect of common therapies in that cases on Se level in blood.

MATERIALS AND METHODS

Samples of venous blood were collected from 1. healthy individuals originally enrolled in epidemiological programs and blood donors 2. patients with established diagnosis of several kinds of malignant disease and cardiovascular failure. Four ml of venous blood were taken by syringe heparinized with 0.1 ml of heparin and stored at -20°C for Se determination in whole blood, or immediately centrifuged for 10 min. at 3500 r/min. to separate blood plasma. The erythrocytes were washed 3 time's with 0.9 % NaCl and then resuspended in it (1:1). Plasma and suspension of erythrocytes were stored at -20°C .

All stored samples were wet ashed first with concentrated $\text{HNO}_3/\text{HClO}_4$ mixture and than with 6M HCl, in borsilicate test tubes placed in an aluminium block heater. The digested material stored in 6M HCl till used for estimating Se concentration.

The Se concentrations were determined by gaseous hydride generation atomic absorption spectrometry (AAS), using a Perkin Elmer 5000 atomic absorption spectrometer equipped with MHS-10 vapour generator accessory.

Analytical procedures were validated using certified reference material IAEA A-12 - Human blood, Contox 0148 level I - Human serum).

The statistic were made with SPSS statistic program.

RESULTS AND DISCUSSION

Results of investigation showed that the mean value determined for Se in whole blood, plasma, and erythrocytes in our population (Table 1) are lower than the values reported in other countries (Maksimovic et al., 1992, Djujic, 1996). In some communities Se plasma content is very low (below $27\text{ }\mu\text{g/L}$) that is very similar with endemic Keshan disease area in China. Indeed, in those communities an increased incidence of cardiovascular and some kind of malignant diseases was found. The very low blood Se content was also connected with areas with Balkan endemic nephropathy (Maksimovic and Djujic, 1998).

Table 1. Se levels in blood, plasma, erythrocytes of Serbian population (1987-1994)

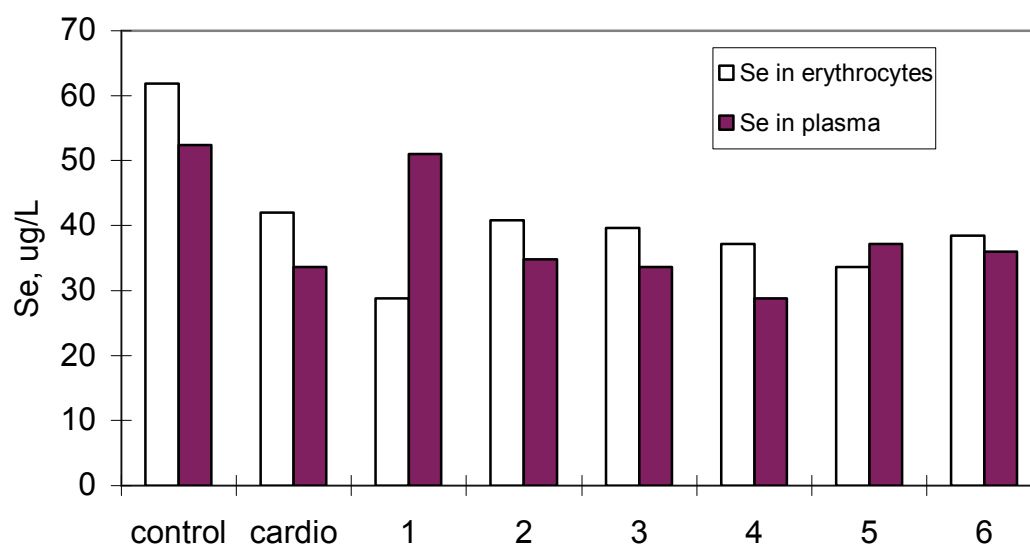
Sample	n	Se, $\mu\text{g/l}$
Whole blood	462	58.2 ± 18.3
Plasma	1456	52.4 ± 12.9
Erythrocytes	1228	61.8 ± 19.6

Our results of measuring the Se blood content in a group of patients with cardiovascular failure, showed lower values (more in the plasma) than in control group of healthy people (Figure 1). The surgical intervention, coronary bypass (knowing that it is Ishaemia-reperfusion oxidative stress), caused the additional fall in Se content in erythrocytes and in blood plasma. The variations in Se level in the individual depends of the clinical severity of heart failure.

Results of estimation of Se levels in the groups of patients suffering from various kinds of cancer showed a lower content of Se in erythrocytes and blood plasma as compared the normal population (figure 1). The lowest Se content in erythrocytes was found in the group of lung cancer patients. Plasma Se content was the lowest in patients of hematological malignancies. The studies among the patients with cancer also showed a greater rate of mortality in the Serbian communities with higher deficiency of Se.

Therapeutic doses of ionizing radiation and anti-tumor drugs that are adequate to eliminate all cancerogenic cells are highly toxic to surrounding healthy tissue and vital functions of the organism. In our case, the data obtained, indicates that oxidative stress induced by common radio and chemotherapy reduce level of Se in blood and also increase parameters of oxidative stress. The variation among the patients were great, depending on the individual state of the organism, previous Se level in patient's blood and the state of illness.

Fig. 1. Se content in erythrocytes and blood plasma of patients with cardiovascular and malignant diseases



cardio - patients with cardiovascular failure, 1 - 6 patients with malignant disease
 1- lung cancers, 2- gastrointestinal cancers, 3- urogenital cancers, 4- hematological cancers, 5- skin cancers and 6- other cancers

CONCLUSIONS

The conclusion of the presented results is that the deficiency of an essential trace element Se in Serbia significantly influences on the health of our population increasing the incidence of cardiovascular and malignant diseases.

The state of illness, cardiovascular or malignant, additionally decreases the Se content in blood deteriorating the antioxidative defense and immunological response of the organism.

The common therapies, surgical intervention, radiation and antitumor drugs contribute the additional decrease in Se content in the blood of patients.

All these cases require the intervention with Se supplementation. The data and our previous investigations (Djujic et al., 1995, Jozanov-Stankov et al., 1998), confirmed the observation that Se supplementation could improve the defense in the organism exposed to oxidative stress (ex. ionizing radiation or antitumor drugs), suggesting that, Se can be an important environmental factor that modifies human health directly or indirectly.

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MINERAL CONTENTS AND SOLUBILITY FROM MEDICINAL HERBS.

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ABSTRACT

Mineral contents and solubility studies were conducted in ten different herb teas used for medical purposes. The samples were dry-ashed and the mineral analysis was done using the ICP-OES. Solubilities were measured at 37°C after 2h incubation. The Ca contents of the medical herbs ranged from 294-3648, Mg 172-716, Fe 4.8-98.4, Zn 1.97-4.71, Cu 0.84-1.60, Mn 2.31-20.95, Ti 0.01-5.12, V 0.01-0.25, and Mo 0.02-0.14 mg / 100g (DM) of samples. The solubility of Ca, Mg, Fe, Zn, Cu and Mn from the herbs varied significantly. Ca solubility ranged from 12.56-30.27, Mg 13.22-60.28, Fe 2.09-23.73, Zn 8.74-36.71, Cu 18.90-60.96 and Mn 5.15-30.38%.

Key words: mineral, solubility, availability

INTRODUCTION

The bioavailability of minerals and trace elements is an essential component of the efforts that are geared towards the world-wide alleviation of mineral deficiency. Human requirements of some trace elements have been established (WHO, 1996). Since these elements are obtained primarily from the diet, their bioavailability, apart from the content, is important in meeting the various levels of requirements by the body. The bioavailability of mineral is depends on variables such as the chemical composition of the diets, the contents of potential enhancers and inhibitors, age, sex and the physiological status of the individuals.

Bioavailability is best estimated with the *in vivo* methods. However, as an alternative to human and animal studies, the availability of minerals and trace elements has also been estimated with the simple, rapid and reproducible *in vitro* method. The chemistry of iron digestion and the interactions of dietary components ultimately determine the bioavailability of Fe to the body. Simulated *in vitro* digestion and the chemical analysis of foods are practical means of approaching the underlying mechanisms that are manifested in bioavailability. the solubility of minerals is a prerequisite for absorption. Hence, factors that maintain minerals in the soluble phase will enhance bioavailability. The research work was to determine the chemical and bioavailable minerals, especially Fe, in a wide range of foodstuffs and beverages.

MATERIALS AND METHODS

Six replicate samples of medical herbs were purchased from different locations in Jena. The herb samples were dried at 105⁰ C to constant weight to determine the dry matter (DM). After dry ashing at 450⁰ C, the samples were dissolved in hydrochloric acid 25% and thinned down with aqua bidest to 2.5%. The mineral contents of the samples were analysed by inductively coupled plasma atomic emission spectrometry – ICP OES (Spectroflame-D, Spectro Analytical Instruments).

The sensitivity of the equipment was estimated by the analyses of the "ARC/CL Total diet reference material – HPD (Kumpulainen and Tahvonen, 1990).

In vitro solubility of minerals from herbs and food samples. Ten grams of thoroughly blended herbs were mixed with 100ml aqua bidest and incubated at 37°C for 2 h. Aliquots of the supernatants were dried later in mineral free crucibles, ashed, dissolved and prepared similarly for the mineral analysis by ICP-OES. The percentage of solubilized minerals was calculated from the total mineral contents of the samples.

RESULTS AND DISCUSSION

The Ca contents of the medical herbs ranged from 294-3648, Mg 172-716, Fe 4.8-98.4, Zn 1.97-4.71, Cu 0.84-1.60, Mn 2.31-20.95, Ti 0.01-5.12, V 0.01-0.25, and Mo 0.02-0.14 mg / 100g (DM) of samples. The solubility of Ca, Mg, Fe, Zn, Cu and Mn from the herbs varied significantly. The Ca solubility ranged from 12.56-30.27, Mg 13.22-60.28, Fe 2.09-23.73, Zn 8.74-36.71, Cu 18.90-60.96 and Mn 5.15-30.38%.

Table 1: Mineral Contents of Some Medical Herbs (mg/100 dry matter)

Herbs	Ca	Mg	Fe	Zn	Cu	Mn	Ti	V	Mo
Peppermint leaves <i>Menthae piperita</i>	1418 ±30	717 ±28	31 ±0.75	3.2 ±0.09	1.05 ±0.05	6.31 ±0.10	0.208 ±0.009	0.042 ±0.001	0.139 ±0.005
Nettel herbage <i>Urtica dioica</i>	1124 ±37	279 ±18	4.9 ±0.11	2.8 ±0.08	1.2 ±0.05	2.3 ±0.06	0.009 ±0.00	0.004 ±0.00	0.016 ±0.001
Horsetail herbage <i>Equisetum arvense</i>	64 ±1.7	216 ±1.6	12 ±0.36	2.0 ±0.02	0.84 ±0.016	8.5 ±0.013	0.010 ±0.001	0.017 ±0.00	0.022 ±0.00
Balm leaves <i>Melissa officinalis</i>	294 ±30	210 ±18	98 ±3.3	2.9 ±0.18	0.84 ±0.07	6.9 ±0.72	5.1 ±0.57	0.24 ±0.03	0.021 ±0.002
Klamath weed <i>Hypericum perforatum</i>	468 ±5.0	172 ±6.7	13 ±0.27	4.2 ±0.03	1.2 ±0.029	21 ±0.24	0.83 ±0.003	0.017 ±0.00	0.023 ±0.00
Valerian root <i>Valeriana officinalis</i>	1533 ±24	593 ±4.3	32 ±0.91	3.0 ±0.07	1.4 ±0.13	8.7 ±0.11	0.91 ±0.11	0.053 ±0.003	0.082 ±0.03
Milfoil herbage <i>Achillea millefolium</i>	898 ±17	342 ±7.9	23 ±1.4	4.2 ±0.26	1.1 ±0.02	4.8 ±0.045	0.82 ±0.048	0.048 ±0.003	0.084 ±0.001
Mixed herbs <i>Species sedativae</i>	3648 ±45	541 ±8.4	24 ±0.92	3.3 ±0.10	1.6 ±0.11	6.6 ±0.09	0.29 ±0.04	0.036 ±0.003	0.14 ±0.002
Fennel leaves <i>Foeniculum vulgare</i>	1731 ±18	403 ±15	51 ±0.76	4.7 ±0.05	0.99 ±0.08	5.5 ±0.04	0.91 ±0.40	0.18 ±0.003	0.030 ±0.00
Sage leaves <i>Salvia officinalis</i>	2122 ±40	572 ±34	19 ±0.65	2.4 ±0.02	0.88 ±0.005	5.3 ±0.14	0.26 ±0.015	0.022 ±0.001	0.03 ±0.001

There are many factors that are responsible for the variations in the mineral composition of foods and beverages. Differences in the mineral contents of plant foods may be due to variety, soil conditions and maturity at harvest. Minerals in animal foods, however, are influenced by age, tissue, sex, nutrition, breed and the amount of activity performed by the animal particularly in the case Fe. Processing procedures, the choice of ingredients and cooking practices contribute to the total and available mineral contents of composite meals, snacks and beverages consumed by population groups. The variations in the chemical availability of minerals from the herbs and food samples analysed could presumably be due to the presence of varying levels of inhibitors and enhancers of availability. Significant

amounts of minerals were solubilised in some of the medical herbs, and these could contribute to the total mineral intake of people who habitually drink the teas.

Table 2. Mineral Solubility of Some Medical Herbs (%).

Herbs	Ca	Mg	Fe	Zn	Cu	Mn
Peppermint leaves	13 0.42	26 0.40	2.1 0.18	12 0.05	30 0.12	10 0.03
Nettel herbage	11 0.16	13 0.09	24 3.9	19 0.39	41 0.79	5.1 0.15
Horsetail herbage	17 1.1	39 1.6	7.8 0.68	26 1.5	61 5.1	23 1.4
Balm leaves	15 0.73	35 0.30	3.5 0.94	19 0.41	59 1.1	23 0.36
Klamath weed	30 0.44	50 0.72	6.9 0.07	37 1.1	58 1.3	30 0.88
Valerian root	22 0.30	48 1.1	5.0 1.7	18 0.54	23 1.7	18 0.19
Milfoil herbage	22 0.64	58 1.5	6.8 0.62	19 0.32	47 4.0	23 0.06
Mixed herbs	19 0.46	60 2.8	3.2 0.26	8.7 0.40	34 2.8	9.6 0.24
Fennel leaves	28 0.15	59 2.1	2.2 0.27	17 0.22	30 2.6	28 0.20
Sage leaves	18 0.73	50 2.2	3.3 0.71	8.9 0.19	19 0.28	22 0.14

Nevertheless, the actual contribution depends on a number of variables that influence the availability of the minerals in the gastrointestinal tract. Some Jamaican herb teas with high polyphenolic contents were reported to reduce Fe absorption in rats (Brown et al.,1990). The Fe absorption from foods consumed with 200-250ml of normally strong tea was reduced by about 60% (Morck et al.,1983). Iron absorption was reduced to a third, and from a vegetable soup to a fourth, when served with tea rather than water (Disler et al.,1975). Furthermore, in a Western breakfast meal, Fe absorption was reduced to 56% by 150ml tea made from 2.5g dry tea (Rossander et al.,1979). The inhibitory effects of tea or tannic acid on Fe absorption from test meals was observed to be dose-dependent (Derman et al.,1977; Siegenberg et al.,1991). The solubility of minerals is a prerequisite for absorption. Hence factors that maintain minerals in the soluble phase of the digestive milieu enhance the bioavailability. In vitro techniques allow studies on the chemical mechanisms that influence the mineral availability. They also have the advantages of cost, speed and reduced variability for screening large numbers of samples. However, the in vitro diffusibility technique was reported not to correlate with in vivo results in detecting the inhibitory effect of tea leaves in a rat diet (Valdez et al.,1992). Several attempts are being made at optimizing and improving the efficiency of various in vitro techniques employed in availability studies (Larsson et al.,1997). The contributions of these herbs in the overall mineral nutrition of the people are being assessed.

CONCLUSIONS

1. The contents and solubilities of minerals from some herb teas varied significantly.
2. The contributions of these minerals to the amount absorbed in the intestinal tract is determined by the synergistic effects of all the variables influencing mineral availability.

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TRANSLOCATION OF HEAVY METALS FROM A SEVERELY POLLUTED BROWN REDDISH SOIL INTO CROPS

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ABSTRACT

The material from a Mollic Brown Reddish soil, severely polluted with heavy metals (Cd, Cu, Pb, and Zn), located around the "Acumulatorul" and "Neferal" industrial enterprises, in the eastern part of Bucuresti city. Lettuce, soybean, and alfalfa were used as test plants. The results concerning both the height and mass of plants and those relating to the translocation of heavy metals from soil into plants and their accumulation in the polluted plants showed that the severity of the phenomenon varied depending on the nature of plant and chemical element. The Cd translocation rate was higher as compared with the Cu, Pb, and Zn translocation. The phosphorus fertilisation may determine an accentuation of heavy metal accumulation in plants. Lettuce accumulated the highest amounts of heavy metals, which means that cultivation of this plant on such soils must be avoided; in other words, the soils polluted with heavy metals are not suitable for lettuce.

Key words: heavy metals, lettuce, soybean, alfalfa, translocation, accumulation, soil pollution, plant pollution.

INTRODUCTION

The pollution pressure, during several decades, on the environment in the eastern part of Bucuresti city, by the daily emissions of toxic gases loaded with particulates of heavy metals from the technological processes in the two industrial enterprises ("Acumulatorul" and "Neferal") has had as a final result a severe soil pollution. Thus, the detailed investigations carried out in this area (Lăcătușu et al., 1998) revealed that the soil is loaded and polluted with heavy metals on 1.784 hectares, of which severe polluted soils, with values of content in the Am horizon higher than the maximum allowable limits (MAL), are on a land area of 390 hectares for Pb, 87 hectares for Cu, and 15 hectares for Zn.

The rest of the area includes soils having heavy metal contents between the MAL and the value of the pedogeochemical background. The Cd pollution is not uniform, and for this reason it was not possible to delineate homogenous mapping units from the viewpoint of the content of this element, the content values being however lower than the MAL proposed by Kloeke (1980).

In order to assess the translocation of the polluting chemical elements from the severely polluted soil to some crops, an experiment was made in the greenhouse, using a soil material taken from the Am horizon of the Mollic Reddish Brown soil near the eastern part of the "Neferal" enterprise, the most polluted area. As test plants, three species, differentiated from the viewpoint of the chemical element absorption capacity, were selected. The importance of different plants in the transfer of heavy metals in the trophic web was recently emphasised by Metz et al. (1997).

MATERIALS AND METHODS

The experiment was carried out in small pots containing 1 kg soil, using as test plants lettuce, soybean, and alfalfa. To have a comparison test, another experiment was carried out, on a soil material from a similar soil, located 3 km far from the emission source, considered as control.

Seven treatments were set up, such as: V_1 - non-polluted soil (control); V_2 - polluted soil; V_3 - polluted soil, fertilised with 120 ppm N + 80 ppm P_2O_5 ; V_4 - polluted soil, fertilised with 120 ppm N + superphosphate (180 ppm P_2O_5); V_5 - polluted soil, fertilised with 120 ppm N, 80 ppm P, and limed with 1,5 g $CaCO_3$ per pot; V_6 - polluted soil, fertilised with 120 ppm N, 80 ppm P, and artificially polluted with 10 ppm Cd + 100 ppm Cu + 1,000 ppm Pb; V_7 - polluted soil, fertilised with 120 ppm N, 80 ppm P, artificially polluted as in the case of V_6 , but limed as in the case of V_5 .

The polluted soil is more acid, with 0.58 pH units, and has a heavy metal content 1.7 (Cd), 7.5 (Cu), 19.6 (Pb), and 7.2 (Zn) times higher than in the case of the non-polluted soil. The other chemical parameters have close values.

During the crop growing period, the soil moisture in the pot was maintained at 60 per cent of the water capacity of soil. In this period, biometric measurements were carried out. The final sampling of plants was carried out at the following phenophases: 8-10 leaves in the case of lettuce; 4 branches in the case of soybean; before blossoming in the case of alfalfa.

The samples for analysis were formed by mixing the individual samples collected during the growing period, that is three individual samples in the case of lettuce and two individual samples in the case of soybean and alfalfa.

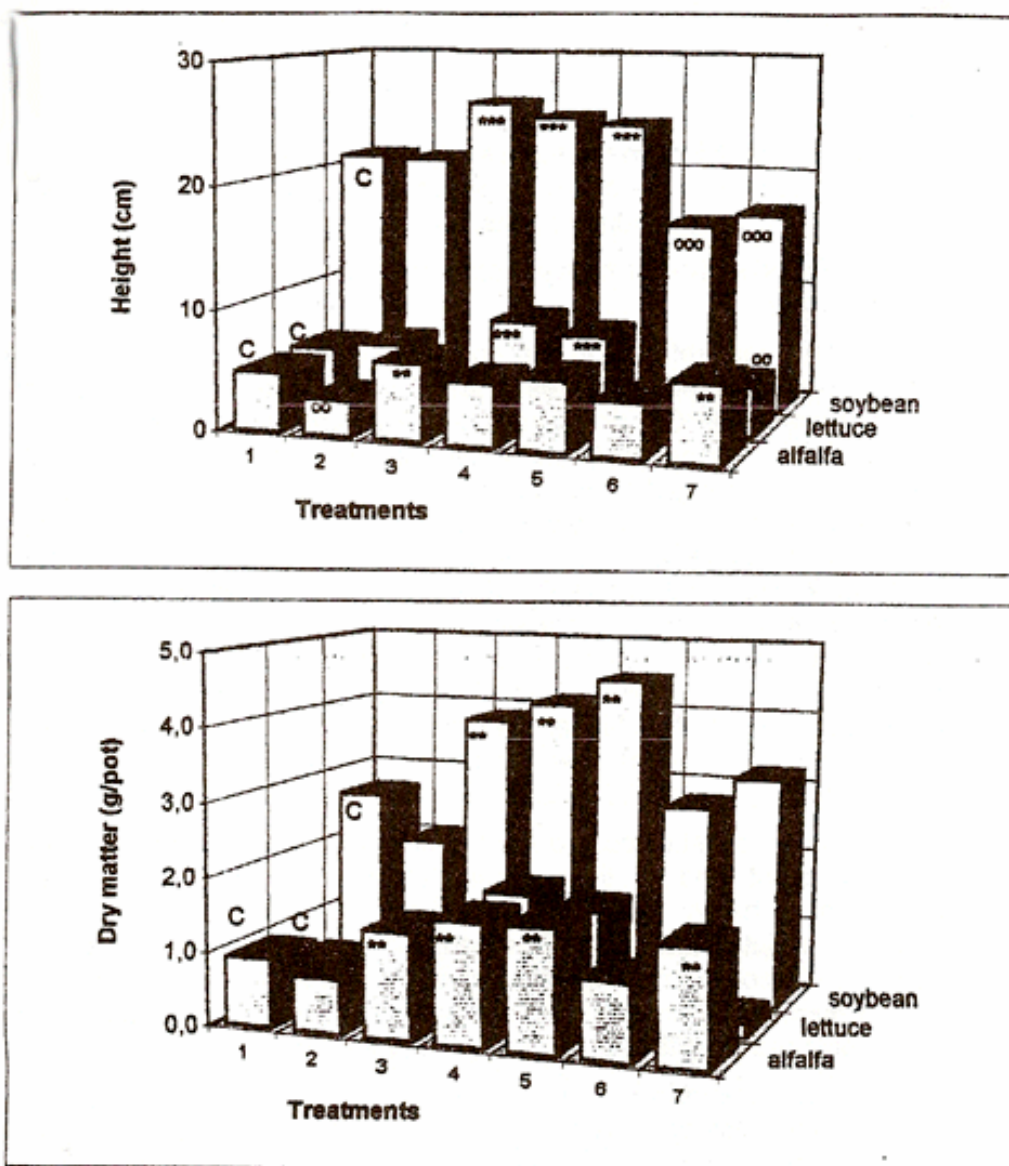
In the laboratory, in the hydrochloric solution obtained by rendering soluble the ash obtained by calcinating at 450° the dry matter of plants, the content of heavy metals (Cd, Cu, Pb, Zn) was measured using the atomic absorption spectrometry.

The main chemical indicators of soil were established taking into account pH in water, mobile P and K (soluble forms in ammonium acetate - lactate at pH 3.7) and mobile forms of heavy metals, soluble in CH_3COONH_4 - EDTA at pH 7.0. The measurements were made by spectrophotometry (P), flamephotometry (K) and atomic absorption spectrometry (heavy metals).

RESULTS AND DISCUSSIONS

Influence of heavy metals on development of lettuce, soybean, and alfalfa

Differences between the treatments were not observed at the beginning of the growing period, the sprouting being simultaneous. A week after the sprouting, some differences began to occur such as colouring and growing of lettuce plants in the sense of the appearance of a yellowish hue with lightly reddish tints and of some smaller and narrower leaves in the cases of treatments V_2 (polluted soil), V_3 (polluted soil, fertilised with NP) and V_8 (double polluted soil). These aspects were accentuated during the growing period, finally leading to the disappearance of lettuce in V_3 and V_6 treatments. The pollution with Pb and Cd negatively influenced also the growing of soybean plants, these ones presenting weak stems, small, slightly stiffened and swirled leaves; on the contrary, as concerns the alfalfa plants, a tendency to stimulate the development in the cases of the Pb and Cd treatments under the conditions of fertilisation and liming occurred, the plants being more vigorous and greener.



- Treatments:
1. Non-polluted soil (control) (C)
 2. Polluted soil
 3. Polluted soil + N_{120} , P_{80}
 4. Polluted soil + N_{120} , P_{180}
 5. Polluted soil + N_{120} , P_{80} , and $CaCO_3$
 6. Artificially polluted soil with Cd (10 ppm), Cu (100 ppm), and Pb (1,000 ppm)
 7. Artificially polluted soil with Cd (10 ppm), Cu (100 ppm), and Pb (1,000 ppm) + $CaCO_3$

Fig. 1. Height and content of dry matter of plants depending on their nature and kind of treatment

The height and the dry matter content of the plants (Figure 1) in the V_2 treatment (polluted soil) were lower as compared with the V_1 treatment (non-polluted soil). Fertilisation with nitrogen and phosphorus, and liming with calcium carbonate determined significant yield increases with all the three species. Accentuation of soil pollution by applying heavy metals (V_6) led to decrease of yield (soybean, alfalfa), until the disappearance of plants (lettuce). Liming of this severely polluted soil (V_7) caused a light invigoration of plants, as compared with those in the V_6 treatment.

Translocation of heavy metals from soil into plant and their accumulation in polluted plants

The data relating to the contents of heavy metals in the three species of plants are presented in figures 2 and 3. Their examination shows that significant concentrations of Cd occurred only with artificially polluted treatments, while significant increases of Cu, Pb, and Zn contents were observed with all the treatments. This situation is due to the lower degree of soil pollution with Cd, as compared with the other heavy metals. A discordant note, by much increased accumulation, is presented by plants in the artificially polluted treatments, as well as those in V_4 treatment, where the rate of phosphorus was two times higher than the optimum rate. The double rate was applied having in view to transfer some higher amounts of heavy metals from soil into plant, hypothesis which by and large was demonstrated. By examining these two figures it may be also observed the capacity of lettuce to accumulate higher amounts of Cd, Cu, and Pb, as compared to the other two species. At the same time, the preferential accumulation of Zn by alfalfa can be observed.

In order to better outline the translocation phenomenon of heavy metals from soil into plant and of their accumulation in polluted plants, the translocation coefficient of heavy metals from soil into plant and accumulation index of heavy metals in polluted plants were defined and computed. The first represents the ratio between the concentration of a chemical element in plant and in soil (mobile form), and the second represents the ratio between the chemical element content in plants from a polluted treatment and the non-polluted control.

The computed values of these two parameters are presented in Table 1. Their examination emphasises significant differences concerning the translocation and accumulation of heavy metals in plants depending on the nature of the investigated plants and chemical elements. Therefore, the increased translocation of Cd is obvious as compared with the other analysed chemical elements (Cu, Pb, Zn) for all the three tested plant species. If the Cd accumulation is separated according to each species it is clearly observed the increased speed of transfer and the higher accumulation of the element in plants of lettuce as compared with the plants of alfalfa and soybean. The feature of lettuce to accumulate heavy metals was pointed out in specific literature (Bergmann, 1993). In other words, lettuce looks like having a higher affinity for heavy metals. In fact the above succession represents the decreasing order of the translocation and accumulation of Cd in the three plant species.

The examination of the values specific to the seven experiment treatments reveals, in spite of the fact that the transfer speed is lower in the case of artificially

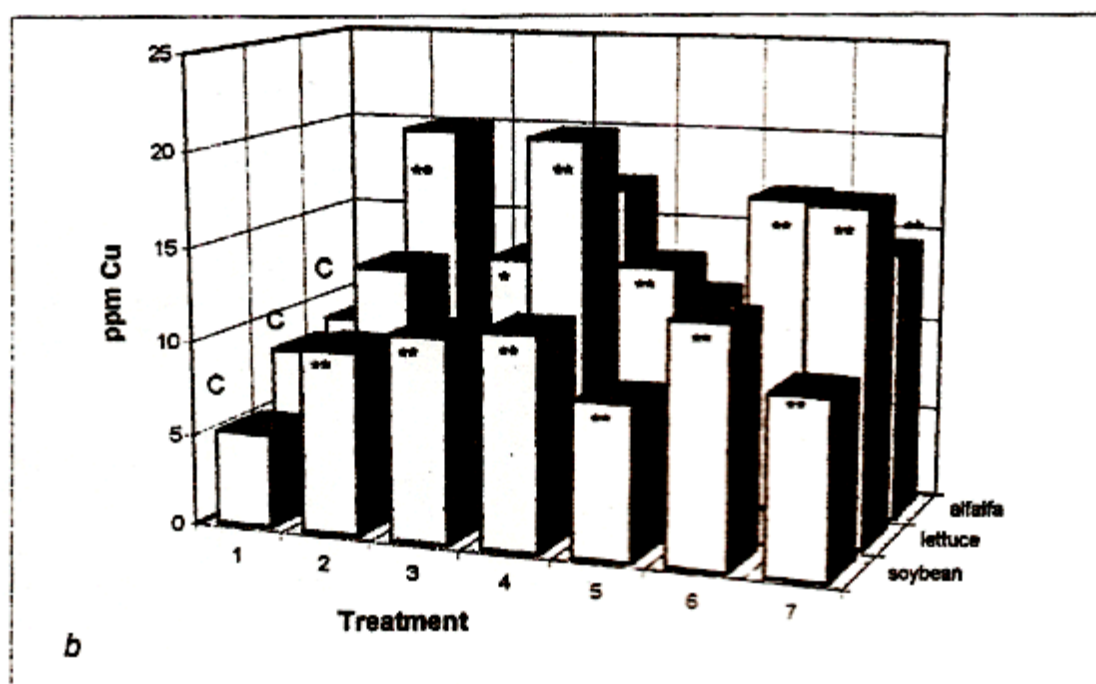
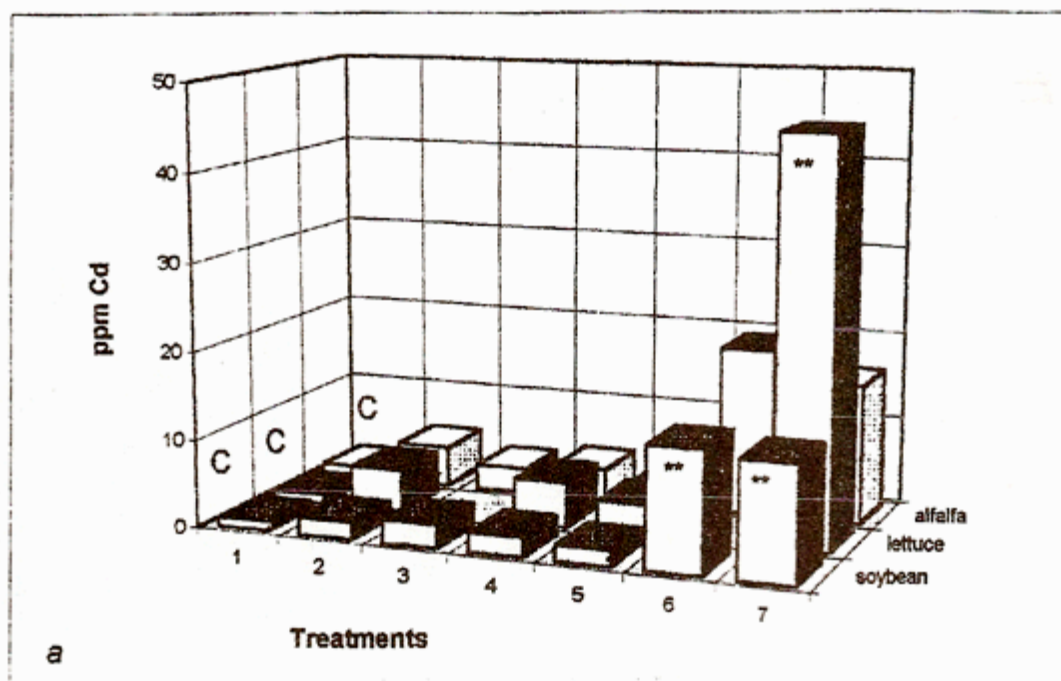


Fig. 2. Variation of Cd (a) and Cu (b) content of plants depending on their nature and kind of treatment

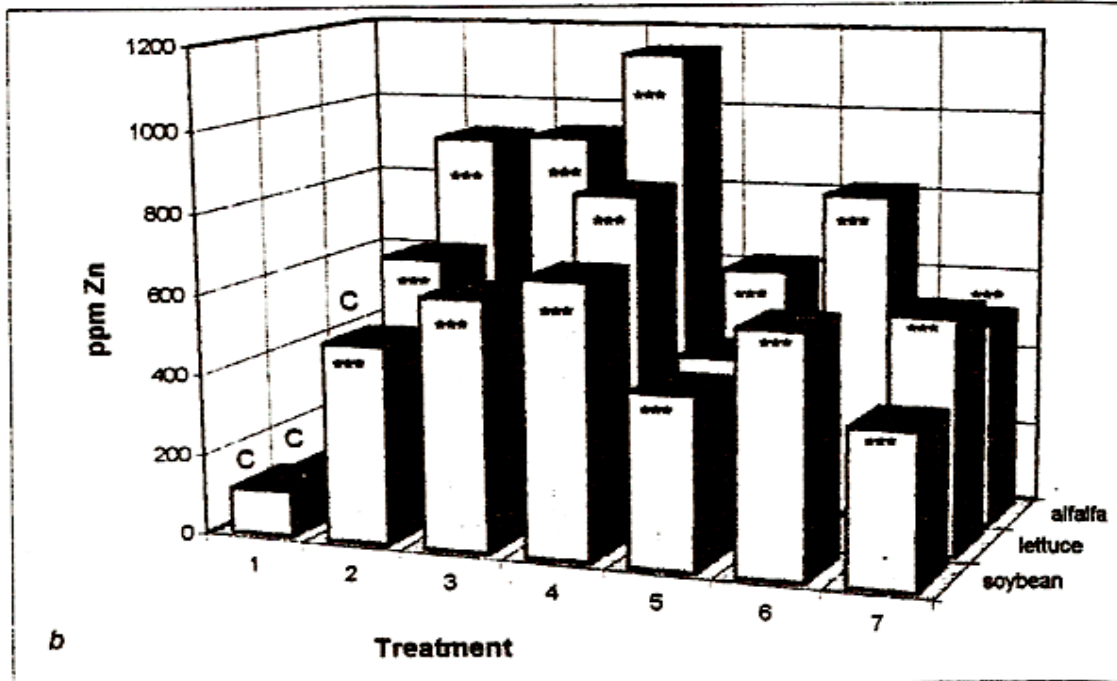
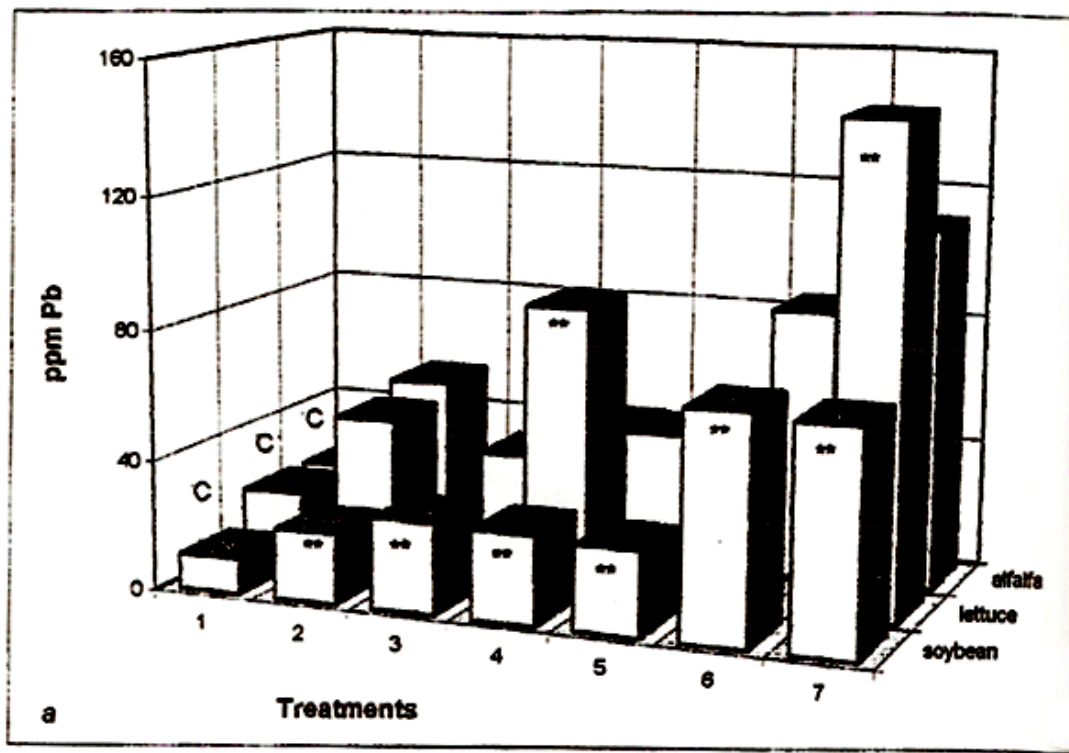


Fig. 3. Variation of Pb (a) and Zn (b) content of plants depending on their nature and kind of treatment

Table 1. Values of translocation coefficient (T.C.) of heavy metals from soil into plants and of accumulation index (A.I.) of heavy metals in polluted plants

	Lettuce		Soybean		Alfalfa	
Treatment	C.T.	I.A.	C.T.	I.A.	C.T.	I.A.
	Cd					
1	7.62		6.62		10.25	
2	10.33	4.80	4.32	1.79	9.33	3.71
3			5.48	2.48	5.94	2.32
4	10.85	5.15	4.51	2.06	7.99	2.98
5	7.89	3.50	3.87	1.68	6.28	2.35
6			2.12	12.77	3.06	15.37
7	7.42	45.73	2.11	12.25	2.48	12.77
	Cu					
1	1.96		0.99		2.13	
2	0.24	1.59	0.17	1.96	0.34	2.22
3			0.19	2.17	0.22	1.45
4	0.35	2.49	0.21	2.27	0.35	1.91
5	0.29	1.71	0.15	1.65	0.22	1.27
6			0.16	2.51	0.21	1.89
7	0.24	2.15	0.13	1.86	0.19	1.61
	Pb					
1	2.34		1.31		2.05	
2	0.17	2.19	0.08	2.00	0.18	2.41
3			0.10	2.59	0.11	1.41
4	0.13	1.70	0.11	2.53	0.15	1.96
5	0.12	2.34	0.10	2.41	0.12	1.38
6			0.05	6.43	0.05	3.87
7	0.10	6.73	0.04	6.28	0.08	5.12
	Zn					
1	22.65		21.20		30.70	
2	5.70	6.85	4.32	4.44	8.17	6.22
3			5.41	5.61	7.77	6.31
4	6.93	8.78	5.77	6.13	9.83	7.75
5	4.34	4.69	4.15	3.83	5.99	4.14
6			5.01	5.33	6.98	5.50
7	6.15	6.04	3.38	3.38	5.17	3.40

polluted treatments, the accumulation of heavy metals in plants within these treatments. The explanation of the phenomenon is given by the capacity factor, that is: the transfer from soil of some high contents of heavy metals during a small interval of time. The high capacity of Cd transfer in the plants within the artificially

polluted treatment, without liming with CaCO_3 , caused lethal effects on plants at short time after springing up.

The comparison of the values specific to the four chemical elements in the case of the three plant species emphasises the following aspects:

- the capacity of lettuce to accumulate heavy metals regardless of the nature of the chemical element;
- the passivity of soybean to the supply of heavy metals, and
- the capacity of alfalfa to accumulate more Zn when the plants grow under soil conditions characterised by a high phosphorus content;
- liming increases the accumulation of heavy metals

As the lettuce represents a food of vegetal origin, its growing should be generally forbidden on polluted soils, and especially on soils polluted with heavy metals.

CONCLUSIONS

1. Mollic Reddish Brown soil near the "Acumulatorul" and "Neferal" enterprises, located in the eastern part of Bucureşti city, is severely polluted with Pb, Cu, Zn, and Cd. Concentration of these chemical elements, in Am horizon, is 19.6, 7.5, 7.2, and 1.7 times, respectively, higher than in a control soil in the non-polluted area.

2. Heavy metals in severely polluted soil have determined inhibition of plant growth, while the balanced fertilisation with N and P, and liming have contributed to obtain significant increases of yield.

3. Over-fertilisation with phosphorus may cause an accumulation of heavy metals in plants.

4. The speed of Cd translocation from soil into plants was higher than in the cases of Cu, Pb, and Zn translocation.

5. Lettuce accumulates, in short time, great amounts of heavy metals, so that the soils polluted with heavy metals are unsuitable for this crop.

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ZINC COMPLEX COMPOUNDS WITH 1-TRIARYLMETHYLBENZIMIDAZOLES

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ABSTRACT

In order to study the influence of the coordination with Zn on antifugal and/or antibacterial activities of some 1-triarylmethylbenzimidazoles (L) were synthesized and characterized new complex compounds ZnL_2Cl_2 and their free ligands. The present paper reports an original pathways for synthesis of these complex compounds by the reaction of bis(benzimidazolato)zinc- $(C_7H_5N_2)_2Zn$ –with triarylchloromethanes in boiling benzene.

1-Triarylmethylbenzimidazoles (L) were easily prepared by treating the corresponding complex compounds (ZnL_2Cl_2) with ammonia. The two aboved-mentioned reactions represent also a new route of synthesis of 1-triarylmethylbenzimidazoles very different from those previously described. The elemental analyses, IR, ^1H - and ^{13}C -NMR spectra is discussed.

Key words: 1-triarylmethylbenzimidazoles, Zn-complex compounds, antimicrobial, antifugal and antibacterial activity.

INTRODUCTION

The transition metal complex compounds are known to play an important role in many biological processes. Antimicrobial activity of some Zn-complex compounds with organic ligands and the improvement of this activity by Zn-coordination are described in literature (Rajesh Nagar N., 1989; Samus et al., 1989; Anjaneyulu et al., 1985; Pujar et al., 1987).

In order to study the influence of the coordination with Zn on antifugal and/or antibacterial activities of some 1-triarylmethylbenzimidazoles (L) were synthesized and characterized new complex compounds (ZnL_2Cl_2) and their free ligands.

MATERIALS AND METODS

Zn-complex compounds with –triarylmethylbenzimidazoles (III_{a-c}) were synthesized by the reaction of bis(benzimidazolato)zinc (I) with triarylchloromethanes (II_{a-c}) (Scheme 1).

Materials:

Triphenylchloromethane of 99% purity (Australanal) (II_a), 4-methoxy-triphenylchloromethane of 98% purity (Flukal) (II_c), 1-Chloro-2-(chlorodiphenylmethyl)benzene with benzene (II_b) of 98% purity was obtained by Friedel-Crafts reaction of 1-Chloro-2-(dichlorodiphenylmethyl)benzene with benzene. The crude product was recrystallised from benzene. Bis(benzimidazolato)zinc- $(C_7H_5N_2)_2Zn$ (Cordes and Walter, 1968) are obtained by reaction of ammoniacal solution of benzimidazole with $ZnSO_4$ ammoniacal solution (molar ratio 2:1) at 20-25 °C; purity 99% yield 95%.

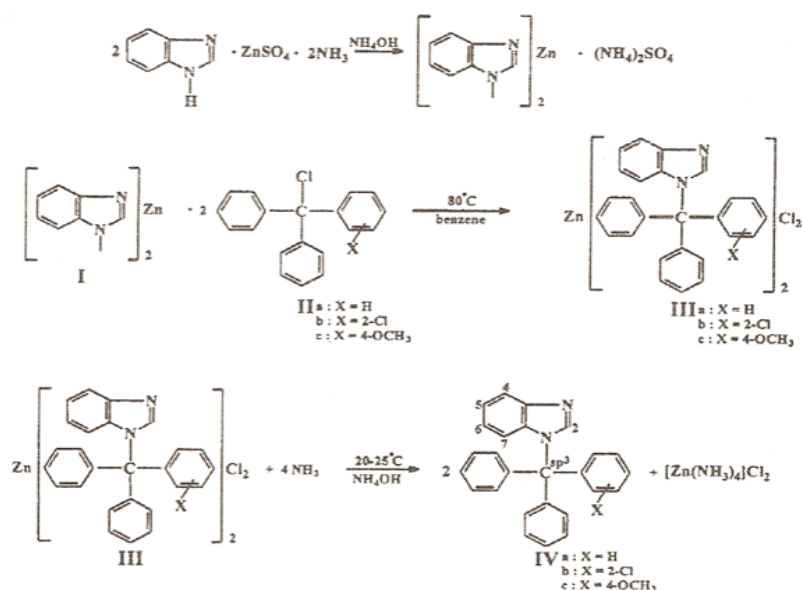
Bis(benzimidazolato)zinc was assessed by potentiometric titration using solution of 0.1 N HClO_4 in acetic acid and by elemental analysis (C,H,N).

The complex compounds ZnL_2Cl_2 (III_{a-c}) and 1-triarylmethylbenzimidazoles (IV_{a-c}) were assessed by elemental analysis (C,H,N) and Schroniger method (Cl); the purity was controlled by TLC.

Melting points determined on a heating plate microscope are uncorrected.

The IR spectra were recorded on a Perkin-Elmer spectrometer in KBr pellet ($4000\text{-}600\text{ cm}^{-1}$ spectral range)

The NMR spectra were recorded on a Varian Gemini 300 Bb spectrometer at 300 MHz and 75 MHz for ^1H and ^{13}C nuclei, respectively. The NMR spectra were recorded in CDCl_3 and chemical shifts referenced to TMS as internal standard.



Scheme 1.

RESULTS AND DISCUSSIONS

Starting from the results obtained earlier (Lăzărescu et al., 1995) concerning N-alkylation of imidazole ring from bis(benzimidazolato)zinc with triarylchloromethanes, were studied the conditions of the similar reaction for bis(benzimidazolato)zinc (I).

Compound (I) and triarylchloromethanes (II_{a-c}) reacted in boiling benzene to give the corresponding Zn complex compounds (III_{a-c}) with 1-triaryl-methylbenzimidazoles in good yields.

This procedure represents a new pathway for the synthesis of complex compounds ZnL_2Cl_2 , where L is a benzimidazole derivative. The procedure previously described involves ligands preparation and their reaction with ZnCl_2 . (Slyusarenks et al., 1974).

Zn complex compounds (III_{a-c}) are not described in literature.

High purity ligands (IV_{a-c}) are easily obtained by reaction of corresponding complex compounds (III_{a-c}) with ammonia in good yields.

1-Triarylmethylbenzimidazoles (II_b and II_c) are not described in literature.

The elemental analysis, melting points and yields of the synthesized complex compounds (III_{a-c}) and ligands (IV_{a-c}) are presented in Table 1:

Table 1: Yields and analytical results of the complex compounds III_{a-c} and IV_{a-c}

Nr.	Molecular formula	M.P. ^{a)} °C	TLC ^{b)} (R _f)	Elemental chemical analysis				Yield %
				Calcd%		Found%		
				C	H	Cl	N	
III _a	C ₅₂ H ₄₀ Cl ₂ N ₄ Zn	176-178	0.74	72.86 72.99	4.70 4.11	8.27 8.07	6.53 6.00	77.5
III _b	C ₅₂ H ₃₈ Cl ₄ N ₄ Zn	243-246	0.70	67.44 67.00	4.13 3.85	15.30 14.93	6.05 5.90	78.0
III _c	C ₅₄ H ₄₄ Cl ₂ N ₄ O ₂ Zn	160-164 (dec)	0.73	70.69 71.00	4.83 4.50	7.73 7.79	6.10 6.50	70.0
IV _a	C ₂₆ H ₂₀ N ₂	180.5-181.5 ^{c)}	0.62					97.0
IV _b	C ₂₆ H ₁₉ ClN ₂	162-165	0.60	79.08 79.25	4.85 4.60	8.98 8.86	7.09 7.50	90.0
IV _c	C ₂₇ H ₂₂ N ₂ O	180-181	0.60	83.05 83.20	5.67 5.50		7.17 7.25	80.0

^{a)} uncorrected^{b)} Kieselgel 60 F₂₅₄; eluent = CH₂Cl₂ : CH₃OH : CCl₄ (5:2:1, v/v); UV, I₂^{c)} Lit. : m.p.= 180-181 °C.

The IR spectral data are presented in Table 2 and Table 2.1:

Table 2: Spectral data of the complex compounds III_{a-c} and IV_{a-c}

Spectral range: 4000-600 cm ⁻¹	Bound	Observation
3120-3110 cm ⁻¹	ν _{CH}	(IM)
3080-3020 cm ⁻¹	ν _{CH}	(Bz)
1600-1350 cm ⁻¹	ν _{C=C} , ν _{C=N}	
1300-880 cm ⁻¹	δ _{CH}	(IM) and (Bz) in plan ring bending
820-690 cm ⁻¹	γ _{CH}	(Bz)
640 cm ⁻¹	Ring torsion	(IM)

Table 2.1: The influence of coordination of the compounds III_{a-c} and IV_{a-c} in IR spectra

III _{a-c}	IV _{a-c}	Assignment
910-915 cm ⁻¹	880 cm ⁻¹	IM- in plan ring bending
1230-1240 cm ⁻¹	1215 cm ⁻¹	δ _{CH} (BZ)

(IM)-imidazole ring

(Bz)-benzene ring

The structures of the new compounds were confirmed by ^1H and ^{13}C -NMR (Tables 3 and 4):

Table 3: ^1H -NMR Shifts of the complex compounds III_{a-c} and IV_{a-c}.

Compound			^1H -NMR (CDCl ₃ ; δ ppm, J, Hz)				
Nr.	X	H-2	H-4 (H-7)		H-5 (H-6)		OCH ₃
III _a	H	8.40 s	7.90 d	6.48 d	7.13 t	6.95 t	-
			J=8.2	J=8.3	J=7.4	J=8.2	
III _b	2-Cl	8.58 s	7.95 d	6.44 d		6.92 t	-
			J=8.0	J=8.2	J=7.7	J=8.0	
III _c	4-OCH ₃	8.38 s	7.91 d	6.48 d		7.13 t	3.77
			J=8.3	J=8.3	J=7.3	J=8.3	
IV _a	H	7.89 s	7.78 d	6.47 d		6.89 t	-
			J=8.2	J=8.2	J=7.4	J=8.0	
IV _b	2-Cl	8.00 s	7.80 d	6.42 d		6.87 t	-
			J=8.0	J=8.3	J=7.4	J=8.0	
IV _c	4-OCH ₃	7.87 s	7.78 d	6.47 d		6.92 t	3.76
			J=8.0	J=8.3	J=7.3	J=8.3	

Table 4: ^{13}C -NMR Shifts of the complex compounds III_{a-c} and IV_{a-c}

Compound		^{13}C -NMR (CDCl ₃ ; δ ppm)						
Nr.	X	C ^{SP3}	2	4	5	6	7	OCH ₃
III _a	H	76.80	145.26	118.90	123.80	124.12	116.10	-
III _b	2-Cl	76.80	146.15	119.00	123.87	124.05	115.27	-
III _c	4-OCH ₃	76.50	145.25	118.95	123.84	124.10	116.16	55.30
IV _a	H	75.40	144.13	120.30	122.00	122.30	115.40	-
IV _b	2-Cl	75.54	144.77	120.27	122.00	122.36	114.69	-
IV _c	4-OCH ₃	75.10	144.12	120.24	121.95	122.27	115.42	55.25

For assignment of chemical shifts were used ^1H and ^{13}C -NMR normal and bidimensional HETCOR spectra. The influence of coordination on ^1H and ^{13}C -NMR spectra is illustrated in Fig. 1:

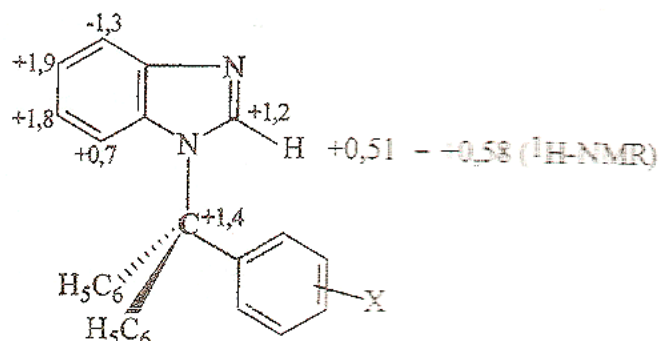


Fig. 1. The influence of coordination

The values within the formula in fig. 1 represent the shifts differences ($\Delta\delta$, ppm) between complex compounds (δ^{CX}) and free ligands (δ^{L}):

$$\Delta\delta, \text{ ppm} = \delta^{\text{CX}} - \delta^{\text{L}}$$

Considering the high polarity of the $\text{N} \rightarrow \text{Zn}$ bond, one might expect an overall deshielding effect on coordination. However, an examination of fig. 1 reveals both deshielding ($\Delta\delta > 0$) and shielding ($\Delta\delta < 0$) effects. The shielding effect should be due to a through space influence of the ZnCl_2 moiety.

CONCLUSIONS

1. New Zn complex compounds with 1-triarylmethylbenzimidazoles (ZnL_2Cl_2) and their free ligands (L) were synthesized.
2. A new pathway was elaborated for the synthesis of these Zn complex compounds by N-alkylation of benzimidazole in bis(benzimidazolato)zinc with triarylchloromethanes. The free ligands (L) were prepared by treating the corresponding complex compounds with ammonia. The two above-mentioned reactions represent also a new route of synthesis of 1-1-triarylmethylbenzimidazoles very different from those previously described in literature.
3. The preliminary microbiological studies show a significantly antibacterial activity for some of these Zn complex compounds.

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THE SANGUIN FERROPROTEINS IN THE SEVERE MECHANICAL TRAUMA

(In experiment)

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ABSTRACT

Using the electron paramagnetic method the changes in the quantity of transferrin in the severe mechanical trauma of soft tissues at different duration of trauma action has been evaluated. In the described affection it is compared in ferroproteins quantity with hemoglobin changes.

Key words: blood, hemoglobin, transferrin, severe mechanical trauma.

INTRODUCTION

Lately ferroproteins have been in the center of attention of scientists. Their structure, physical and chemical characteristics and their biologic role in physiological state and (Worwood, 1989; Oshtran et. al., 1985; Rusu et. al., 1990) pathological state has been determined (Halliwell al., 1988; Smokizyn, 1988)

An essential interest for biology and medicine presents the sanguine ferroproteins in the extreme state accompanied with the hipoxi and oxygen stress. In this respect we have studied the hemoglobin (Hb) and transferrin (TF) in the severe mechanical trauma.

MATERIALS AND METHODS

The experiments have been carried out on the Westal, adult, male rats. (mass 180-220gr.) The severe mechanical trauma was modelled by squeezing rat's back leg soft tissues (Kulaghin, 1979). All animals were divided into 4 groups: 15, 30, 60, 240 minutes squeezing.

The quantity of TF was estimated by electron-paramagnetic resonance method. In the samples of frozen blood the signal of TF was marked at $g = 4,1140,005$ As standard served the spectrum of Cr^{+3} ions of ruby monocrystal. Hb was evaluated with the traditional hemoglobin cynite method. Erythrocytes were determined with the unified method of calculating the red cells in the Goreaev camera. These data are given in the table. The figures' veracity was assessed statistically by using the Student's t-criterion.

Lot Results	Control	15 min of trauma action	30 min of trauma action	60 min of trauma action	240 min of trauma action
TF (UC)	28.032± 1.942	29.168± 2.899	38.977± 6.005	27.040± 2.235	*22.791± 1.191
Total Hb ×10 gr./l	13.10± 0.15	**15.49± 0.2	**14.86± 0.2	*14.59± 0.2	13.64± 0.3
Eryth. ×10 ¹² /l	7.4±0.1	7.4±0.2	7.5±0.1	7.9±0.1	8.1±0.2

The result's veracity: * $p \leq 0.05$; ** $p \leq 0.01$

RESULTS AND DISCUSSIONS

The severe mechanical trauma is characterized by similar trends in the modifications of sanguine ferroproteins quantity. Thus, in the initial stage of metabolic response to the action of harmful agent (15-30 minutes) the quantity of total Hb increases as well as the quantity of TF. The Hb rises to the maximum at 15 minutes with 18,3% in comparison with the control. This is earlier than TF. TF increases with 38% only at the 30 minutes of trauma action.

In the advanced stages of trauma (60 and 240 minutes) the quantity of hemoproteins decrease gradually, being more sensitive in these periods. The TF quantity is almost at the normal level in the third group (60 minutes), but in the fourth group it falls under the physiological level (22,79 UC, $p < 0,05$). The quantity of total Hb slowly falls down and at the last group achieves the normal level (13,6 gr/l). Simultaneously with the total Hb decreasing gradually increase the numbers of red sanguine cells.

During the severe mechanical trauma the blood, component of which is Hb, having disturbance in circulation is able to perform for a while its basic function, the oxygen transportation. This system has a great possibility of adaptation and Hb has an important role in this respect. The early periods of trauma is accompanied by the increasing of total Hb and in the last periods with the multiplication of red cells number.

The obtained results confirm the data of previous research [Monteiro H.P. et. al 1988] which show that TF is a protein relatively resistant to the stress and real modifications of its quantity occur only at the advanced stages of trauma (240 minutes).

CONCLUSIONS

The severe mechanical trauma of soft tissues affects the sanguine ferroproteins located in plasma - TF and in red cells too - Hb.

The evaluation of the affection depends on function of respective protein and the role of iron in the their composition. It is possible to assume that the proteins, where iron accomplish a concrete metabolic function, are more sensitive while the affection of proteins that have the function of iron transportation occur much later. It is possible that the described phenomenon depends on the place of syntheses of proteins too and on the degree of affection of respective organ.

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MOLYBDENUM CONTENT IN THE SOILS OF BANAT: GEOGEN BACKGROUND, FERTILISING OR POLLUTANT POTENTIAL

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ABSTRACT

The goal of the paper is to present the contents of total and mobil (assimilable) molybdenum of the main types of soils in Banat, as well as 2 maps representing the distribution of this microelement in these soils. For the acid soils there is also presented the distribution of molybdenum on the soil's profile, discussing the principal factors that influence its mobility.

Key words: molybdenum content, total and mobile forms

INTRODUCTION

Applying soil liming and mineral fertilizers more and more concentrated and pure, as well as reducing the weight of natural fertilizers in the total of nutritive elements, determines the necessity to know also the content of microelements from soil, as a natural source to assure the nutrition of plants.

Microelements are considered to be those chemical elements necessary for plants in very small quantities, of about 10^{-2} - 10^{-5} % related to dry substance. Excepting iron and manganese, which from geological respectively pedological point of view belong to the major elements, the other microelements (B, Cr, Mo, Co, Ni, Zn, Cd, V, F, I) are found in soils in very small amounts (generally beneath 0.0001%) being included in the group of minor elements (Băjescu and Chiriac, 1984; Lăcătușu et al., 1986).

Molybdenum is present in the majority of the rocks of the earth's crust in very small amounts, what makes difficult the laboratory analysis. Therefore there are relatively little data about the abundance of this element in the soils of Roumania (Băjescu and Chiriac, 1984). Researches on molybdenum content as well as on other microelements, total and mobile forms, in soils, started in 1978, by investigating the soils of the experimental fields of OSPA Timișoara, further on extended in Timiș, Arad and Caraș-Severin counties. There is presented a review on the multitude of the obtained analytical data, represented on 2 maps of the repartition of this microelement in the soils of Banat.

MATERIALS AND METHODS

Molybdenum is difficult to determine directly by emission spectrography, being also necessary the extraction with solvents (Borlan and Hera, 1973). The most data referring to the abundance of this microelement were obtained by spectrophotometric methods, which suit best for routine determinations (Butler and Mathews, 1966). Holten (Davidescu et al., 1988) described a polarographic method for determining molybdenum in rocks and ores with a detection limit of 0.01%.

Atomic absorption spectroscopy in flame presents an insufficient sensibility for the direct determination in the acid solution resulted after mineralization and moving off of silica. It can be grown by concentration in steps, implying extraction with organic solvents of the molybdenum-oxichinoline complex (Edge et al., 1962) or α -benzoinoxine (Gergen and Pușcă, 1979).

Using electrothermic atomisation, the performances of the methods based on atomic absorption increase, but it implies a more sophisticated apparatus. Among the spectrophotometric methods those ones that use ditiol and tiocianate are mostly used (Butler and Mathews, 1966).

Passing of total molybdenum content from soil to solution, in the purpose of dosage, can be realised by (Gergen and Pușcă, 1987):

- a) wet proceeding, which consists in soil disintegration at warm with a concentrated mineral acid or a mixture of mineral acids;
- b) dry proceeding, which consists in the fusion of soil with a flux and the solubilisation of the fusion in a mineral acid;
- c) combined proceeding, which consists in soil calcination and then the dissolving of the residue with a mixture of mineral acids.

In the analysis that were done we used the wet proceeding, with perchloric acid 60% at warm, which presents the advantage that oxidases also the organic matter of the soils and makes insoluble, very good, the silica (Gergen and Pușcă, 1987). Soil molybdenum, existing generally as molybdates absorbed by minerals and soils colloids or in the cristaline structure of some sulphoxidic minerals, is passed through the solution by soil disintegration with perchloric acid 60%, at warm. At the soils with a superior humus content (> 5%) the previous oxidation with concentrated nitric acid is needed.

RESULTS AND DISCUSSIONS

Characterisation of molybdenum spreading, total and mobil forms in the soils of Banat

In order to characterise the spreading of molybdenum total forms, there were analysed and interpreted 116 soil samples, harvested from the most representative soils of Banat. Analytical results were statistically processed and mean values and ranges are presented in Table 1.

Table 1: Molybdenum content, total forms in the principal soils of Banat (superior horizon, 0-20 cm)

Types of soil and localisation	Nr. of samples	Mean value (ppm)	Range (ppm)
Typical chernozem, Tomnatic	12	3.28 $\pm$ 0.3	1.50-4.58
Gleizated chernozem, Biled	8	2.18 $\pm$ 0.2	1.12-3.86
Cambical chernozem, Vinga	12	1.01 $\pm$ 0.1	0.60-2.10
Brown clay-illuvial soil, Visag	10	0.81 $\pm$ 0.1	0.40-1.40
Brown clay illuvial soil, Orțișoara	8	0.95 $\pm$ 0.1	0.55-1.44
Vertisil, Valcani	14	2.62 $\pm$ 0.3	1.11-3.42
Alluvial soil, Lunca Timișului	28	3.31 $\pm$ 0.3	1.01-5.21
White luvisil, Făget	12	0.76 $\pm$ 0.1	0.22-1.12
Brown acid soil, Valea Minișului	6	0.48 $\pm$ 0.1	0.24-0.80
Litosil, Carașova	6	0.54 $\pm$ 0.1	0.30-0.81

In order to characterise the spreading of molybdenum in mobile forms there were analysed and interpreted 154 soil samples harvested from the most representative soils of Banat. Analytical results were statistically processed and mean values and ranges are presented in Table 2.

Table 2: Molybdenum content, mobil forms (to be extract in Tamm reactive) in the principal soils of Banat (horizon, 0-20 cm)

Types of soil and localisation	Nr. of samples	Mean value (ppm)	Range (ppm)
Chernozem, Tomnatic	12	0.08+/-0.01	0.02-0.15
Chernozem, Calacea	16	0.09+/-0.01	0.02-0.17
Gleizated chernozem, Biled	8	0.10+/-0.01	0.02-0.20
Gleizated chernozem, Lovrin	16	0.08+/-0.01	0.02-0.16
Brown clay, Visag	10	0.10+/-0.01	0.02-0.22
Brown clay, Sănandrei	8	0.15+/-0.02	0.04-0.27
Brown clay, Caraşova	6	0.14+/-0.01	0.03-0.26
White luvisoil, Făget	12	0.05+/-0.01	0.01-0.10
White luvisoil, Caransebeş	6	0.04+/-0.01	0.01-0.11
Verisil, Valcani	14	0.25+/-0.02	0.11-0.45
Alluvial soil, Lunca Timişului	28	0.15+/-0.02	0.08-0.18
Gleizated soil, Bozovici	6	0.10+/-0.01	0.04-0.16
Litosoil, Reşiţa	6	0.04+/-0.01	0.01-0.08
Solonet, Diniş	6	0.29+/-0.03	0.18-0.40

The corelative maps of the repartition of molybdenum, total forms (figure 1) and mobile forms (figure 2) were made up by extrapolation after the research on the over 100 analyzed soil samples, which cover the most part of the soil types, of the relief and of the deposits of parenteral rocks from Banat. The graphic display on levels of molybdenum content was made by superposition of the geologic, soils, parenteral rocks, relief and climate maps.

In this way new areals were created characterised by analytical similitudes which in the majority of cases were explained through the interference of disintegration, alteration, transport, sedimentation processes with the bioaccumulative or eluvial-iluvial ones. For more details regarding the supplying states with molybdenum in mobile form, there were researched thoroughly, by analysis on agrochemical samples, some areals from two dominant pedoclimatic zones: the zone of low plane (Câmpia Torontalului) and the zone of hills (Dealurile Lipovei), Bethausen areal.

The rocks with molybdenum have a reduced resistance at mechanical disintegration and at chemical alteration. The resulted oxomolybdenic compounds that result are carried through the solution and moved away from the origin zones (mountains and hills). A first modality of geogen accumulation of molybdenum in total form is realised in the depression basins (corridors, tectonic and erosional depressions) where the solutions of hipergene alteration mix with river waters, rich in calcium, accelerating the precipitation of molybdenum salts. In well drained soils from the loess plane of Banat, molybdenum forms stable complexes with oxygen and calcium abundance maintains it enriching the sediment.

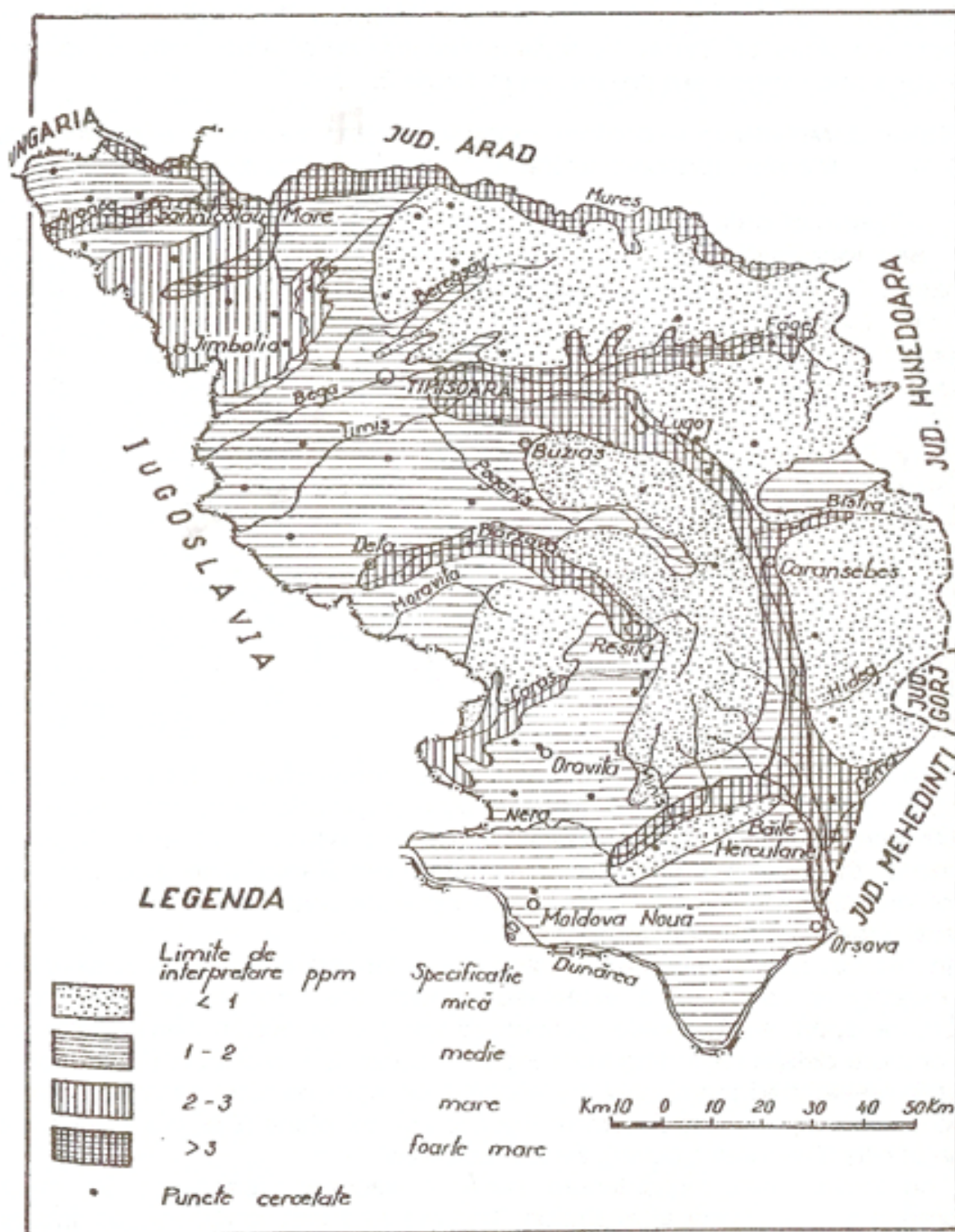


Fig. 1: Molybdenum supply, total forms, of the soils of Banat (depth 0-40 cm)

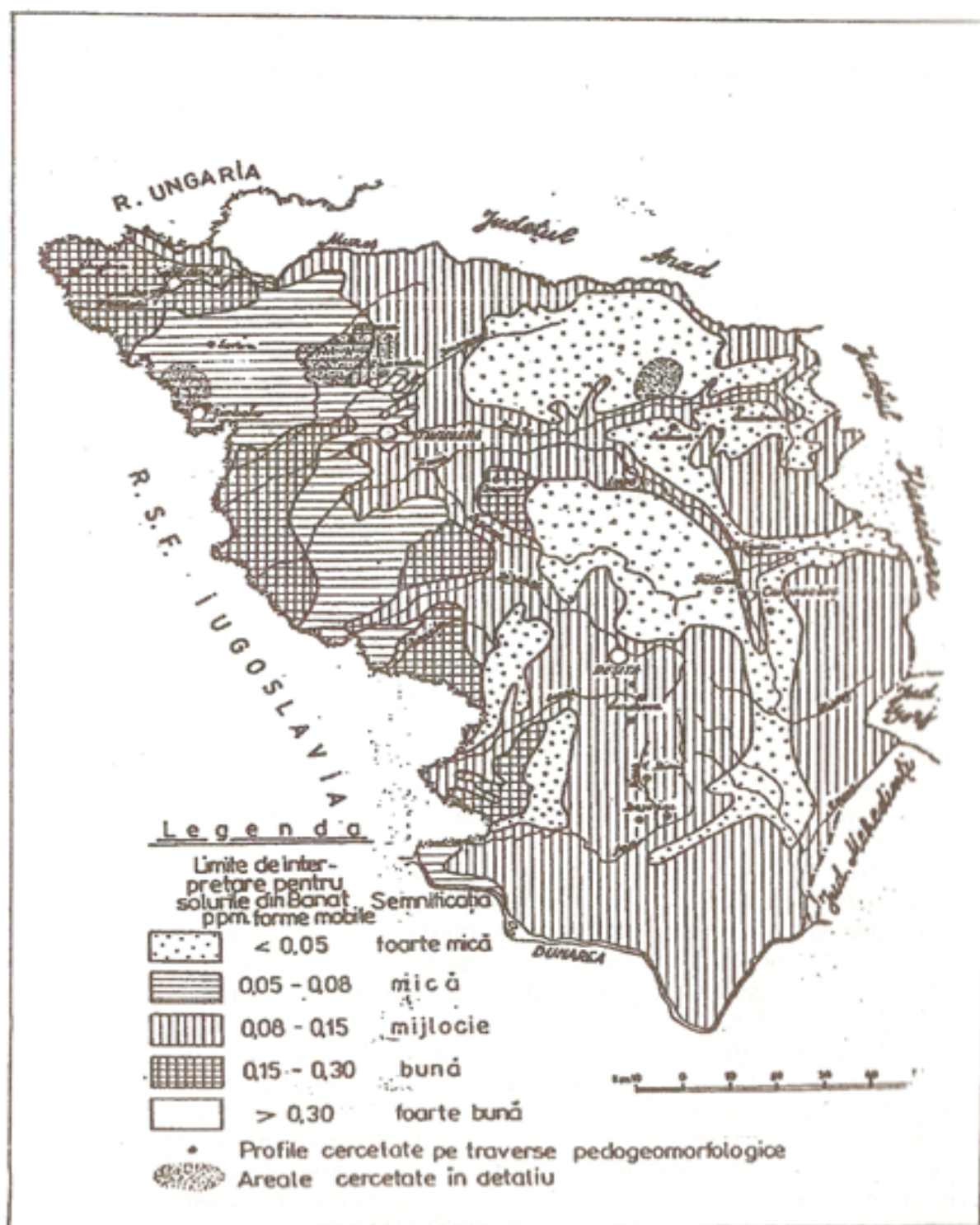


Fig. 2: Molybdenum supply, mobil forms, of the soils of Banat (depth 0-20 cm)

Researches made on the distribution of mobile molybdenum on profile (Băjescu and Chiriac, 1984; Lăcătușu et al., 1986) indicate accumulations in the A horizon of all researched soils, with the tendency of decreasing of contents on profile at chernozem, vertisoils and reaccumulations in the B horizon at clay luvisols or in the soils which suffer of excessive pluvial humidity depending on the intensity of iluviation (figure 3). Generally, the eluvial horizons are very poored in this element.

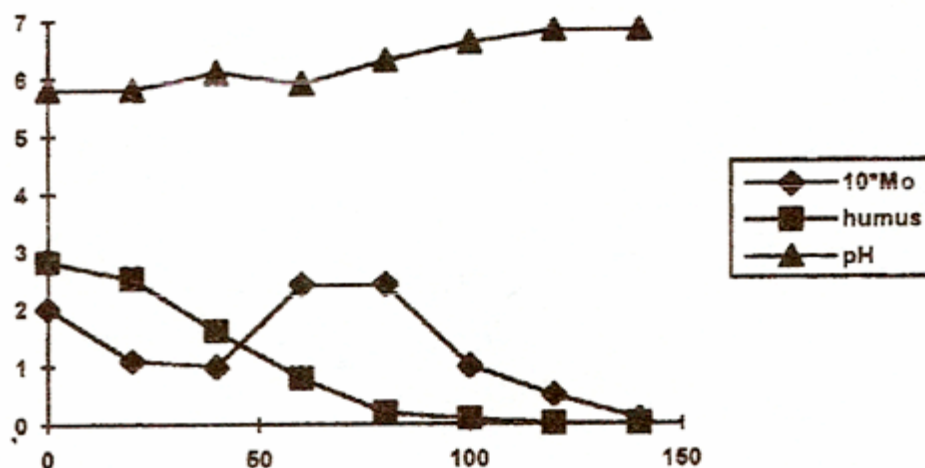


Fig. 3: Profile repartition of molybdenum in the pseudogleic soil typical at Bethausen (after Gergen et al., 1992; Janos and Gergen, 1982)

Undertaken researches (Gergen et al., 1992) show that in the ploughed podzolic soils with excessive humidity from the eastern and south-eastern part of Banat there is a very reduced content of mobile molybdenum, explained after Borlan (Gergen et al., 1985) by its blocking by the Fe and Al oxides as well as by the reduced native content in this microelement (Holten, 1961; Hutchkinson, 1972).

In such conditions there is the premise that on this soils frequently appears the molybdenum deficiency, and the appliance of nitrogen fertilizers with acid physiological reaction will increase the probability of appearance of molybdenum deficiency states at sensitive plants, facts that were observed on the ground (Janos et al., 1995; Janos, 1994).

Lăcătușu (Holten, 1961; Janos and Goian, 1995) asserts that the humus, cantitatively as well as qualitatively, has a benefic role in molybdenum accumulations and adding organic fertilizers contributed to this accumulations. In this context it can be concluded that the little highest values of mobil molybdenum content in the low plane and in a part of the high plane can be associated with the higher humus contents from these areals, generally occupied by chernozems (Geffrey, 1983; Lăcătușu and Gherlase, 1992). There also exists a strong binding between molybdenum contents (mostly total molybdenum) and clay content, the clay soils, especially the vertisol ones, having higher quantities of molybdenum (Lăcătușu et al., 1986).

Beside the specific properties of the soils and solidification rocks, exterior influences of the environment intervene on the accumulations and mobilities of molybdenum. On a varied pedogenetic background, molybdenum can be also influenced by the local climate conditions. Lăcătușu (Holten, 1961) appreciates that on a background weak in molybdenum, its mobility reduces very much in conditions of cold and cloudy weather, fact that confirms climatical too, the probability of the appearance of molybdenum deficiency in arable soils from the central and eastern Banat hills regions. In spite of the fact that in total and mobile forms it is present in soils in reduced quantities, and its areal distribution is relatively uniform, it plays nevertheless an important role in plant nutrition, contributing by molybdoenzymes to the assimilation of different nitrogen forms.

Having a strong affinity to sesquioxides, molybdenum retention is reduced once the pH increases. Calcarization of acid soils increases molybdenum mobility and improves the nutrition of plants with this element (Lăcătușu et al., 1987). In order to correlate better the influence of soils reaction on the mobility and accesibility of this microelement for plants, in the agrochemical practice a characteristic synthetic indicator was introduced, obtained by the relation (Gergen et al., 1985):

$$I_{Mo} = pH + 10Mo$$

For values of the indicator $I_{Mo} > 7.6$, characteristic for the little debasified soils (chernozem, vertisoils) a part of the brown soils from the western part of Banat, it can be considered that the probability of the appearance of deficiency in molybdenum is reduced.

Podsols, including acid mountain soils, present higher deficiency probabilities ($I_{Mo} < 6.5$). Between this two extreme domaines are situated the soils from the piemountain plains or the low plains, with moderate debasified soils (brown clay, brown reddish) with I_{Mo} values between 6.6 and 7.5 where the appearance of molybdenum deficiency is isolated, being influenced by the other environmental or antropic factors.

CONCLUSIONS

After analysing more than 200 soil samples of the most representative soils of Banat, 2 maps were realised representing the distribution of molybdenum in total and mobile forms in that soils. The obtained maps have inherent limitations due to the work scale, the lack of some experimental data and the reduced volume of analysis and fulcrums. Anyway, they can direct the future researches, especially in the zones where the obtained values raise pollution or disequilibrium problems in the nutrition of plants.

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ISOENZYMIC FORMS OF N-ACETYL- β -D-GLUCOSAMINIDASE IN THE URINE OF THE INDIVIDUALS EXPOSED TO MERCURY

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ABSTRACT

Mercury compounds are toxic environmental pollutants and cumulative poisons concentrating in the kidney. In order to find a parameter appropriate for early discovery of an injured kidney in individuals exposed to mercury effect, the activities of isoenzymic forms of urinary N-acetyl- β -D-glucosaminidase (NAG, EC 3.2.1.30) were being determined in this work. For the separation and determination of the activities of isoenzymic forms a simple, fast and reproducible method was developed, suitable for toxicological and clinical practice. The analysis of the obtained isoenzymic urinary NAG profiles indicated that the B form contribution to the total NAG activity is statistically considerably increased under mercury effect ($p < 0.05$) compared to the control group. The increase in total NAG activity is correlated with the B form activity ($r = 0.583$). Since the NAG B form is bound with lysosomal membrane its increase in urine is an early parameter indicating the injuries of lysosomes. The contributions of the A ($64.13 \pm 16.51\%$) and M (21.32 ± 6.32) forms activity to the total NAG activity do not change considerably compared to the controls

Key words: N-acetyl- β -D-glucosaminidase, Isoenzymes, Mercury, Nephrotoxicity.

INTRODUCTION

Although very toxic, inorganic mercury compounds have various applications and dealing with them is inevitable. Mercury is easily absorbed by skin, and gastrointestinal and respiratory tracts and is selectively concentrated in kidneys, which are particularly sensitive to its toxic effect (Bomhard et al., 1985; Rosenman et al., 1986; Eti et al., 1995). The assessment of renal injury based upon serum creatinine or blood urea nitrogen levels is insensitive, since these tests show abnormal findings only when major impairment of renal excretory function has developed. Other measures of renal function such as creatinine clearance, p-aminohippuric acid clearance, urinary concentrating ability, proteinuria, glycosuria may be somewhat more sensitive but are technically not satisfactory for use of screening tests (Meyer et al., 1984). In contrast, urinary enzyme analysis is an extremely sensitive indicator of renal injury. N-acetyl- β -D-glucosaminidase (NAG) is a lysosomal enzyme present in renal tubular cells. NAG can be assayed easily and reproducibly and has been shown to be a sensitive indicator of early renal injury (Mandic et al., 1995). Some results (Boogaard et al., 1996) suggest that after exposure to mercury at levels below the biological exposure index, a transient increase in NAG can be observed, but is not an early indicator of developing renal dysfunction.

Since in the individuals exposed to mercury the maximum concentration is found in lysosomal tubular cells of kidneys and as the isoenzymic NAG forms in lysozymes are differently bound, it is supposed that the activity of the individual isoenzymic forms would be a sensitive parameter for renal injuries to be established. Therefore, in this work, the isoenzymic forms of urinary NAG were separated and the contributions of the activity of each form to the total NAG activity were determined.

MATERIALS AND METHODS

The total urine NAG activity and activities of isoenzymic forms in the control group ($n = 10$) and in group exposed to mercury ($n = 40$) were determined.

For the determination of NAG activity a synthetic substrate 2-methoxy-4-(2'-nitrovinyl)-phenyl-N-acetyl- β -D-glucosaminide (MNP-GlcNAC) was used. The substrate solution was freshly made by dissolving the substrate in water warmed at 90°C. Optimal concentration of substrate was 1.5 mmol/l. Activity of NAG was assayed by adding 100 μ l of urine sample to 1000 μ l of the substrate solution and 500 μ l of 0.1 mol/l citrate buffer, pH = 4.5. After 30 min at 37°C the enzyme reaction was stopped by adding 500 μ l of 0.1 mol/l carbonate buffer, pH = 9.5, and the absorbance read at 505 nm. Reagent blanks were performed for each determination. A calibration curve was constructed with known concentrations (from 0.025 to 0.200 mmol/l) of 2-methoxy-4-(2'-nitrovinyl)-phenol (MNP) ($y = 1.275x - 0.00089$; $r=0.9996$). One unit of enzymatic activity (U) represents the amount of enzyme which hydrolyses one μ mol of substrate per min at 37°C ($U/l = \mu\text{mol}/\text{min}/l$).

To separate isoenzyme NAG forms a column filled with DEAE-cellulose was used. The flow through the column was 40 ml per hour, and the eluted fractions from the column were 4 ml each. The volume of urine used for separation was 50 ml. Prior to that serum was concentrated and dialysed for 24 hours against 0.01 mol/l phosphate buffer, pH = 7.0 at 4°C. The B form was eluted by 0.01 mol/l phosphate buffer pH = 7.0 and A form by using a linear concentration gradient 0-0.3 mol/l NaCl in the same buffer. The activity of NAG in fractions (400 μ l fractions) was assayed by MNP-GlcNAc as substrate (calibration curve: $y = 0.854x + 0.00499$, $r = 0.999$).

Statistical analysis was performed by Student's t-test. Linear regression analysis and Pearson's correlation coefficient were used for comparison of biochemical variables. The results were expressed as means $\pm$ SD

RESULTS AND DISCUSSIONS

It has already been pointed earlier to the significance of the determination of the urinary NAG activity for the estimation of nephrotoxic mercury effect in the individuals periodically or permanently exposed to its effect. In this work, a group of workers was chosen whose mean value of mercury concentration in urine ($0.110 \pm 0.115 \mu\text{mol}/l$) was almost equal to a maximum allowed concentration (MAC, $0.100 \mu\text{mol}/l$), and the mean value of mercury concentration in blood ($0.127 \pm 0.117 \mu\text{mol}/l$) lower than a maximum allowed ($0.170 \mu\text{mol}/l$) - Fig. 1. Urinary NAG activity in this group was $1.80 \pm 1.08 \text{ IU}/l/\text{min}$ and statistically it did not differ considerably from the control group ($1.43 \pm 0.88 \text{ IU}/l/\text{min}$). Since between the urinary NAG activity and mercury concentration in urine there was found no correlation, in our further work we have tried to find out a sensitive parameter for the nephrotoxic mercury effect by means of the separation of the urinary NAG isoenzymic forms.

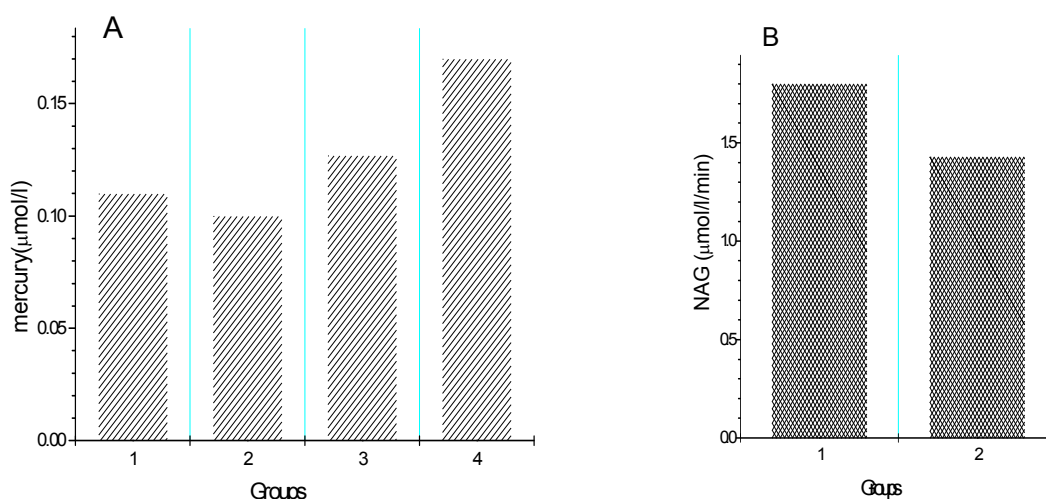


Fig. 1 A. Mercury concentrations: in urine exposed (1) and nonexposed persons (2) in blood exposed (3) and nonexposed (4) subjects
B. Urinary NAG activities in exposed (1) and nonexposed subjects (2)

The isoenzymic NAG forms were separated on a column filled with DEAE cellulose. Before placing it on the column urine was concentrated 20 to 30 times by means of ultrafiltration on PM -10 membrane and then dialysed against 0.01 mol/l phosphate buffer pH = 7.0 during 24 hours. The separation procedure of the isoenzymic forms is described in the Material and Methods section. Elution NAG diagram is shown in Fig. 2 and the percentage contribution of isoenzymic forms to total NAG activity in the control group and in the individuals exposed to mercury effect is shown in Table 1.

Table 1. NAG-activity, isoenzyme content (expressed as percentage of total activity) from mercury exposed and nonexposed persons

Group	B	A	M
Mercury nonexposed	1.75 ± 1.26	67.23 ± 10.21	18.20 ± 3.65
Mercury exposed	2.14 ± 1.67	64.13 ± 16.51	21.32 ± 6.32
I*	1.06 ± 0.51	63.67 ± 18.59	22.96 ± 8.83
II**	4.65 ± 3.23	68.76 ± 14.80	20.01 ± 4.09

Values are expressed as the mean ± S.D.

*I - group of individuals exposed to mercury effect whose urinary NAG activity was within normal values

**I - group of individuals exposed to mercury effect whose urinary NAG activity was higher than the range of normal values

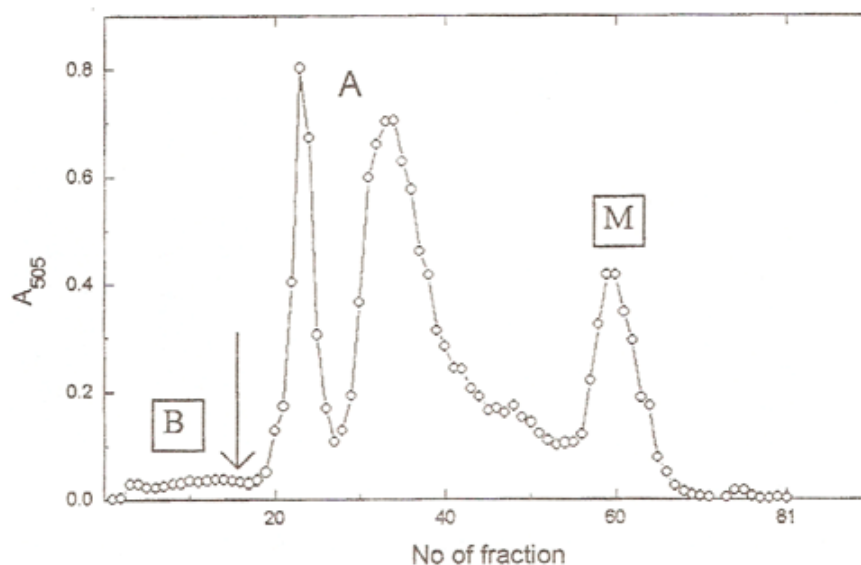


Fig. 2. Isoenzyme profiles following separation of DEAE-cellulose chromatography of urine samples from exposed person to mercury. S↓ indicates the salt gradient which was initiated after 60 ml of eluate. A, B, and M are different isoenzyme peaks.

In urine of the nonexposed individuals to mercury effect the contribution of isoenzymic A, B and M forms is 67.23%, 1.75% and 18.2%, respectively. M form of NAG is more acidic compared to A form and binds more strongly with DEAE cellulose.

The B form activity ($2.14\% \pm 1.67\%$) and M form activity ($21.32 \pm 6.32\%$) contributions to total NAG activity in the individuals exposed to mercury effect are increased compared to the control group ($1.75 \pm 1.26\%$; $18.2 \pm 3.65\%$). If the urine samples (group II, Table 1) where total NAG activity ($3.22 \pm 0.97 \text{ IU/l/min}$) statistically is considerably increased compared to the control ($p < 0.001$) are singled out, a considerable increase in B form activity (4.65 ± 3.23 , $p < 0.01$) to total activity noticed. A good correlation is also found between the total NAG activity and B form activity ($r = 0.583$, $y = 1.02x - 0.09$) in the whole group of the exposed individuals. Since the NAG B form is bound with lysosomal membrane its increase in urine is an early parameter indicating the injuries of kidney tubular cells under mercury effect. On the basis of the results obtained it is concluded that the isoenzymic NAG profiles, particularly the B form activity, represent a more sensitive parameter in establishing the nephrotoxic mercury activity than does the total NAG activity.

In order to make the determination of the activity of isoenzymic NAG forms suitable for the routine analysis, the method for the separation of isoenzymic forms on a small column filled with DEAE cellulose (dimensions 0.8 cm x 6 cm) was worked out. On the basis of the elution NAG profiles, obtained on the big column, it has been established that maximum A form is eluted with 0.175 mol/l NaCl in 0.01 mol/l phosphate buffer, pH = 7.0, and NAG M form with 0.4 mol/l NaCl in the same buffer. Several various elution concentrations and buffer volumes were examined and it was

found that the optimum separation of the NAG isoenzymic forms was achieved under the following conditions:

- 150 – 500 μ l dialyzate placed on the column;
- B form eluates with 12 ml 0.01 mol/l phosphate buffer, pH = 7.0;
- A form eluates with 16 ml 0.175 mol/l NaCl in 0.01 mol/l phosphate buffer, pH = 7.0;
- M form eluates with 8 ml 0.4 mol/l NaCl in phosphate buffer, pH = 7.0;

The above separation conditions were applied in urine samples in which isoenzymic profiles were already done on a big column. A special agreement in the contributions of NAG isoenzymic forms obtained. The NAG elution profile obtained on the small column filled with DEAE cellulose is shown in Fig. 3 for the same sample which is shown in Fig. 2. The percentage contributions of B, A and M forms on the big (0.17%; 79.92%; 20.08%) and on the small column (0.23%; 78.85%; 20.92%) are approximately equal.

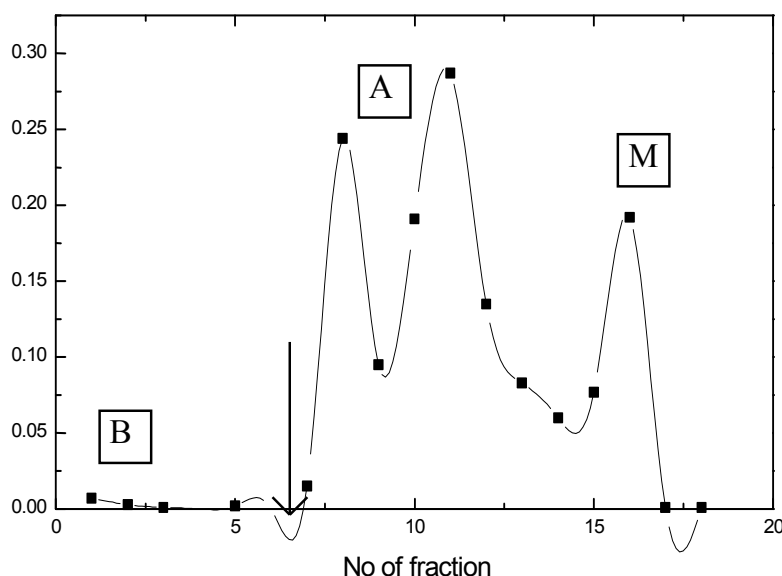


Fig. 3 Isoenzyme profiles following separation of DEAE-cellulose chromatography on the small column of urine samples from exposed person to mercury.

As the above described method for the separation of the urinary NAG isoenzymic forms is simple, fast and reproducible, it is proposed for use in toxicological and clinical practice.

CONCLUSIONS

1. The determination of the urinary NAG activity in the individuals exposed to mercury effect shows that urinary NAG is sensitive parameter of nephrotoxic mercury effect. However, in the individuals permanently exposed to mercury effect in lower dosages and whose mercury concentrations in urine and blood are approximately equal MAC, urinary NAG proves to be insufficiently sensitive.

2. In order to find out a more sensitive parameter, the possibility of determining the activity of urinary NAG isoenzymic forms was examined. A fast, simple and

reproducible method for the separation of NAG isoenzymic forms on DEAE cellulose was worked out, and applied on urine samples of 40 individuals exposed to mercury effect and 10 individuals of the control group.

3. The results of the analysis of NAG isoenzymic profiles show that the contribution of the B form activity to the total NAG activity is statistically considerably increased compared to the control group. Therefore, it is proposed to use the activity of NAG B form as an early parameter in determining tubular injuries.

4. Statistically significant differences in the activities of NAG A and M isoenzymic forms were not found between the individuals exposed to mercury effect and those in the control group.

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APPLICATIONS OF THIN-LAYER CHROMATOGRAPHY IN THE SEPARATION OF SOME METALLIC CATIONS WITH TOXICOGEN POTENTIAL

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ABSTRACT

A mixture of toxic cations was separated on thin layers with silicagel R impregnated with piperasine 20% and silicagel chemical modified with mercaptopropiltrimetroxisilan mixed with cellulose MN (Macherey-Nagel) (1:1, g/g), using as a mobile phase piperasine 20% - methanol-acetic acid (30:65:5, v/v), respectively buthanol - benzen- acetic acid (50:30:20, v/v).

Key words: Thin-layer chromatography – metallic ions.

INTRODUCTION

During the last period, chromatographie on thin layer together with another spectrometric techniques is more and more used in inorganic analysis (Alstodt and Dewald, 1992; Asolkar et al. 1992). By this technique the metal content in river and seas waters (Baranovska et al., 1992; Bobkic, 1992) in human placenta (Asolkar et al. 1992) and the toxic cations (De and Chakroborty, 1981; Dey et al., 1981) were analysed.

MATERIALS AND METHODS

Cations were separated on alumine plates (Gaibakoan et al., 1989), silicagel (Asolkar et al. 1992; Gupta, 1995; Jain, 1990) cellulose (Janjic, 1994; Măruțoiu, 1996), PEI-cellulose (Mohammad and Fatima, 1987), carboxymethyl-cellulose (Mohammad and Khan, 1993) PAB-cellulose (Mohammad et al., 1994), diethyl-2-(hydroxypropyl)aminoethylcellulose (Myiasoedova et al., 1986), organic ions exchangers (Oguma, 1975; Pirisic-Janjic et al., 1991) anorganic ions exchangers (Rayand and Kauffman, 1994), impregnated silicagel (Dey et al., 1981; Sharma et al., 1993; Shimizu et al., 1990) and chemical modified phases (Asolkar et al. 1992; Shimizu et al., 1991).

The mobil phases used at cations separation were correlated with the used stationary phases. Thus, in the case of alumine was used chlorhidric acid mixed with tin chlorure and sodium tiocianate in different concentrations (Gaiabakoan et al., 1989). The plates with silicagel were developed with chlorhidric acid, nitric acid or sulphuric acid in different concentrations (Gupta, 1995) toluen-chloroform (50:1, v/v), benzen-methylizopropilcetone (50:1, v/v) (Asolkar et al. 1992), acetone-ethylacetate-benzen (7:1:3, v/v), buthylamine- acetone-formic acid (2:6:2, v/v) (Husain and Ishaghy, 1992). Cellulose was eluated with buthanol-chlorhidric acid 12N-dioxane (5:1:4, v/v), ethanol-isobu-

thanol-concentrated chlorhidric acid-water (60:30:5:5, v/v) (Măruțoiu et al., 1996). The plates with Pei-cellulose were developed with sulphuric acid 0.1 mol/dm³ - dioxane (7:3, 1:1, 3:7, v/v) (Mohammad and Fatima, 1987) and PAB- cellulose was developed with sulphuric acid-ammonia sulphate (0.01:0.1:1, mol/dm³) (Mohammad et al., 1994).

The plates prepared with ions exchangers were developed with chlorhidric acid in methanol, chlorhidric acid and acetic-chlorhidric acid (Oguma, 1975), dioxane-nitric acid 0.1M (8:12, v/v) (Rajand and Kauffman, 1994).

Elution of impregnated silicagel was done with nitric acid in different concentrations (Sharma et al., 1993); piridine-benzen-acetic acid-water (6:5:8:4, v/v), buthanol - benzen - formic acid (5:10:9, v/v) and piridine-benzen-acetic acid-water (5:5:4:1, v/v) (Shimizu et al., 1991).

The chemical modified stationary phases were developed with acetone-trifluoroacetylacetone (90:10, v/v) (Shimizu et al., 1991) and methanol-water-acetic acid (50:30:4, v/v) (Asolkar et al., 1992).

Cations detection was done by pulverisation on developed plates with some reactives:

- solution 5% of diethyltiocarbamate of sodium in ethylic alcohol (Măruțoiu et al., 1996)
- water solution 0.02% of arsenazo III (Gupta et al., 1995)
- alcoholic solution 1% of 8-hydroxyquinoline and exposure to ammonia vapors (Sharma et al., 1993)
- solution of red alizarine, solution of dimethylglyoxine in ethanol, solution of ammonia tiocianate in ethanol, solution of diphenylcarbazine, solution of ferrocianure, solution of tin chlorure, solution of ditizone, rodizonate and ammonia sulphure (Măruțoiu, 1987)
- solution 1% of PAN in ethylic alcohol, solution 1% of p-dimethylamino-benzilidenrodamine in alcohol, solution 2% of 8-hydroxyquinoline in cloroform and UV examination at $\lambda = 254\text{nm}$ for Fe^{3+} , Co^{2+} , Cu^{2+} , Pb^{2+} , Ni^{2+} , Pt^{4+} , Ag^{+} , Bi^{3+} , Hg^{2+} (Janjic et al., 1994).
- solution 0.1% β -naphtol in methanol (Shimizu et al., 1991)
- alcoholic solution 0.1 of pyridine-2-aldehyde-2-furoilhidrazone and rubeanic acid (Shimizu et al., 1994).

In this work we wished to separate a mixture of toxic cations on chromatographic plates prepared of cellulose MN (Macherey-Nagel) and silicagel chemical modified with mercaptopropiltrimetoxisilan, as well as on plates with R silicagel impregnated with a solution 20% of piperazine.

RESULTS AND DISCUSSIONS

Cromatographic plates with R silicagel, cellulose MN (Macherey-Nagel) and cellulose MN mixed with R silicagel chemical modified with mercaptopropiltrimetoxisilan (1:1, g/g) were prepared by solvation in distilled water of the organic liant CM-cellulose and agar-agar (1:1, g/g) in a concentration of 3% (Volymets et al., 1986); after that the R silicagel and MN cellulose powders were added in mixture with R silicagel chemical modified with mercaptopropiltri-

metoxisilan. The obtained paste was layed on glass plates 20x20x0.3 cm, 0.3 mm thick, with a special device.

Plates impregnation with R silicagel was realised by eluation with a solution 20% piperazine in distilled water for 4 hours.

Standard solutions 0.1% in distilled water of the studied cations (see Table 1) have been dropped on the chromatographic plates with Brand micropipettes. The used mobile phases were mixtures of piperazine 20%-methanol-acetic acid (30:65:5, v/v) for the plates with R silicagel, cellulose MN and the mixture cellulose MN-R silicagel chemical modified with mercaptopropiltrimetoxisilan and buthanol-benzen-acetic acid (50:30:20, v/v) for R silicagel and R silicagel impregnated with piperazine 20%.

After developing, the plates are kept for 15 minutes in a niche and after that are sprayed with a ditizone visualizing solution 0.1% in ethilic alcohol. Results are presented in table 1.

Table 1: Values $R_f \times 100$ of the studied toxic cations

Nr. crt.	Studied cations	$R_f \times 100$				
		Mobil phase A	Mobil phase A	Mobil phase A	Mobil phase B	Mobil phase B
		R Silicagel	MN Cellulose	MN-SMC Cellulose (1:1, g/g)	R Silicagel	R Silicagel impregnated with PIP
1.	Cr ³⁺	70	84	80	25	63
2.	Cd ²⁺	70	85	0	27	48
3.	Pb ²⁺	60	73	10	21	54
4.	Bi ³⁺	35	54	25	22	38
5.	Hg ²⁺	90	70	72; 50	84	69
6.	Co ²⁺	80	90	80	13	84
7.	Cu ²⁺	75	70	12	34	32

Mobil phase A: piperazine 20%-methanol-acetic acid (30:65:5, v/v)

Mobil phase B: buthanol-benzen-acetic acid (50:30:20, v/v)

SMC - Silicagel chemical modified

PIP - Piperazine

CONCLUSIONS

Using piperazine as a complexing agent, either in mobile phase or by impregnation of the stationary phase it can be seen from table 1 that the best results are obtained on layers of R silicagel chemical modified with mercaptopropiltrimetoxisilan in MN cellulose mixture (1:1, v/v) using as a mobile phase solution of piperazine 20%-methanol-acetic acid (30:65:5, v/v) and R silicagel impregnated with piperazine 20% and as mobile phase buthanol-benzen-acetic acid (50:30:20, v/v).

At the separation on MN cellulose layers mixed with silicagel chemical modified with mercaptopropiltrimetoxisilan for the Hg ion appear two distinct spots due to the appearance of two complexes of Hg with piperazine and the -SH group from the chemical modified silicagel.

Using the two separations on R silicagel impregnated with piperazine and MN cellulose mixed with chemical modified silicagel all the seven toxic cations can be separated and identified.

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PRECONCENTRATION AND SEPARATION BY TLC OF SOME TOXIC CATIONS FROM THE SOMEȘ RIVER WATER

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ABSTRACT

A series of toxic cations from the water of river Someș in Cluj-Napoca city, were preconcentrated on a column filled with impregnated silicagel with 2-acetyl-amino-5-mercapto-1,3,4 thiadiazole (2AAMTD).

A sample from this water, in a volume of 1 litter was passed through a column filled with impregnated silicagel. The cations retained on the column were eluted with 20 ml of nitric acid 4N. From the solution eluted were spotted manually spots in a volume of 5 ml with fine Brand micropipettes. The plates used for cromatography were glass plates coated with diethylaminoethylcellulose. The same volume of pure cations solutions were spotted on the same plate.

The mobile phase was methanol : sulphuric acid (9 : 1, v/v) and the detection was made with alcoholic solution (0.1%) of pyridine 2-aldehyde-2-furoylhydrazone and under UV light.

According to this were separated and identified the following toxic cations: Fe^{2+} , Co^{2+} , Ni^{2+} , Cd^{2+} , Cu^{2+} , Pb^{2+} , Zn^{2+} , Cr^{3+} .

Key words: thin layer cromatography, toxic cations, Someș river water.

INTRODUCTION

The 2 acetyl-amino-5-mercapto-1,3,4 thiadiazole (2 AAMTD) loaded on the silica gel was used as preconcentration reagent for Cu (II), Cd (II), Ni (II), Pb (II), Zn (II), Fe (II), Co(II) and Cr (III) prior to Atomic Absorption Spectrometric etermination. Both "in the series" and column methods were used for the preconcentration of the above methods. the absorption – elution cycle was repeated four times with no observable decline in the efficiency of the absorbent. As there are no observable effects due to changes in volume of samples solution up to a volume of 1 litter, a concentration factor of 20 can be achived. The method has been applied to the samples of Someș River water followed by TLC.

MATERIALS AND METHODS

Preparation on Silica gel impre with 2 AAMTDgnated

For impregnation we used Macherey-Nagel Silicagel 60 silica gel for column chromatography (0.05-0.2 mm). 100 g silica gel was mixed with 100 ml of 2AAMTD 2% alcoholic solution. After 5 min. of stirring, the mixture was kept for 24 h at 4 °C, afterwards was filted and dried in vacuum. For the quantitative determination of 2-acetyl-amino-5-mercapto-1,3,4 thiadiazole fixed on silica gel, the elementary analyses was used.

Preconcentration procedure

For the determination of the optimal conditions, pH and the necessary time for ions changing, we used the "in the series" procedure. 50 ml of 0.001% solution of Cu (II), Cd (II), Ni (II), Pb (II), Zn (II), Fe (II), Co(II) and Cr (III) was stirred with 0.5 g of impregnated silica gel from 5 to 300 seconds after which every solution was filtered. The concentration in the filtered solution was determined by AAS (Mohammed and Majid Khan, 1993; Baranowska et al, 1992; Asolkar et al, 1992; Volynets et al, 1989, Stephens, 1980; Deshmukhand and Kharat, 1989; American Cyanamid, American Patent, 1958).

For the quantitative determination of recovered metal ions were used the "on column" procedure. Glass columns (ϕ 12 mm), were filled with 1g impregnated silicagel washed with NH_3 2.5×10^{-3} M solution till pH= 7.5-8. For each cation 50 ml of 0.001% solution was passed through the columns with approx. 1 ml/min. To determine the retained quantity of cations the columns were eluted with 20 ml of HNO_3 of different concentrations (1N, 2N, 4N and 6N). The effluent was collected on 25 ml calibrated flasks, brought up to sign with deionized water, in order to determine the ion content by AAS. In order to separate and identify by TLC the above named cations from the Someş River water, 1000 ml of water from a place from our town, were eluted on a column filled with 5g of impregnated silica gel. After elution the column was washed with 50 ml of nitric acid 4N. (Deshmukhand and Kharat, 1990; Ajnal et al, 1989; Stake et al, 1986; Nagahiro et al, 1990; Popper et al, 1964; Roman et al, 1995).

Thin layer chromatography

Thin layer chromatography (TLC) was performed on 20x20 cm home made glass plates coated with diethylamino-octyl cellulose using a mixture of 2% Agar-Agar. Plates were spotted manually, 2 cm from the bottom edge and 1 cm apart, by means of fine Brand micropipettes 3 μl /spot of river water.

Chromatography was performed by ascending development through 15 cm., in N type chambers, with methanol : sulphuric acid (9:1, v/v) as mobile phase. Pyridine-2-aldehyde-2-furoylhydrazone (0.1%) and UV light at 366 nm (Camag lamp) were used for cations visualization: Cd (II), Cu (II), Pb (II), Ni (II), Zn (II), Fe (II), Co (II), Cr (III).

RESULTS AND DISCUSSIONS

2 AAMTD is a yellow crystalline powder with solubilisation both in water and in dilute acids. The elementary analyses confirm the theoretical results and the IR analyses confirm the molecule structure, as it is shown in table1.

Table1: The elementary analyses of 2-acetylamino-5-mercapto-1,3,4 thiadizole

Element	Theoretic results	Practical results
C	28.42	28.68
H	2.87	2.80
N	23.98	23.74
O	9.13	-
S	35.60	35.70

The elementary analyses shows that the range of fixed quantity of 2 AAMTD on silicagel is between 1.5-2.0%. After "in series" determination we notice that the reaction between Me (II) and 2 AAMTD impregnated on silica gel is going on with a high speed and over 95% of Me(II) had been fixed after 5 min. of stirring. The effect on shaking time on the adsorption is shown in Table 2.

Table 2: Effects on stirring time on the adsorption; quantity of Me (II) and Me (III) fixed on 2 AAMTD (%)

Stirring time	5 (sec)	15 (sec)	20 (sec)	30 (sec)	60 (sec)
Quantity of cation retained	%	%	%	%	%
Cd	79.20	79.86	80.25	85.15	89.90
Cr	79.50	79.95	80.60	86.16	90.50
Cu	78.98	79.95	80.70	86.30	90.78
Ni	79.62	79.85	80.35	85.96	88.98
Zn	79.15	79.60	80.15	84.92	89.50
Pb	79.64	79.80	80.74	84.81	88.80
Fe	78.35	79.70	80.90	85.30	89.30
Co	79.25	79.99	81.03	86.20	90.10

The fixing capacity of Me (II) on 2AAMTD is influenced also by pH, in a pH range between 5-10.

The best Me (II) recovery was obtained by using HNO₃ at different concentration as eluting solutions. The best results for cation recovery were obtained with HNO₃ 4N, except for Cu (II), where the HNO₃ 6N was used as it is shown in table 3.

Table 3: The results of the cations recovery

Coefficient of recovery (%)	HNO ₃ 1N	HNO ₃ 2N	HNO ₃ 4N	HNO ₃ 6N
Cd (II)	96.56	98.13	98.54	95.85
Cu (II)	76.14	44.90	85.92	90.44
Pb (II)	88.80	86.00	96.94	76.20
Ni (II)	37.32	44.26	81.20	57.84
Zn (II)	48.04	52.62	73.50	54.76
Fe (II)	62.20	70.10	87.90	90.30
Co (II)	44.70	60.35	87.90	82.15
Cr (III)	52.70	73.18	94.20	80.30

The effect of preconcentration is relevant when passing 1000 ml containing 1 µg/ml of Me (II) through a column filled with 5 g silica gel impregnated with 2AAMTD after eluting with 50 ml HNO₃ 4N, the Me (II) concentration increased of about 15-20 times. The results are illustrated in table 4.

Table 4: The preconcentration results of 1000ml solution which contain 1 µl Me/ml

Cation	Cd	Cr	Cu	Ni	Zn	Pb
µg/Me/ml	19.64	18.88	19.38	16.24	14.10	17.0

The plates were pulverisation with pyridine-2-aldehyde-2-furoylhydrazone and under UV light at 366 nm the meall the mentioned cations were identified as it is shown in table 5. The spots of water samples shows the presence of other cations which are not identified.

Table 5: The $R_f \times 100$ values for the cations separated on DEAE-cellulose R, with methanol : sulphuric acid (9:1, v/v) as mobile phase

No.	Compound	$R_f \times 100$	Vizualization
1	Cd (II)	57	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
2	Cu (II)	28	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
3	Pb (II)	4	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
4	Ni (II)	67	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
5	Zn (II)	38	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
6	Fe (II)	98	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
7	Co (II)	72	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)
8	Cr (III)	64	pyridine-2-aldehyde-2-furoylhydrazone (0.1%)

CONCLUSIONS

The 2 AAMTD impregnated on silica gel can be used for the determination of trace amounts of free dissolved metal ions from waste water or natural waters, too (Cenan et al, 1997).

The proposed method can be applicable to the enrichment of other elements and be combined with highly sensitive determination method.

Thin layer chromatography can be used when no apparatus is available.

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OXIDATIVE STRESS EVALUATION IN EXPERIMENTAL AND CLINICAL ASTHMA

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ABSTRACT

It was studied the intensity of the oxidative stress in ovalbumin sensitised rats and in asthmatic children. Ten Sprague- Dawley male rats were sensitised in 1,7,14 and 21 days with 0.5 ml ovalbumin solution s.c and challenge in 28 days. On isolated rat trachea the acetylcholine dose-effect response was performed (10^{-5} – 10^{-4} M). The ovalbumin sensitisation increased the acetylcholine constrictor effect for all doses (mean value 25). In clinical study, the asthmatic children (mild form) were investigated. The enzymatic antioxidant capacity was studied with two parameters: superoxidedismutase (S.O.D.) and glutathione-reduced form (G.S.H.). We observed the enhancement of oxidative stress in sensitised animals and also in asthmatic children. Superoxidedismutase and glutathione-reduced form significantly decreased in sensitised animals in correlation with oxidative stress intensity and tracheal contractility.

Key words: acetylcholine, isolated trachea, ovalbumin, asthma, superoxidedismutase, glutathione-reduced form.

INTRODUCTION

Oxidative stress has been reported to induce the constriction of the airway smooth muscle in experimental and clinical asthma.

Free radicals are chemicals species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. They are produced continuously in cells either as accidental by-products of metabolism.

The most important reactants in free radical biochemistry in aerobic cells are oxygen and its radical derivates: superoxide, hydroxyl radical, hydrogen peroxide and transition metals.

Pathological free radicals reactions are uncontrolled, abnormal free radicals reaction occurring in the cells. Such reactions may be induced by physiological free radicals reactions if these escape from their control mechanism and also by external physical or chemical agents.

At the molecular level, oxygen toxicity is attributed to the reactions between cellular components and the free radicals formed from oxygen.

The compounds, which block free radicals reactions by trapping the radicals, are known as radical scavengers. Compounds, which provide protection against the toxic effects of the reactive oxygen species, are termed antioxidants. The following natural antioxidants are present in the membranes vitamins C, A, E and K, selenium and thiol-containing compounds glutathione.

The oxidative stress could be evaluated by decreased antioxidant capacity, expressed in this study by two parameters: superoxidedismutase and glutathione-reduced form.

In this study we established a correlation between the enhance of the acetylcholine constrictor effect and the intensity of oxidative stress, expressed by the decreased antioxidant capacity in rats sensitized with ovalbumin and in asthmatic children.

MATERIALS AND METHODS

Experimental asthma:

We used male, Sprague-Dawley rats (250-300g) separated in two groups: control group (10) and sensitised group (10).

Preparations were put in an organ bath containing 50 ml Krebs-Henselleit solution at 37 Celsius degree, continuously gassed with a mixture of 95% O₂ and 5% CO₂ in order to maintain oxygen tension and a pH of 7.4.

A force transducer for recording isometric contraction, displayed on a xy inscriptor, has been used in order to follow up tracheal smooth muscle contractility.

After an equilibrium period of 60 min with 6 intermediate changes of solution, a dose-response curve was performed using acetylcholine concentrations from 10⁻⁵ to 10⁻⁴ M.

For each study we have performed 4 to 6 experiments. Statistical analysis included calculation of mean values, standard deviation and Student's test.

Sensitisation protocol:

We used 0.5-ml ovalbumin solution subcutaneous (sc.) - in 1,7,14,21 days and 0.5-ml ovalbumin solution sc. - challenge in 28 days; the animals were sacrificed after 24 hours and we collected blood for biochemical tests.

Clinical asthma:

From asthmatic children-mild form, with age between 6-10 years old, we collected blood for the evaluation of the antioxidant capacity expressed by superoxidedismutase and glutathione-reduced form.

Biochemical Techniques:

Superoxidedismutase (S.O.D.): in a redox system, in presence of O²⁻, the indicator Tetrazolium-Blue-Nitro was reduced to Formazan, a coloured product which has maximum absorption at 560 nm. In the presence of S.O.D. activity, the number of O²⁻ radicals decreased and the Formazan production diminished.

Glutathione - reduced form (G.S.H.): glutathione from blood sample reacted with dissolved 5,5'-thiobis (2-nitrobenzoic acid) (DTNB) and resulted a coloured product which has maximum absorption at 420 nm.

RESULTS AND DISCUSSION

The acetylcholine constrictor effect increased in sensitised animals for all doses (mean value 25) (fig 1).

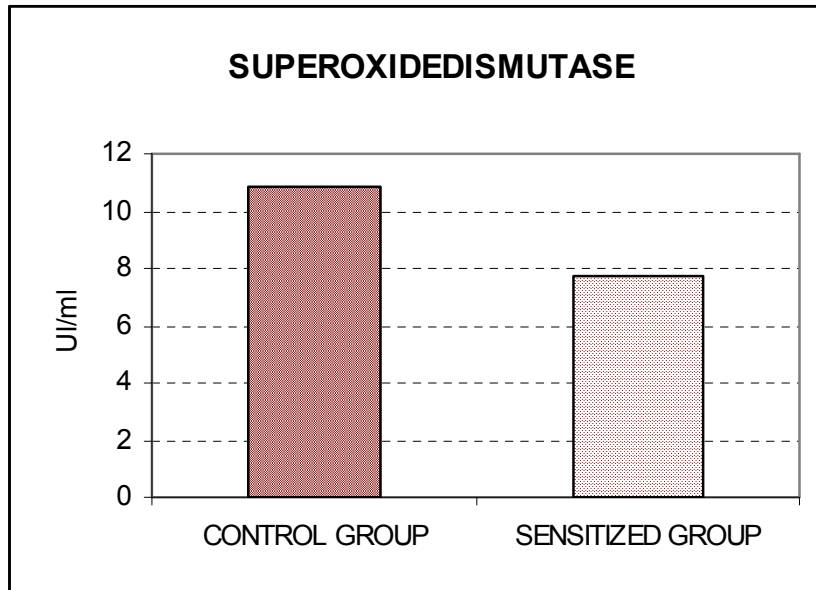


Fig. 1. Acetylcholine contractile effect for control and sensitized group.

The enzymatic antioxidant capacity expressed by S.O.D. and G.S.H. significantly decreased in sensitised group and in asthmatic children comparatively with control group:

S.O.D.: the control value was 10.72 U/ml and in sensitised group; it significantly decreased at 7.82 U/ml ($p < 0.01$) (fig 2)

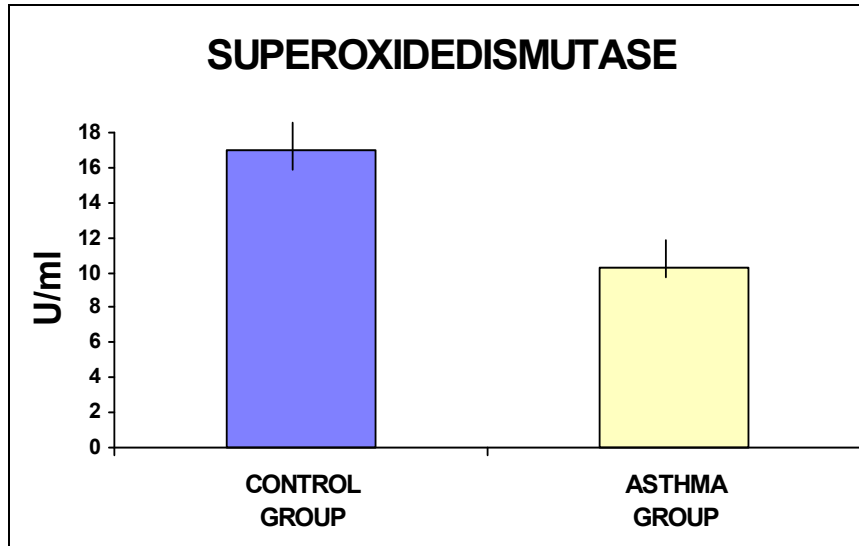


Fig. 2. The activity of superoxidedismutase for control and sensitized group.

In asthmatic children, the SOD value decreased at 10.28 U/ml, comparatively with the control value: 17.03 U/ml ($p < 0.001$) (fig 3).

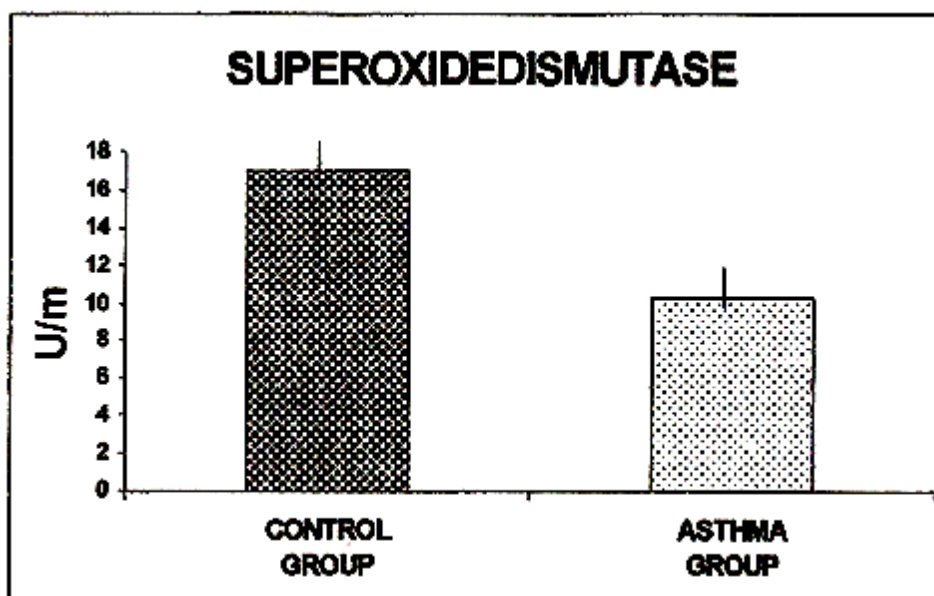


Fig. 3. The activity of superoxidedismutase for control and asthma group.

Cells have developed a comprehensive array of antioxidant defences to prevent free radical formation or limit their damaging effects. These include enzymes to decompose peroxides, proteins to sequester transition metals and a range of compounds to scavenge free radicals.

Enzymes also take part in the cellular defence against the free radicals. SOD is a metallo-enzyme, which contains manganese, iron, or copper and zinc. It transforms the superoxide anion radical to H_2O_2 , which is eliminated by peroxidase or catalase. An extracellular tetrameric SOD also protects the extracellular space from the pathological free radicals reactions.

A good cooperation between SOD and enzymes that detoxify H_2O_2 , catalase and Se-dependent GSH-Px is important for an efficient first line of defence for metabolised the generated H_2O_2 .

G.S.H.: the control value was 601 $\mu M/l$ in sensitised group and it significantly decreased at 336 $\mu M/l$ ($p < 0.001$) (fig 4).

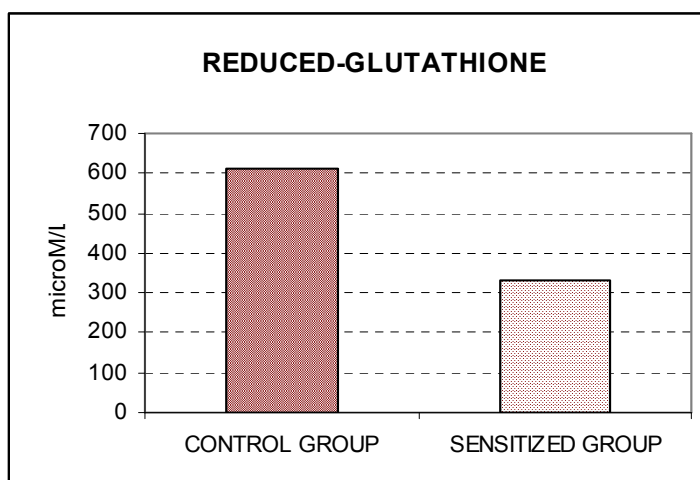


Fig. 4. The activity of glutathion-reduced form for control and sensitized group.

In clinical asthma, the GSH value decreased at 272 $\mu M/l$, comparatively with the control value: 300 $\mu M/l$ ($p < 0.01$) – fig. 5.

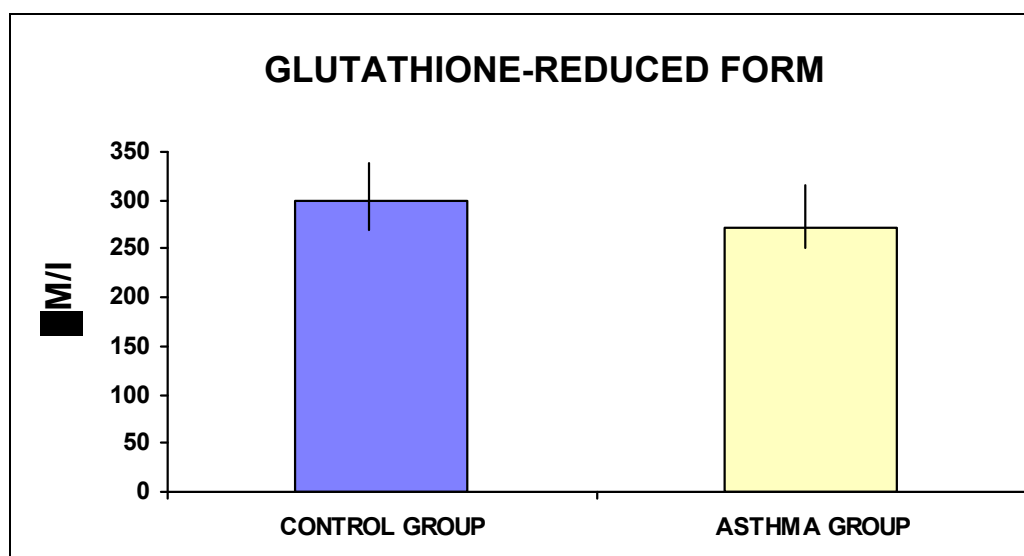


Fig. 5. The activity of glutathione-reduced form for control and asthma group.

Glutathione functions are involved in an important number of cellular processes. One of its functions is to detoxify reactive intermediates formed spontaneously or enzymically.

In the enzymically processes, the enzymes involved are GSH-transferases and GSH-peroxidases. Both peroxidases catalyze the reaction in which hydroperoxides are reduced too less reactive alcohol's. In this reaction GSH acts as a hydrogen-donor.

It's oxidised form; HSSG is reduced again by GSH – reductase, using NADPH as a substrate.

CONCLUSIONS

1. We observed the enhancement of tracheal contractility and oxidative stress in sensitised animals.
2. The parameters, which expressed the enzymatic antioxidant capacity: S.O.D. and G.S.H. significantly decreased in sensitised group and in asthmatic children.
3. The enzymatic antioxidant capacity decreasing was correlated with oxidative stress intensity
4. We observed a correlation between the enhance of tracheal contractility and oxidative stress intensity in ovalbumin sensitised rats.

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“IN VIVO” TESTING OF BIOCOMPATIBILITY OF TITANIUM CLIPS USED IN LAPAROSCOPIC SURGERY

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ABSTRACT

We used four groups of Whistar white rats, both sexes, with weight between 360 and 380 g, each group being composed of 10 animals. Titanium clips and threads (produced by ETHICON firm and romanian products) were introduced in peritoneal cavity by fixing on epiploon. Rats subdued of the experiment were sacrificed after 1,2,4 and 6 months. The results of this experimental study show that both titanium clips can be used in laparoscopic surgery with similary results.

Key words: titanium clips, biocompatibility, experimental

INTRODUCTION

Development of laparoscopic surgery required “in vivo” searching of bioincompatibility of titanium clips on laboratory animals. For this reason we accomplished a study using experience animals about the effects determined by implantation titanium clips at level of peritoneal cavity, taking as witness the reaction determined by Nylon 10 and Vicryl 3-0 threads. The study included a macro- and microscopic searching of the tissues on the surface related to the implant, as well of tissues from another regions of the peritoneal cavity, for a period of six months.

MATERIALS AND METHODS

We used four groups of Wistar white rats, both sexes, with weight between 360 and 380g, each group being composed of 10 animals. Titanium clips and threads were introduced in peritoneal cavity by fixing on epiploon. At rats from first two groups were fixed titanium clips produced in our country respectively titanium clips produced by Ethicon firm. At rats from the 3rd and 4th groups were fixed Nylon 10 threads, respectively Vicryl 3-0 threads. At all animals it was used general intraperitoneal anesthesia with Tropanal 500mg, 0.1ml/100g weight. The approach of peritoneal cavity was made by median laparotomy and then titanium clips and threads were applied on epiploon. The closing of abdominal cavity was made by wound suture with Nylon 8 threads at the level of the muscle aponeuretic layer and then at the skin level. The postsurgery evolution was favorable for all animals. Rats subdued of the experiment were sacrificed after 1,2,4 and 6 months. It was macroscopically examined the aspect of peritoneal cavity and then it was taken samples both from the place where the clips and threads were applied and from sites situated at a distance from the implant place. All samples were histopathologically analyzed.

RESULTS AND DISCUSSIONS

A. Macroscopic features

At rats from first two groups the aspect of peritoneal cavity was normal, except one case from 1st group which presented a little zone (approximately 1.5/1cm) of fibrous induration, which contained the clip in the middle. This change is explained by a more difficult application of the clip and by consecutive producing of haemotoma.

At rats from 3rd and 4th groups, macroscopic changes were minimal, both at the fixing place of threads on epiploon and at a distance. The liver, spleen, stomach and guts presented normal aspects.

B. Microscopic features

I. 1st group of rats

a) 1 and 2 months postsurgery

- intense hyperemic reaction in the loose connective tissue around the foreign material;
- cellular infiltrate composed of polymorphonuclear leukocytes;
- moderate fibrous reaction

b) 4 and 6 months postsurgery

- mild inflammatory reaction;
- mild fibrous reaction.

II. 2nd group of rats

a) 1 and 2 months postsurgery

- a lower hyperemic reaction comparatively with the first group;
- cellular infiltrate composed of polymorphonuclear leukocytes;
- presence of fibrin.

b) 4 and 6 months postsurgery

- inflammatory and fibrous reactions were more reduced comparatively with the first group.

III. 3rd group of rats

a) one month postsurgery

- numerous granulomas of foreign body in the loose connective tissue of the epiploon;
- granulomas were constituted of nodular masses of granulation tissue, including a great number of multinucleated giant cells around threads, lymphocy plasmocytes, a few granulocytes and fibrilar connective tissue which was pretty well represented.

b) 2 and 4 months postsurgery

- presence of resorption granulomas;
- fibrillar connective reaction;
- mild inflammatory reaction (a few lymphocytes and plasmocytes);
- autolysis of giant cells;
- nuclear and cell debris.

c) 6 months postsurgery

- minimal inflammatory reaction;
- minimal fibrous reaction;
- total hydrolysis of Vicryl 3-0 threads.

CONCLUSIONS

Tested clips presented a good biological tolerance. The biocompatibility of titanium clips were proved by:

- the favourable postsurgery evolution;
- the normal aspect of peritoneal cavity;
- the absence of macroscopic changes at the level of the implant place; except one case from 1st group which presented a little zone of fibrous anduration;
- microscopically, there were no granulomas of foreign body, comparatively with witness groups;
- inflammatory reaction and fibrous reaction were minimal. At rats from 3rd group these reactions were produced comparatively with animals from 1st group. These differences are expalined by greater size of autochtonous clips and by the chemical composition, autochtonous clips being more pure.

The results of this experimental study show that titanium clips can be used in laparoscopic surgery.

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THE INFLUENCE OF THE TAR EXPOSURE ON THE COPPER CONTENT OF SOIL, PLANTS AND HUMAN IN ROSITZ AREA (GERMANY)

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ABSTRACT

Copper is both an essential and potential toxic element for plants, animals and human. The decontamination of tar-exposed soil by combustion at 1200 °C increased the Cu content of soil residues from 12 to 53 mg/kg. The Cu content in the soil decreased insignificantly from 17 to 7.2 mg/kg air-dried soil with increasing depth. There are not significantly difference between Cu content from control, contaminated and decontaminated soil used in the pot trials with oats, mustard and spinach. The tar-exposed soil has not been effected the Cu content of the 3 plant species.

The analysis of several fruits and vegetables from house gardens also showed that the tar exposure did not affect their Cu content compared with the loess control area. It was demonstrated with the duplicate method at 7 women and 7 men from Rositz and 10 women and 10 men from Jena in 7 subsequent days that people in Rositz consumed 1.4 respectively 1,6 mg Cu/day and those from Jena 1.4 respectively 1.3 mg Cu/day. The WHO, FAO and IAEA recommended 1.0 mg Cu/day for women and 1.2 mg Cu/day for men.

Key words: tar exposure, copper content

INTRODUCTION

Copper is widely distributed in nature in elementary state, sulfides, arsenates, chlorides and carbonates. The concentration of copper in the earth's crust is generally estimated at 50 mg/kg, it tends to be the highest in the ferromagnesium minerals, such as the basalts pyropene and biotite, where its average is 140 mg/kg (Scheinberg H., 1991). The movement of the relatively high concentration of copper from the earth's crust into the soil depends on weathering, the processes of soil formation, drainage, oxidation-reduction potentials, the amount of organic matter in the soil and, perhaps the most important, the pH. Copper is concentrated in the clay mineral fractions enriched in organic carbon. The medium level of Cu concentrations varies from 13 mg Cu/kg up to 24 mg Cu/kg being the highest for Kastanozems and Chernozems and the least for Podzols and Histosols (Kabata Pendias and Pendias, 1992). Agricultural soils with 100 mg Cu/kg or a limit of 2-3 kg Cu/ha/ year are considered acceptable (Hoffman G. 1990). The appearance of copper deficiency in agricultural and horticultural soils was comprehensive described by Bergmann (1992). Plants contain 4-20 mg Cu/kg dry weight. Some plant species have a great tolerance to increased concentrations of Cu and may accumulate extremely high amounts of this metal in their tissue. Deficiency in plants, though rare, has been reported and usually associated with

less than 5 mg Cu/kg in sunflowers, spinach and lucerne (Anke et al. 1994). Copper is also widely distributed in the human body and takes part in many processes associated with the development of young organisms (e.g. the synthesis of the complex proteins of collagenous tissues in the skeleton and the myelinization of nerve fibres), so it should be present in the daily diet (Eastwood 1997).

MATERIALS AND METHODS

The investigation based on the analysis of :

- samples of contaminated soil
- samples of decontaminated soil at 1000-1200°C
- oat, mustard and spinach samples in the pot trial with different soils
- fruits, vegetables and spices from the tar contaminated living area Rositz in comparison with Jena.
- food duplicates on adults (7 men, 7 women) from the tar contaminated area and from the control area (10 men, 10 women).

The copper analysis was carried out by means of the optical emission spectroscopy with inductively coupled plasma (ICP-OES). The soil samples were prepared with aqua-regia (Hoffmann 1991). The samples of plants material and foods duplicate were dried to constant weight at 105°C and dry-ashed afterwards at 450°C. The samples were brought in solution through mineralisation with 25% hydrochloric acid.

RESULTS AND DISCUSSIONS

The Copper content of the Rositz soil

The investigated samples proceed from six particularly tar contaminated station in an industrial area (table 1). The soil samples contained in average 12 mg Cu/kg dry matter. The copper concentration varied between 7,2 and 21 mg/kg, insignificantly ($F_p > 0.05$).

Table 1: The Copper content of the contaminated soil from Rositz

Station	$\bar{x}$	s	%
1(4)	15	14	125
2(6)	10	4.2	83
3(5)	21	20	175
4(4)	9.4	1.2	78
5(4)	8.0	2.0	67
6(4)	7.2	2.0	6,0
$\bar{x}$	12	11	100
Fp	> 0.05		-

All copper concentrations from soil samples are in admitted limits. Industrial areas with loess contained in average 5 to 30 mg/kg Cu (Kabata Pendias and Pendias, 1992). The highest copper concentration in soil in industrial area is 56 mg/kg, under the maximum admitted limit of 100 mg/kg for agricultural soils.

The Copper concentration in different soil horizons from Rositz

Soil samples were collected from different horizons for 5m depth. The copper concentration decreased with the depth (table 2). The copper accumulation in top horizon of soil is characteristic for this trace element. This phenomenon is an effect of various factors, but above all, Cu concentration in surface soils reflects the bioaccumulation of the metal and also anthropogenic sources of copper. The explanation is general available and corresponds also for Rositz. The high standard deviation for upper soil of 17, reflects a different anthropogenic copper emissions.

Table 2: The Copper content of the contaminated soil from Rositz in different soil horizons (mg/kg dry matter; n = replicates number)

Depth, m(n)	$\bar{x}$	s	%
0-1m (9)	17	17	142
1-2m (5)	12	3.1	100
2-3m (5)	9.0	4.0	75
3-4m (4)	8.7	1.5	72
4-5m (4)	7.2	1.7	42
(27)	12	11	100
Fp	> 0.05		-

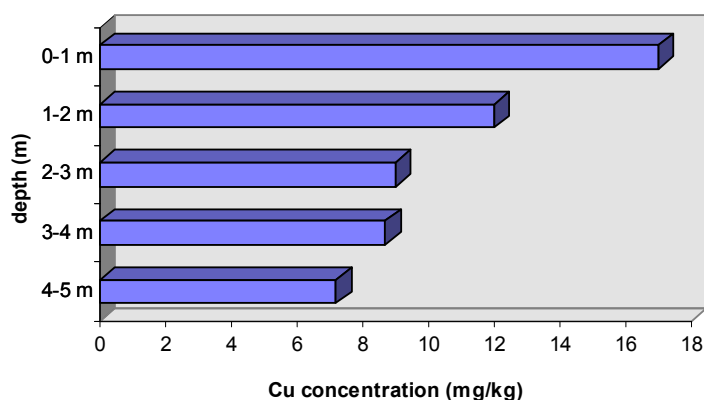


Fig.1: The Cu distribution in different soil horizons

The thermic decontamination of soil for all stations and depth determines increase of copper concentration, more as fourfold, but also, under the maximum admitted limit (table 3). Both, the tar pollution and organic matter of Rositz soil, lead to an enrichment of copper.

Table 3: The Copper content of the contaminated and decontaminated soil from Rositz (mg/kg)

N;n	Contaminated soil		decontaminated soil		p	% ¹⁾
	s	$\bar{x}$	$\bar{x}$	s		
27:6	11	12	53	17	<0.001	442

1) contaminated soil = 100%

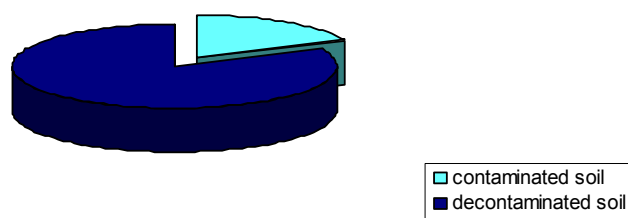


Fig.2: The Cu content of contaminated and decontaminated soil from Rositz (mg/kg)

The Copper content in the pot trial with different soils

The copper transfer from soil in plants was analysed for three different species under controlled conditions in green house and it was repeated six times. In the pot trial, the control soil contained in average less copper than all other soils: tar contaminated soil from Rositz, his decontaminated soil and two mixed soils from control and tar contaminated soil, respectively decontaminated soil (table 4). All differents in the copper content of six soils are significantly.

Table 4: The Copper content in the pot trial with different soils (mg/kg dry matter)

Kind of soil	$\bar{x}$	s	% ¹⁾
Control soil (6)	14	0.59	100
Contaminated soil (6)	22	1.6	157
Decontaminated soil (6)	32	1.4	229
50% contaminated soil + 50% control soil (6)	19	0.58	136
50% decontaminated soil + 50% control soil (6)	24	1.22	171
Fp	<0.001		-

1) control soil = 100%; other soil = x%

The tar contaminated upper soil from industrial polluted area of Rositz contained 22 mg Cu/kg, a typical copper concentration for loess, while the control soil contained 14 mg Cu/kg, this corresponds for the sand soil type. The decontaminated soil accumulated 32 mg Cu/kg, more than twofold of copper content in control soil. The standard deviation of copper content in the pot trial with different soils was low. This demonstrated a homogeneity of utilized soils.

The effect of oat, mustard and spinach in the tar contaminated soil from Rositz and his decontaminated soil on the copper transfer in the food chain

1.Oat

Surprising, the green oat in panicle in the control soil with the least copper concentration, accumulated relative much copper in the green mass due to the pH-value (table 5). It was remarked a good bioavailability of copper in a soil with a pH of 5.5 and one moderate in contaminated soil from Rositz with 7.3 pH.

Table 5: The copper content of oat grown on different kind of soil (mg/kg DM)

Kind of soil	$\bar{x}$	s	% ¹⁾
Control soil	13	3.6	100
Contaminated soil	11	0.99	85
Decontaminated soil	16	8.6	123
50% contaminated soil + 50% control soil	10	0.5	77
50% decontaminated soil + 50% control soil	13	1.2	100
Fp	>0.05		-

1) control soil = 100%; other soil = x%

The relatively more copper content in the oat grown on decontaminated soil was due to his slow development. The young plants accumulated more copper per kg dry matter than those matures (Anke et al. , 1994).

2. Mustard

The copper intake of mustard like a second culture in the same soil, enriched with roots mass, followed a similar rule such as the oat (table 6). Again, the mustard grown on the tar contaminated soil from Rositz, enriched with roots mass, the decontaminated soil and mixed soils, contained significantly less copper that the control soil.

His neutral pH-value was the cause of the lower bioavailability of the copper in this type of soil.

Table 6: The copper content of mustard in pot trial (mg/kg DM)

Kind of soil	$\bar{x}$	s	%
Control soil	11	5.4	100
Contaminated soil	8.4	0.75	76
Decontaminated soil	7.3	1.7	66
50% contaminated soil + 50% control soil	9.2	1.0	91
50% decontaminated soil + 50% control soil	6.8	1.2	62
Fp	>0.05		-

3. Spinach

Like a third culture plant, was spinach which again, in control soil accumulated the most copper. The spinach grown on all the other soil variants accumulated only 39 up to 78% copper (table 7).

Table 7: The copper content of spinach in the pot trials (mg/kg DM)

Kind of soil	$\bar{x}$	s	%
Control soil	23	1.5	100
Contaminated soil	15	5.4	65
Decontaminated soil	9.0	1.7	39
50% contaminated soil + 50% control soil	18	2.7	78
50% decontaminated soil + 50% control soil	15	3.1	65
Fp	>0.05		-

The spinach from the intensive enrichment with roots decontaminated soil, was developed especially good, without to normalize his copper amount. The copper content of spinach grown on all the other soil variants was differentiated insignificantly. The danger of copper load is excluded for the plants grown on the tar contaminated soil, respective his decontaminated soil from the pot trial with oat, mustard and spinach.

The copper content in different fruits, vegetables and spices from Rositz and the control area-Jena

As a result of fruits, vegetables and spices analysis, only chive, leek and kohlrabi from Rositz contain a high copper amount comparative with those from the control area (table 8). The different copper content between the control area and Rositz remained in average insignificantly. Theirs copper content is normal (Rohrig, 1998).

Table 8: The copper content in different fruits, vegetables and spices from Rositz and control area (mg/kg DM)

Sort (n;n)	Control area		Rositz		p	% ¹⁾
	s	$\bar{x}$	$\bar{x}$	s		
Chives (23;11)	2.0	5.3	10	9.8	> 0.05	189
leeks (7;7)	2.3	5,9	8.1	4.2	> 0.05	162
Kohlrabi (8;12)	2.1	4.1	5.3	1.9	> 0.05	129
Apples (24;15)	0.93	2.6	2.8	1.0	> 0.05	107
Carrots (24;13)	2.7	5.4	5.5	4.1	> 0.05	102
pears (2;8)	3.6	6.4	5.3	1.3	> 0.05	83
Anions (23;8)	2.5	5.5	4.2	1.3	> 0.05	76
Beans (11;6)	1.9	7.4	5.0	1.6	< 0.05	68
Cucumbers (15;7)	4.0	1.3	8.7	1.8	< 0.05	67
Potatoes (25;13)	3.2	6.4	4.2	1.2	< 0.05	66

1)control area = 100%; Rositz = x%

The copper intake of adults

The adults from Rositz (both sex), have been ingested in November and December significantly more copper in foods and beverages than those from Jena, in January (table 9). This isn't because of great dry matter winter consumption, but probable of local influences (e.g. copper content of drinking water, self-prepared beverages) (Anke et al. 1997). The copper content in drinking water in Germany is of 12 µg/l. The both sex inhabitants from Rositz consumed food with 34% more copper than those from Jena.

Table 9: The copper intake of adults from Jena and Rositz (mg/kg DM)

Living areas (n;n)	Women		Men		p	%(1)
	s	$\bar{x}$	$\bar{x}$	s		
Jena (70;70)	8	3.8	3.2	1.2	> 0.05	84
Rositz (49;49)	2.4	5.1	4.3	1.8	> 0.05	84
P	<0.001		< 0.001		-	
%(2)	134		134			

1) women = 100%; men = x%; 2) Jena = 100%; Rositz = x%

The men from Rositz and Jena consumed in average with 16% less copper in dry-matter than the women. The difference remained insignificantly. This insignificantly difference appeared because of the women preferences for cocoa, chocolate and all the cocoa produces (Rohrig 1998).

The least daily copper consumption by women is 0.9 mg and 1.0 mg by men and the highest is 2.7mg by women and 2.1 mg by men. For the adults human it was recommended by the WHO/FAO/IAEA, in average, the intake of 1.0 mg Cu/day by women and 1.2 mg Cu/day by men (Anonym, 1989). This recommendation of copper consumption was exceeded as well by the tested inhabitants from Rositz as by the control group from Jena (table 10).

Table 10: The mean copper intake of adults from Jena and Rositz (mg/day)

Living areas (n;n)	Women		Men		p	% ¹⁾
	S	$\bar{x}$	$\bar{x}$	s		
Jena (70;70)	1.2	1.4	1.3	0.57	> 0.05	93
Rositz (49;49)	0.8	1.4	1.6	0.88	> 0.05	114
P	>0.05		< 0.05		-	
% ²⁾	100		123			

%¹⁾women = 100%; men = x%; %²⁾Jena = 100%; Rositz = x%

The appearance of a copper deficiency is excluded at the inhabitants from the tar contaminated area-Rositz. Also, a chronic copper intake isn't expected.

CONCLUSIONS

1. There is not an excessive copper in soil, plants and human population
2. The copper concentration of soils, plants as well as the copper intake by the Rositz inhabitants are not so high and not so low as to affected the human health.
3. The inhabitants from Rositz, both sex, consumed in average more copper than the other tested inhabitants of Germany. The cause of this consumption is not known.
4. Concerning his copper amount the thermic decontaminated soil can be used as building material.

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STUDIES REGARDING THE FIXING OF TIN IONS (II) ON PECTINS OF THE CELL MEMBRANE OF SUGAR BEET

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ABSTRACT

Studies concerning the fixing of tin (II) cations on the pectins of sugar beet draff, made in comparison with those for Ca(II), Cu(II), Zn(II) and Cd(II) ions, revealed the following affinity scale of pectic substances ("in muro") $\text{Sn}^{2+} \sim \text{Cu}^{2+} > \text{Cd}^{2+} \sim \text{Zn}^{2+} \gg \text{Ca}^{2+}$. Using the experimental data and the Langmuir model there were estimated the values of maximum quantities of tin that can be detained by the pectins of sugar beet draff.

Key words: sugar beet draff, pectines, metallic cations, tin, bisorption

INTRODUCTION

Industrial era involved an intensification on the international market of the demand of raw materials, including metals (Bădăraș et al., 1999), determining an increase on waste materials that arrive in the environment. After the classification made by Galvin (1996), among the 25 most met metals in industrial waters, copper, cadmium, tin and zinc are considered to be current toxic. The pollution potential of these metals eliminated in the environment does not disappear, being amplified during the transfers of matter in the trophic chain.

is one of the newest physico-chemical methods of treating industrial waters polluted with metallic cations (Brauckmann, 1990; Tollier and Robin, 1979). The criteria for an ideal biosorbant are: to be cheap, not expansive obtainement; rapid ion fixation; the form, mechanic properties of the biosorbant to be compatible with a continuos utilisation; to be selective, the desorbition process to train a minimum degradation of the biosorbant.

Due to its high content in pectines, sugar beet draff (an abundant subproduct of sugar industry) is a potential biosorbant (Nriagu, 1996; Thellier and Ripoll, 1992). Pectines (figure 1 - after Thibault et al., 1993) having a high density of anionic charges are considered polyelectrolytes that can fix metallic cations, conferring the vegetal membranes properties of ion exchangers, governed by Donnan's laws (Nriagu, 1996; Dronnet et al., 1996). The main active component of the biosorbant, in this case, is the galacturonic acid.

In this paper we proposed to evaluate by three different methods (Lutwick et al., 1988) the fixing capacity of the pectines from sugar beet draff of tin cations, having also in view the influence of the dispersing environment conditions. On this way, almost theoretically, it could be appreciated if sugar beet draff can be a biosorbant for tin (II) cations.

MATERIALS AND METHODS

The characteristics of the used layers is presented in tabel 1. Draff acidification was made after Bertin method (Thellier and Ripoll, 1992). Saponification of draff was done by cooling a suspension of acidified draff (20g/l) at 4°C with pH adjusted at 13 by adding a solution of NaOH 1M, followed by a 2 hours stirring, neutralisation with a solution Hcl 0,1M, washing and drying.

Metallic cations were obtained from solutions of $\text{Sn}(\text{NO}_3)_2$, $\text{Cu}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{Zn}(\text{NO}_3)_2 \cdot 7\text{H}_2\text{O}$.

Tabel 1: Galacturonic acid, arabinose content and some physico-chemical parameters of the used layers (α = dissociation coefficient)

Specification	Draff	Saponified draff	Used method
Galacturonic acid (mg/g)	220	205	Blumenkranz and Asboe Hansen
Arabinosis	229	226	Thelier and Ripoll
Methyl-esterification degree (GM) (%)	52	< 2	Voragen
Acetylation degree (GA) (%)	52	< 1	Voragen
Cation exchange capacity (CSC) (meq/g)	0,55	1,13	$\text{C.S.C.} = \text{AcGal} \cdot (1 - \text{GM}) / 176,16$
Linear density of maximum charge ξ (for $\alpha=1$)	0,773	1,578	$\xi = \alpha \cdot 1,61 \cdot (1 - \text{GM} / 100)$

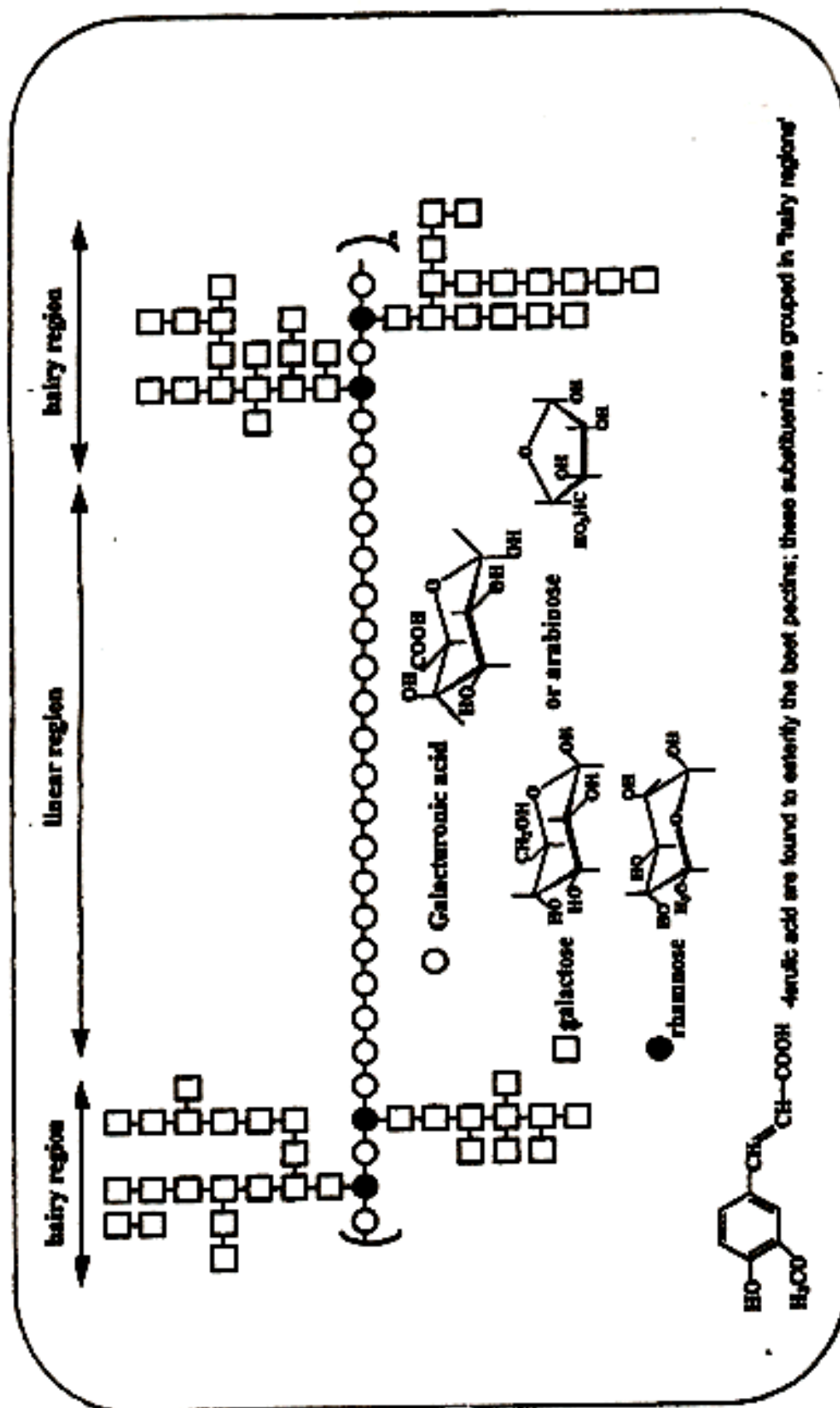
The selectivity of the pectines from draff for the studied cations was estimated with the help of two methods: pH-metric method (Lutwick et al., 1988) and the method of fixing isotherms (Lutwick et al., 1988, Dronnet et al., 1996).

In order to estimate from the quantitative point of view the fixing capacity of cations on the used layers, the Langmuir isotherms were used. These were obtained with the experimental data and using the following equation:

$$\frac{[\text{Me}]_{\text{final}}}{[\text{Me}]_A} = \frac{1}{\text{Me}_{A\text{max}} K_L} + \frac{[\text{Me}]_{\text{final}}}{\text{Me}_{A\text{max}}}$$

where: $[\text{Me}]_A$ = concentration in fixed metal related to layers' mass
 $\text{Me}_{A\text{max}}$ = maximum fixing capacity (maximum amount of metal fixed on a gram of substance)
 K_L = absorption equilibrium constant
 $[\text{Me}]_{\text{final}}$ = residual concentration of metal at equilibrium

We used the Langmuir model because it was also used in the case of other biosorbants (Brady and Duncan, 1994a; Brady et al., 1994b; Chang and Hong, 1996; Crist et al., 1988; Holan et al., 1993; Hu et al., 1996; Marshall et al., 1993; Namasivayam and Yamuna, 1995).



RESULTS AND DISCUSSIONS

Selectivity of draff for the cations Sn(II), Cu(II), Cd(II), Zn(II), Ca(II) - pH-metric method

The evolution of pH differences registered after adding increasing quantities of metallic cations to a draff suspension with a concentration of 2 meq/l, brought to an initial pH of 5.1 is presented in fig. 2, both using the "continuous" method (the cation solution was added in small quantities in the same quantity of draff suspension) and using the "in batch" method (a series of suspensions were prepared with different ratios $[Me^{2+}]_{total} / [Cp]$). The last method was used because the kinetics of fixation of cations by draff is not exactly known.

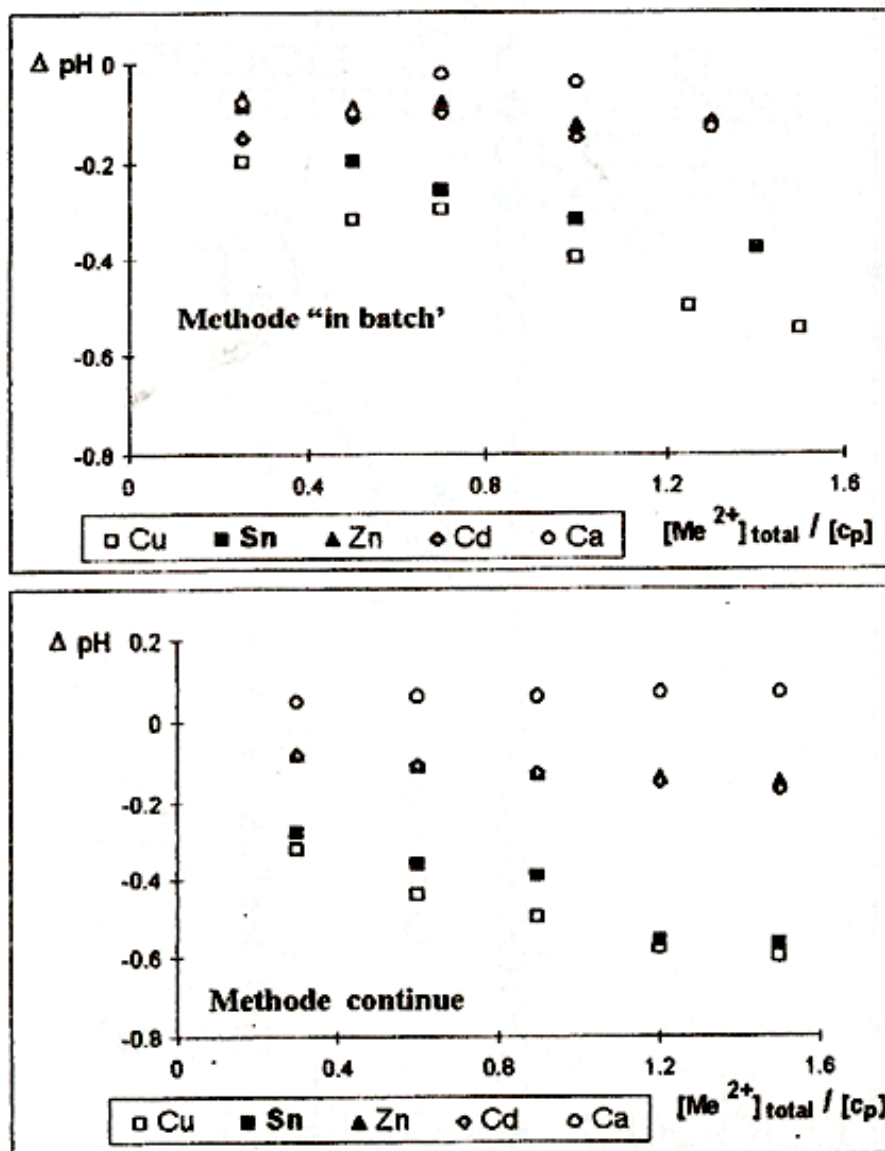


Fig. 2. Evolution of pH, corrected for dilution, for the sugar beet draff suspension (2 meq/l) at an initial pH of 5.1, in sodium nitrate 0.1M environment

The graphic was obtained representing ΔpH related to the value of the ratio $[Me^{2+}]_{total} / [Cp]$, $[Me^{2+}]_{total}$ = total concentration of the cations, $[Cp]$ = pectine concentration, exactly the number of equivalents of carboxyl groups dissociated in one liter of solution). As it can be observed in this figure, the used cations may be classified in two groups: one group contains the Cu^{2+} and Sn^{2+} ions and the other

group Zn^{2+} , Cd^{2+} and Ca^{2+} ions. In the case of the ions of the first group once the content of cations added at the pectine solution increases (increased values of the ratio $[\text{Me}^{2+}]_{\text{total}} / [\text{Cp}]$), much bigger amplitudes of pH can be registered than in the case of Zn^{2+} , Cd^{2+} and Ca^{2+} . The fixing capacity of the tested ions on the pectines from sugar beet draff decreases from copper ions to calcium ions, tin ions having a higher fixing capacity as compared to those of calcium, zinc and cadmium.

The two methods of adding metallic cations solutions to draff suspensions permit the determination of the same selectivity sequence that is: $\text{Cu}^{2+} > \text{Sn}^{2+} \gg \text{Cd}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+}$. Anyway it is more obvious by the continuous method.

Affinity of draff for tin (II) cations - method of fixing isotherms

It was studied the absorption of metallic cations on unmanufactured draff (suspension in distilled water) in quantities of 3.36, 14.55 g/l (that is 2 and 8 meq/l). Figure 3 represents the ratio $[\text{Me}^{2+}]_{\text{fixed}} / [\text{Cp}]$ related to $[\text{Me}^{2+}]_{\text{total}} / [\text{Cp}]$ in the case of the absorption of metallic cations on unmanufactured draff (suspension in distilled water) in quantities of 3.36, 14.55 g/l (that is 2 and 8 meq/l).

For a ratio $[\text{Me}^{2+}]_{\text{total}} / [\text{Cp}] = 1$ the fixing degree is multiplied by 4; 3 and 1.6 for Ca^{2+} , Cd^{2+} and Zn^{2+} . If the draff content is 4 times bigger, while for Sn^{2+} and Cu^{2+} ions the fixing degree is multiplied only by 0.92 and 1.2 if the suspension contains 14.55g/j draff compared with that with a 4 times less concentration. The fixing level of this 4 cations is very high (0.8) even at a father draff content, while the fixing level of the first 3 cations in this conditions is low (< 0.4).

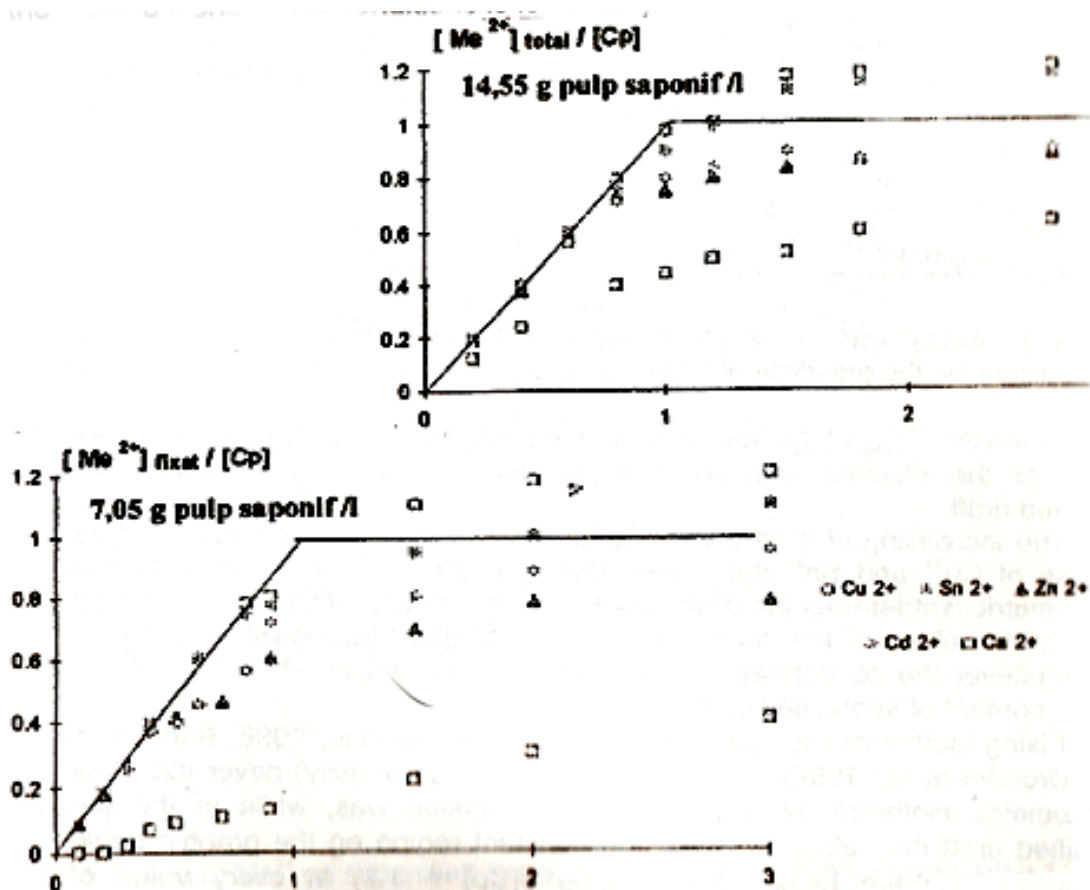


Fig. 3. Fixing of Me^{2+} on sugar beet draff (different concentrations of suspensions) in the presence of NaNO_3 solution 0.1M; (-----) stichiometric isotherm

there were used suspensions with a content of 7.05 g/l (8meq/l) and 14.55 g/l (16.5 meq/l), the cationic exchange capacity being 1.13.

The representations of $[Me^{2+}]_{fixed} / [Cp] = f([Me^{2+}]_{total} / [Cp])$ for all studied cations are grouped in figure 4.

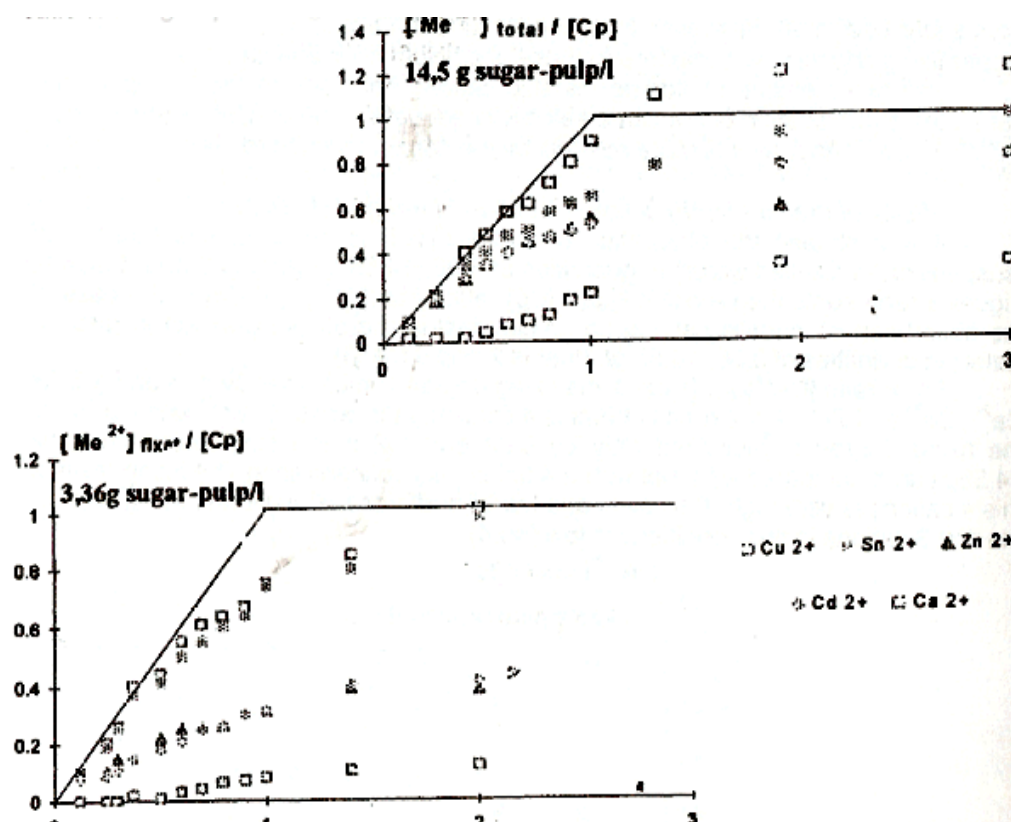


Fig. 4. Fixing of Me^{2+} on saponified sugar beet draff (different concentrations of suspensions) in the presence of $NaNO_3$ solution 0.1M; (-----) stichiometric isotherm

When $[Me^{2+}]_{total} = [Cp]$ the values of the ratio $[Me^{2+}]_{fixed} / [Cp]$ are lower than the plateau of the isotherm for all cations at any concentration of saponified draff.

The increasing of draff content means an increasing of the fixing degree. In the case of Cu^{2+} and Sn^{2+} the values from the graphic are closer to that of the isotherm as the draff concentration is bigger. These can even exceed the constant values of this isotherm at a ratio of $([Me^{2+}]_{fixed} / [Cp])$ of about 1.2 for Cu^{2+} , whatever the concentration of suspension and of 1.05 -1.15 for Sn^{2+} related with the content of saponified draff.

Fixing isotherms for pectines (Bădărașu and Neamțu, 1998; Bădărașu et al., 1999; Dronnet et al., 1996) (layers with higher charge density) never exceeded the isotherm, whatever the studied cation was, while in the case of saponified draff the values at which the constant region of the graphic begins for Cu^{2+} biosorption are higher than 1 ($[Cu^{2+}]/[Cp] = 1.2$) at every value of the concentration of suspension.

The more pronounced affinity of pectines “in muro” compared to that of soluble pectines from sugar beet could be explained by the different distance between the functional groups that could fix the metallic cations. Thus, in the case of pectines from the vegetal wall (saponified draff or that brought into an acid form), these positions are probably closer than at soluble pectines (substances more dispersed in the suspensions used) ((Bădăraș and Neamțu, 1998; Bădăraș et al., 1999; Dronnet et al., 1996). Another explanation would be the existence of some types of bindings that imply more than two carboxyl groups at one divalent cation (chelating, complexation) and/or a phenomenon of surface absorption.

Modelling of biosorbption

In figure 5 are presented all Langmuir representations for the cations whose biosorbption was studied on simple and saponified draff. Experimental results can be represented after the model of Langmuir isotherm whatever the concentration and type of layer, excepting the fixation of Ca^{2+} .

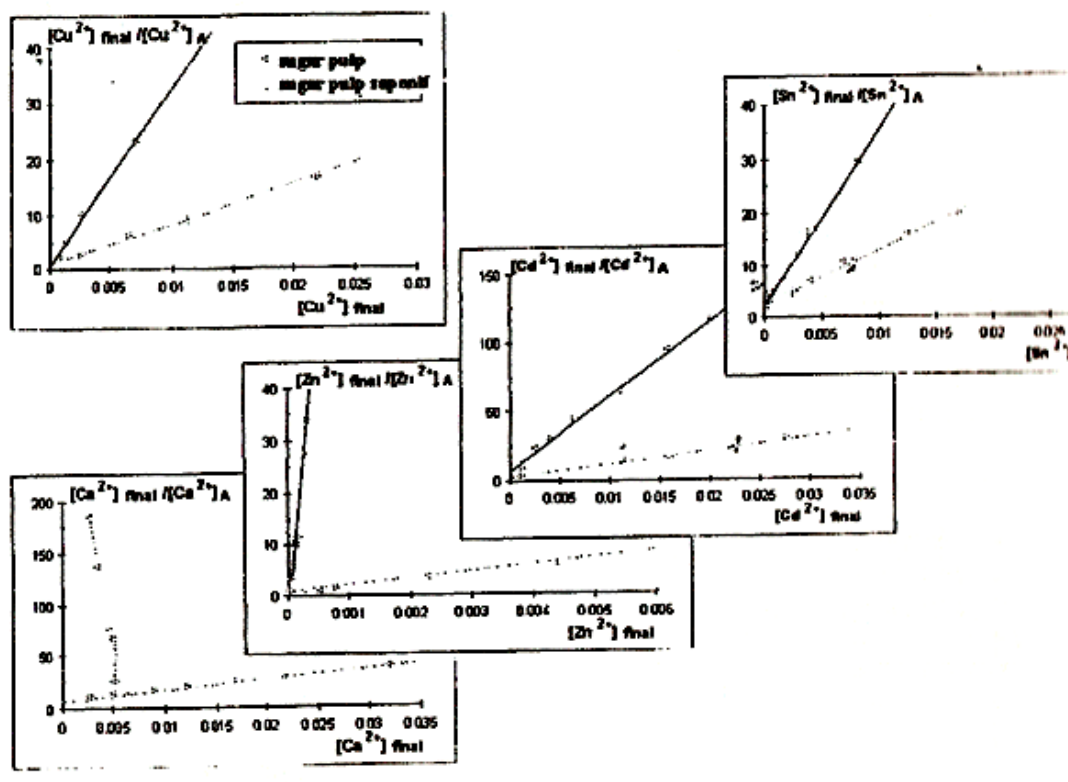


Fig. 5. Fixing of Me^{2+} on saponified and simple sugar beet draff (concentration 14.55g/l). Langmuir isotherms obtained with experimental data (linear form of equation)

In the case of fixing on the saponified draff, the obtained isotherms registered much higher values than on simple draff. The inclination of the “line” described by the representation $[Me^{2+}]_{final}/[Me^{2+}]_A$ related to $[Me]_{final}$ is as greater as the fixation of ions is weaker. The inclination is negative in the case of fixing Ca^{2+} on draff, excluding in this case all possibilities of adjustment with the help of Langmuir model.

The parameters of the Langmuir equation determined with the isotherms from fig. 5 are grouped in 2, both for simple and saponified draff. These data reveal the fixing of Me^{2+} on sugar beet draff in acid and saponified form.

Also, the ratio Me_{Amax}/CSC corresponds at the maximum number of fixed cations related at the number of groups from layer that can fix cations. Value 1 for this ratio designates a perfect stoichiometry of fixation against the ionic groups (carboxyl functions), this means 1 cation Me^{2+} is fixed on two groups COO^- .

Values of the ratios Me_{Amax}/CSC in the case of Cu^{2+} and Sn^{2+} ions are greater than 1 for a concentration of simple draff of 14.55 g/l and for any concentration of saponified draff suspension, demonstrating in this way that the simple electrostatic bond between two carboxyl functions and a divalent cation is not the only one implied in the fixation of these cations.

Percentage of metal fixed on the layer

From the values Me_{Amax} determined by the Langmuir equation, the mass percentage of metallic cations fixed on the layer was evaluated (2 a and b).

The maximum percentage of fixed metal related to the dry substance of the layer are presented in 3.

2. Fixing of Me^{2+} on sugar beet draff in acid and saponified form. Parameters of Langmuir equations

a) Draff

Cation	Ca^{2+}		Zn^{2+}		Cd^{2+}		Sn^{2+}		Cu^{2+}	
Conc. subst. (g/l)	3.64	14.55	3.64	14.55	3.64	14.55	3.64	14.55	3.64	14.55
K_L (l/eq)	-	-	1700	2170	620	750	6610	6570	10970	8200
Me_{Amax} (meq/l)	-	-	0.27	0.49	0.38	0.47	0.46	0.58	0.54	0.62
Me_{Amax}/CSC	-	-	0.49	0.85	0.77	0.86	0.83	1.05	1.00	1.12
r^2	-	-	0.990	0.994	0.988	0.983	0.985	0.980	0.993	0.998

b) Saponified draff

Cation	Ca ²⁺		Zn ²⁺		Cd ²⁺		Sn ²⁺		Cu ²⁺	
Cont. layer (g/l)	7.05	14.55	7.05	14.44	7.05	14.55	7.05	14.55	7.05	14.55
K _L (l/eq)	-	185	1285	1240	1710	1320	4440	2930	4730	3740
Me _{Amax} (meq/l)	-	0.83	0.91	1.01	1.07	1.11	1.17	1.28	1.30	1.35
Me _{Amax} /CSC	-	0.73	0.81	0.89	0.95	1.00	1.04	1.13	1.15	1.20
r ²	-	0.997	0.998	0.999	0.999	0.998	0.999	0.999	0.998	0.998

3. Fixing of Me²⁺ on sugar beet draff in acid and saponified form. Maximum percentage of fixed metal related to the dry substance of the layer

Layer	Draff		Saponified draff	
Content (g/l)	3.64	14.55	7.05	14.55
Ca ²⁺	-	-	-	1.7
Zn ²⁺	0.9	1.6	3.0	3.3
Cd ²⁺	2.1	2.6	6.0	6.8
Sn ²⁺	4.8	6.0	6.9	7.6
Cu ²⁺	1.7	2.0	4.1	4.3

The highest percentages of fixed metal on the layer were registered in the case of tin ions biosorption. Even if the values Me_{Amax} for Sn²⁺ are always lower than that of Cu²⁺ (2) the mass percentage of tin recovering is net superior due to the higher atomic mass compared to that of copper. The values of maximum quantities of tin that can be detained by the pectines from sugar beet draff content brought into an acid form and the saponified one are likely to that established for other biosorbents (Brady and Duncan, 1994a; Brady et al., 1994b; Chang and Hong, 1996; Crist et al., 1988; Holan et al., 1993; Hu et al., 1996; Marshall et al., 1993; Namasivayam and Yamuna, 1995).

CONCLUSIONS

1. Using the pH-metric method the following scale of selectivity was estimated for the tested cations on sugar beet draff $\text{Sn}^{2+} \sim \text{Cu}^{2+} > \text{Cd}^{2+} \sim \text{Zn}^{2+} \gg \text{Ca}^{2+}$
2. Comparing the fixing isotherms it was established the same selectivity of sugar beet draff for the used metallic cations.
3. The level of fixation of tin (II) cations on saponified draff is higher than that of isotherms, these fact can be due to the existence of some binding types that imply more than two carboxyl groups at a divalent cation (chelating, complexation) and/or a surface adsorption phenomenon.

4. Fixing capacity of tin (II) ions on sugar beet draff, evaluated with the Langmuir model proved to be comparing with that of copper (II) ions, but much higher than that of zinc (II), cadmium (II) and calcium (II).

5. The greatest percentage of metal detained both by acid and saponified draff was registered in the case of tin (II) ions biosorption.

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THE INFLUENCE OF SOME BIVALENT CATIONS ON NONSTEROID ANTIINFLAMMATORY DRUGS INDUCED GASTRIC ULCER IN RATS

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ABSTRACT

Bivalent cations have many implications in the human organism, being important both for normal and pathological functioning. In our work we investigated the influence of zinc (Zn), magnesium (Mg), nickel (Ni) and copper (Cu) on the experimental induced gastric ulcer in rats. We worked on five series of rats in which we produced gastric ulcer administrating phenilbutasone (Phb) 0.50 mg/kg by endogastric probe. One hour before the Phb administration we administrated intraperitoneal zinc acexamate (ZnAc), magnesium acexamate (MgAc), copper II formagan (CuF) and respectively nickel chloride (NiCl₂). The obtained data show that Zn significantly decreases the ulcerogenic effect of Phb, while Ni increases it. Cu and Mg compounds don't significantly influence the experimental ulcerogenity. We consider that some bivalent actions, in certain plasmatic level, can have a protective effect against gastric ulcer induced by nonsteroid antiinflammatory (NSAI) drugs.

Key words: phenilbutasone, gastric ulcer, zinc, magnesium, copper, nickel.

INTRODUCTION

The metal bivalent cations are wide spread both in nature and in human organism and they have a lot of complex roles (Lohman and Beyersmann, 1993; Pais I., 1995).

The digestive system is one of the systems in which trace elements have numerous implications. As these cations are present in food and water, digestive tract is the first contacted system in the organism.

Every lack of balance of these cations is involved in the pathogenesis of many diseases (such as spasmophilia, cardiac arrhythmia, blood coagulation diseases, rickets etc.) On the other hand bivalent cations have many therapeutic uses. Thus, magnesium is used in the treatment of some forms of spasmophilia, acute myocardial infarction, supraventricular arrhythmia, stress etc (Classen, 1986, Woods et al, 1992, Smetana, 1997, Durlach et al, 1992).

Zinc compounds are used in the treatment of gastric and duodenal ulcer, they can increase the antibacterial activity of several sulfonamides, they can be used in immunity stimulation etc. (Bulbena et al., 1990).

Therapeutic uses of new compounds containing calcium, copper, iron etc are more and more numerous.

NSAI are some of the most used drugs in the present pharmacotherapeutics. But their aggressivity on the gastric mucous, producing or aggravating gastric ulcer, abdominal pain, digestive bleeding etc. is the most frequent and damaging adverse effect.

In our work we investigated the influence of some compounds containing four bivalent cations on the experimental NSAID induced ulcer in rats.

MATERIALS AND METHODS

We worked on series of 10 adult, male Wistar rats, weighting 190-220 g, bred in the same laboratory conditions and identically fed. All the animals received no food 14 hours before the experiment started, but they received water ad libitum. In order to produce gastric ulcer we administered phenylbutasone (Phb) 50 mg/kg by endogastric probe to all the animals.

The first series was the control one and it received only Phb.

One hour before Phb each of the other series received intraperitoneal a compound containing one of the investigated ions as follows:

- series II received zinc acexamate (ZnAc - fig. 1) 0.5 mEq/kg;

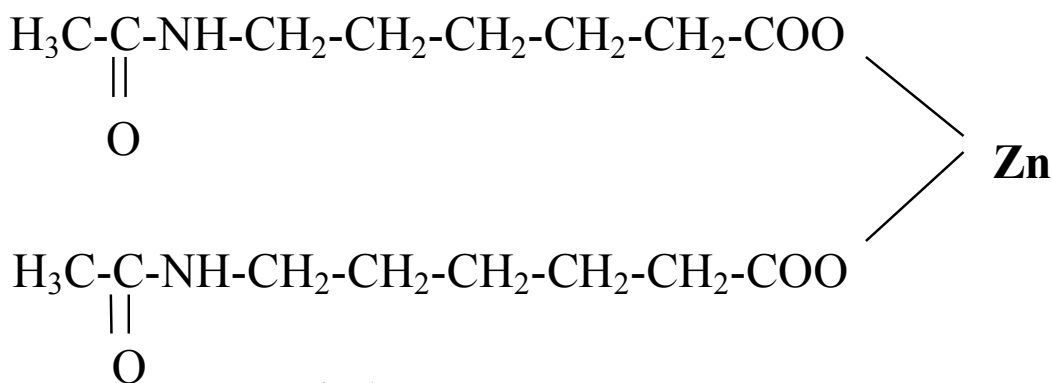


Fig. 1. Zn acexamate

- series III received magnesium acexamate (MgAc - fig. 2) 0.5 mEq/kg;

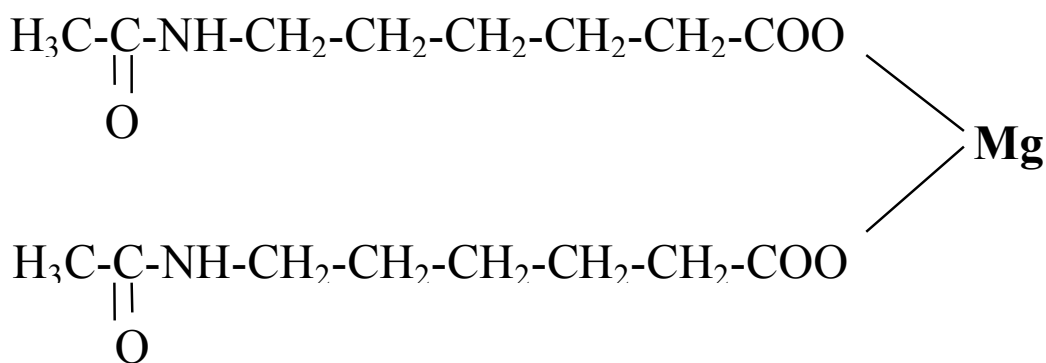


Fig. 2. Mg acexamate

- series IV received copper formagan (CuF - fig.3) 0.5 mEq/kg;

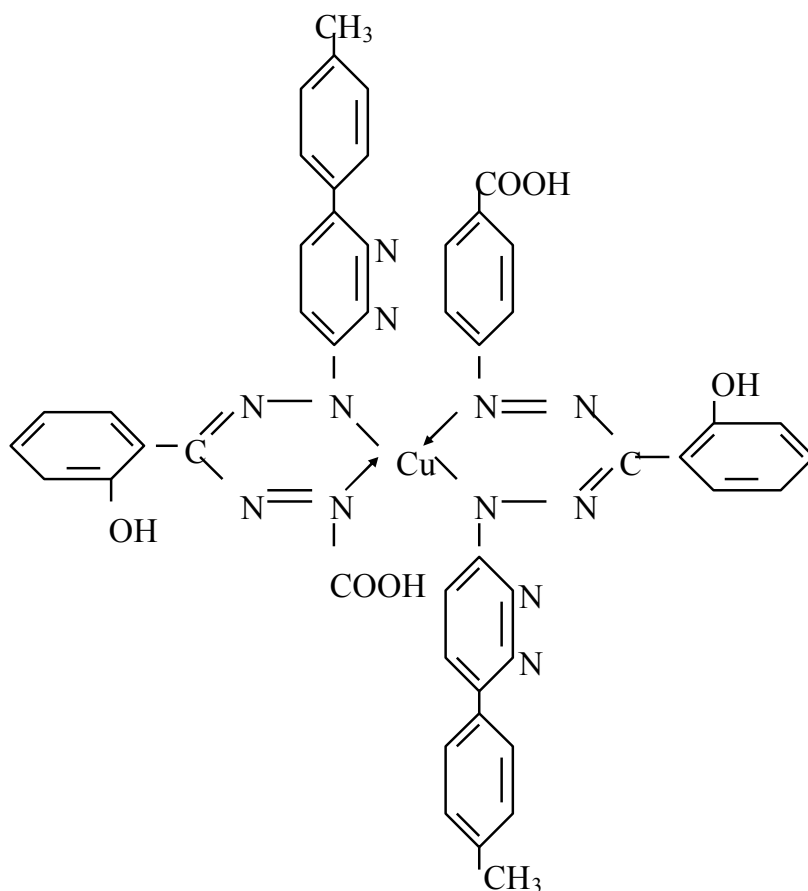


Fig. 3. Copper II formagan

- series V received nickel chloride (NiCl_2) 0.05 mEq/kg.

All the animals were sacrificed six hours after Phb administration by carotid sectioning. We removed the stomachs and examined them both macroscopically and microscopically. We quantified the mucous damage using a 0-4 scale:

- 0 - no lesion;
- 1 - moderate hemorrhage;
- 2 - intense hemorrhage;
- 3 - hemorrhages + 1-3 zones of necrosis;
- 4 - hemorrhages and numerous zones of necrosis.

We also determined the plasmatic levels of the investigated ions in blood samples drawn from the retroorbital plexus at various moments of the experiment using an atomic absorptive spectrometer.

The obtained data were statistically interpreted by means of "t" test.

RESULTS AND DISCUSSIONS

The influence of the tested ions on Phb ulcerogenity can be seen in table 1.

Table 1. The influence of the tested ions on the NSAII induced ulcer in rats

Specification	Control (Phb)	ZnAc+Phb	MgAc+Phb	NiCl ₂ +Phb	CuF+Phb
Gastric score	2.59±0.57	1.31±0.27	2.29±0.31	3.27±0.22	2.42±0.41
P(towards the control group)		<0.01	NS	<0.05	NS

NS = non significant

The plasmatic levels of the investigated ions (at various moments of the experiment) are shown in table 2.

Table 2. Plasmatic level of the investigated ions (mg/l)

Specification	Zn	Mg	Ni	Cu
Initially	14.68	19.2	0.05	*
1 hour	29.98	26	4.3	*
2 hours	27.13	27.1	4.68	2.13
4 hours	27.54	23	4.18	5.87
6 hours	23.86	23.4	3.5	4.61

* undetectable plasmatic level of the investigated ion

Our obtained data show that ZnAc significantly decreases Phb ulcerogenity, thus confirming previous results of other researchers (Cho et al. 1976; Guerniche et al., 1995) and of our own (Nechifor et al., 1990)

Magnesium moderately decreases Phb ulcerogenity, but this effect is not statistically significant. The difference between those two compounds is due to the difference between the two metals because the acexamic acid certainly doesn't influence the ulcerogenity.

Nickel, also used in a much lower concentration than the other two ions, increases the damaging effect of Phb and we consider that, at least partially, this effect could be due to the generation of free radicals. Zinc and partially magnesium, too, can decrease the free radicals generation (Afanasiev et al., 1995). But the opposite effect between zinc (which has the capacity of reducing the lipid peroxidation) and nickel was also noticed in other tissues (Guerniche et al., 1995).

On the other hand we consider that the zinc protective effect at the lysosome membrane level could also be involved in the decrease of NAIS ulcerogenity. There also have been demonstrated the protective effect of zinc against apoptosis induced by various factors and this fact could also be important for gastric protection given by zinc compounds (Sunderman F.W., 1995).

Our results let us consider that there could be a modulating effect of bivalent cations on NAIS ulcerogenic action. This seems to be in concordance with data which showed that other cations (such as manganese etc) can diminish gastric lesions produced by various agents, like ethanol (Ligumsky et al., 1995).

We consider that the enrichment of certain cations (zinc, manganese etc.) intake could have a good preventive effect against various agents induced ulcer.

CONCLUSIONS

- a) In the dosage we used ZnAc significantly decreases Phb ulcerogenity.
- b) NiCl_2 significantly increases the damaging effect of Phb on the gastric mucous.
- c) MgAc and CuF have a statistically insignificant effect on Phb ulcerogenity.
- d) We consider that some bivalent cations could have a modulating role in the NSAID induced gastric ulcer.

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KINASE IMMUNODEFICIENCIES AND THE CALCIUM IONS TRANSMEMBRANE TRANSPORT

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ABSTRACT

In the processes of transmembrane transport, calcium plays an essential role. This fact was revealed in molecular biology and enzymology related to biomembranes. Ionised calcium intervenes also in mechanisms regarding membrane permeability, neuronal excitability, skeletal muscle contraction, energogenesis processes (e.g. activity of Ca-Mg-ATP-ase), hormonal liberation, enzyme activity related to bone mineralisation etc.

Deficiency of some proteins in which transmembrane transport ionised calcium is involved is correlated with some genetic disorder affecting immune system. In this context one can remark that Ca^{2+} ion is important in transmembrane transport and a calcium dyshomeostasy seems to be related with inherited immunodeficiencies like a Burton-tyrosine kinase (Btk) and Zeta-chain associated and phosphorylated tyrosine kinase (ZAP-70) deficiency.

Key words: kinase immunodeficiencies – calcium ions

INTRODUCTION

Calcium is one of the biometals present in the composition of various protides, lipids and carbohydrates. In blood serum around 45% of calcium is associated with proteins (80% with albumins and 20% with globulins); 50% is in free state (unbound, ionised etc.); 5% is bound to substances not associated with proteins.

The complexes and protein-bound calcium act as a reservoir, which maintains the equilibrium of, ionised fractions. Bounded calcium has also an important role in the configuration and function of associated proteins.

Numerous factors may disturb calcium homeostasis, such as: physiological factors, e.g. sex, age, pregnancy, lactation; nutritional factors, e.g.: decreased intake, malnutrition, presence of phytates, phosphates etc.; alcoholism; stress, physical activity, circadian rhythms; genetic factors, e.g.: modifications of membrane proteins, enzyme deficiencies (more specific in case of kinases); modifications in cytoplasmatic proteins etc.

In this paper there will be discussed some aspects regarding Ca^{2+} ions in protein deficiencies and genetic disorders of the immune system with details on some immunodeficiencies involving kinases.

DEFICIENT PROTEINS AND GENETIC DISORDERS

A number of minerals have been shown to be essential in development of immune response in animal. An increasing number of deficiency syndromes are becoming recognised in humans. The Ca^{2+} transmembrane transport seems to be related with an important domain of inherited immunodeficiencies like a Bruton-

tyrosine kinase (Btk) and Zeta-chain associated and phosphorylated tyrosine kinase (ZAP-70) deficiency – Table 1.

Table 1 : Deficient proteins in genetic disorders of the immune system (after Fischer and Leonard, 1995).

PROTEIN	DISORDER
<u>Membrane proteins</u> γ c (IL-2 receptor γ chain) CD 40 ligands fas (CD 95)	- X-linked SCID - Hyper-IgM syndrome - Lymphoproliferative syndrome
<u>Kinases</u> Btk ZAP-70 ATM DNA dependent protein kinase Jak 3	- X-linked agammaglobulinemia - CD8 deficiency - Ataxia-telangiectasia - Murine SCID - SCID
<u>Cytoplasmatic proteins</u> Tap 2 WASP (also nuclear)	- Low CD8 and NK cells - Wiskott-Aldrich Syndrome
<u>Transcription factors and transactivators</u> C II TA RFX 5 Winged helix protein	- MHC-class II deficiency - MHC-class II deficiency - Nude mouse

NOTE: SCID = Severe combined immunodeficiency.

* = Single aminoacid substitution found in patients with recurrent viral infections.

Jak 3 = Janus family tyrosine kinase.

BTK DEFICIENCY

X-linked agammaglobulinemia (XLA) was the first genetic immunodeficiency identified by Bruton. In XLA, there is defective B-cell differentiation, resulting in greatly diminished numbers of mature B cells, while pre-B and particular pro-B cells are detectable. The XLA is associated with mutations of the Btk (Bruton or B-tyrosine kinase) gene (Tsukada et al., 1993; Vetrie et al., 1993). Btk is a tyrosine kinase that is related to Itk/Tek and the newly recognised Bmx kinases.

The SH 2 and SH 3 domains contain a pleckstrin homology domain and a Tec homology domain in the NH₂-terminal tail of the molecule. Btk is expressed at all points in the B-cell differentiation pathway until plasmacells, in which Btk expression is turned off. Btk is expressed and by other haematopoietic lineages, particularly by most cells.

A different mutations of the btk gene (122 found in 157 families) encompassing most parts of the coding portion of the gene, have been described, with no clear correlation between the location of the mutation and clinical phenotype. In mice either a missense mutation in residue 28 or gene inactivation lead to a

milder phenotype than in humans with XLA, with the murine phenotype primarily characterized by a defective response to type 2 T-cell independent antigens.

Given the complexity of the Btk molecule, which can presumably interact with many proteins through its different domains, its precise role in B-cell development remains poorly understood. Some light has been shed from studies showing how the Src family tyrosine Kinase Lyn can activate Btk. By using a two-hybrid system and a vaccinia virus expression system, it was shown that Lyn can transiently interact with Btk and can phosphorylate a tyrosine in the Kinase domain of Btk. It is proposed that by altering the configuration of the kinase domain, this phosphorylation event activates Btk, leading to Btk autophosphorylation (notably of the SH 3 domain) as well as phosphorylation of substrate (the Ca^{2+} seems to be associated with this phosphorylation of the substrate). It is also assumed that Btk activation is preceded by its membrane association, since an active Btk mutant (Btk*) was found associated to the plasma membrane of LB-cells. However, it remains unclear how this scheme can be integrated in signals providing B-cell progression throughout its differentiation pathway.

ZAP-70 DEFICIENCY

Zeta-chain associated and phosphorylated tyrosine kinase (ZAP-70) is a tyrosine kinase selectively expressed in the T-cells that associate with the T-cell receptor zeta-chain. It contains a kinase and two SH 2 domains, and has a similar structure to that of Syk. It is proposed that following activation through T-cell receptor (TcR) triggering of the Src family kinase p56^{lck} or p59^{fyn} and subsequent phosphorylation of the immune receptor tyrosine activation motifs in the cytoplasmic domains of zeta and CD 3 chains, ZAP-70 associates and is then phosphorylated.

A genetic deficiency in ZAP-70 protein has now been recognised in five distinct families (Arpaia et al., 1994; Chan et al., 1994). Mutation affects either the kinase domain or one of the SH2 domains. In all cases, ZAP-70 inactivation results in a severe phenotype characterised by two main features:

- a lack of mature CD 8 T-cells (while CD 4 + CD 8 + thymocytes are detectable).
- a profoundly defective activation of peripheral CD 4 + T-cells that can be overcome by phorbol and calcium ionophore.

The latter finding demonstrates the major role of ZAP-70 in the TcR-mediated signaling cascade. Little tyrosine phosphorylation of cellular proteins can occur in the absence of functional ZAP-70.

Nevertheless, it has been proposed that Syk kinase is strongly enough expressed in thymocytes to partially overcome the ZAP-70 defect, allowing CD 4 + lymphocyte differentiation. CD 8-cell differentiation in this model will not occur because the Lck-mediated signal in CD 8 + cells is expected to be less intense than in CD 4 + cells, and thus insufficient to activate Syk.

Other defects in ZAP-70 protein were found in three patients in whom the T-cell deficiency was associated with eosinophilia. In these cases, the ZAP-70 gene sequence was normal, suggesting a regulatory defect. Rare cases of functional T-cell immunodeficiencies that remain unclear explained have been described in other patients, including defective Ca^{2+} entry and defective IL-2 synthesis. They represent an

interesting model for the further definition of genes of importance in lymphocyte signalling (Macchi et al., 1995).

CONCLUSIONS

1. Calcium dyshomeostasis seems to be related with inherited immunodeficiencies;
2. The transmembrane transport of Ca^{2+} is related with Bruton-tyrosine kinase (Btk);
3. Genetic deficiency in Zeta-chain associated and phosphorylated tyrosine kinase ZAP-70 protein affects the kinase domain or one of the SH 2 domains;
4. Little tyrosine phosphorylation of cellular proteins can occur in the absence of functional ZAP-70;
5. In patients with eosinophilia the ZAP-70 gene sequence was normal.

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HIGH PURITY FERRIC CITRATE- OBTENTION AND CHARACTERISATION

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ABSTRACT

High purity ferric citrate is as substance with a large ranges of use in the food industry, in medicine, in the ink and the photographic industry and as a biochemical reagent.

Key words: medical biochemistry, medicine.

INTRODUCTION

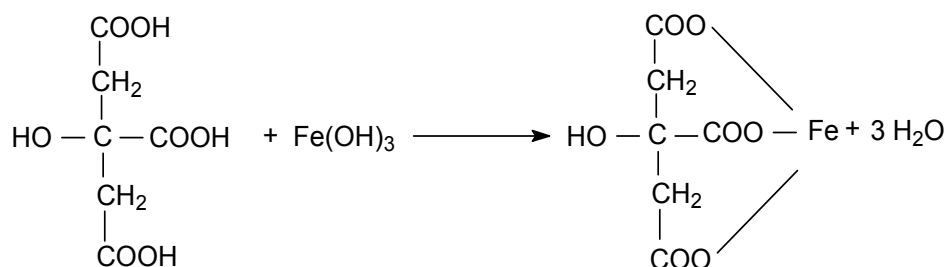
High purity ferric citrate isn't produced in our country. As a result of the performed studies and of the technical means of the high purity ferric citrate synthesising and taking into account the fact that the ferric citrate is not a substance with a definite composition, we intended to obtain a chemical reagent with the following characteristics which satisfy its rances of use.

Table 1: Characteristics of the high purity ferric citrate

Characteristics	Estimate	
Fe content (complexometric)	%	16 -20
Chlorides (Cl ⁻)	% max.	0,01
Sulphates (SO ₄ ²⁻)	% max.	0,03
Ammonia (NH ₄ ⁺)	% max.	0,5
Heavy metals (Pb)	% max.	0,005

MATERIALS AND METHODS

The ferric citrate is obtained through the iron action on the citric acid. The reaction that is supposed to take place is the following:



It's the only method that leads to the obtaining of the ferric citrate with an adequate iron content.

In order to obtain the ferric hydroxide we applied the quantitative method of the trivalent iron with ammonium hydroxide used in gravimetry.

The ferric chloride and the ammonium hydroxide of p.a. quality were used as raw materials.

In order that the ferric hydroxide precipitation to be quantitative (the Fe^{2+} ions form complexes with the ammonia and do not precipitate quantitatively) the ferric chloride solution was treated before the precipitation with hydrogen peroxide for the complete oxidation to Fe^{3+} . The pH of the reaction is comprised between 3 –7 and the drying temperature is 110°C .

It resulted from the experimental achievement, that the optimum technological parameters for the obtaining of the ferric citrate with an adequate iron content are the following:

- the concentration (normality) of the citric acid aqueous solution 3n;
- reaction temperature: 50°C ;
- perfection time of the reaction: 14 hours;
- drying temperature: 100°C ;
- drying time: 14 hours.

A performance efficiency of the ferric citrate synthesis couldn't be given because it was ascertained that during the reaction the quantity of the initial chemical reagent is found again at the end, the problem being only the structural composition of this complex.

RESULTS AND DISCUSSIONS

The purpose of the analytical study was the characterisation of the ferric citrate obtained as a result of the technological studies.

The compound appears in different formulas, its structure being dependent both on the obtaining conditions and on the isolation ones.

For the characterisation and the establishment of its chemical formula, the data of chemical analysis were correlated with a series of structural determinations:

- The carbon was determined through the Schöniger formula – found 21,5 – 22,2%; theoretically 22,7%;
- The iron was determined complexonometric through titration with EDTANa_2 at pH= 2-3 in the presence of the sulphosalicylic acid as indicator agent; Fe content = 16 – 16,5%; theoretical value 16,7%.

From the values of the carbon and iron content the combination ratio Fe(III): citric acid 1:1 is confirmed.

The purity of the ferric citrate was also controlled and this corresponds at limits to the product we wanted to obtain (see table 1).

The IR spectra were noted in KBr dispersion in the $400\text{--}4000\text{ cm}^{-1}$ with a SPEKORD 75 IR and they were compared to that of the free ligand, the citric acid, noted in the same conditions.

Table 2. The IR spectra of ferric citrate

Citric acid	Ferric citrate	Assignment
3500 – 3000 fi*	3500 – 3000 fi	ν H ₂ O associated
1750 fi	1750 u**	ν COOH
-	1600 fi	ν_{as} COO ⁻
-	1410 fi	ν_s COO ⁻
1200 fi	1200 fi	δ H ₂ O
1100 m***	1100 m	δ H ₂ O
900 m	900 m	ν C – CH ₂
-	480 - 470	ν Fe – O – C

* fi very intense

** u shoulder

*** m medium

In the 3500 – 3000 cm⁻¹ range both in the citric acid spectrum and in that of the ferric citrate, a wide intense band appears which is characteristic to that of the valence vibration of the water molecules associated through hydrogen bonds.

The valence vibration band characteristic of the COOH group which appears at 1750 cm⁻¹ in the free ligand, appears in the ferric citrate as a shoulder with a very weak intensity.

At the same time in the ferric citrate spectrum there were set off vibrations bands of ν_{as} and ν_s valence of the COO⁻ group coordinated at ~ 1600 and at 1410 cm⁻¹ respectively.

In the 470 – 480 cm⁻¹ range in the ferric citrate spectrum band characteristic of the Fe – O bond appears.

Therefore the study of the spectra in IR shows that the citric acid coordinates at the Fe (III) ion, through the three COOH groups.

There were also carried out electronic spectra. With the help of these electronic spectra further information concerning the electronic structure and the symmetry of ferric citrate complex was acquired.

There were made notations in the 200 – 700 nm range and the ferric citrate spectrum was compared to that of the free ligand, the citric acid.

Two transition bands at 205 nm and 512 nm were rendered evident.

The band at 205 nm is characteristic of the (II - II^A) ligand, suffering a slight bathochrome displacement from 200 to 250 nm as a result of its coordination to the metallic ion.

The band at 512 nm is a d – d transition band and it can be attributed to the $6A_{1g} - 4E_g$ spin transition, a band characteristic of the Fe(III) (d⁵) ion in an octahedral surrounding.

Thermal stability determinations were also carried out. With its help further information concerning the chemical composition, the geometry of the complex and the kinetics of the decomposition was acquired.

The AT curves point out the fact that the reaction of the thermal decomposition takes place in four stages.

Table 3 Thermal decomposition of ferric citrate

Steps	$T_i^* - T_f^{**}$	Weight loss		Elimination products and residue
		found	theoretically	
I	50 - 115	11,5	10,74	2H ₂ O
II	115 - 280	15,69	16,1	3H ₂ O
III	280 - 360	36,9	39,4	3CO ₂
IV	360 - 500	11		
		23,57	23,88	23,88 (Fe ₂ O ₃)

* T_i – the reaction's starting temperature

** T_f – the reaction's final temperature

The first step can be attributed to the loss of two crystal water molecules.

The second step can be attributed to the loss of three coordination water molecules which attends three of the six Fe(III)(d⁵) coordination positions in an octahedral surrounding.

The third step corresponds to the loss of three CO₂ molecules.

The fourth step corresponds to the loss of the rest of the molecular hydrocarbon, at the end Fe₂O₃ being obtained.

CONCLUSIONS

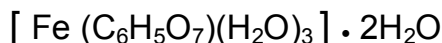
1. The optimum parameters of obtaining the high purity ferric citrate were established.

2. The technological process on laboratory level was elaborated.

3. Studies for the products characterisation from an analytical point of view were carried out, by using work techniques:

- the elementary chemical analysis;
- the IR spectrometry;
- the electronic spectrometry;
- the differential thermic analysis.

4. As a result of the investigations of the pieces of information obtained by means of these methods the following formula was attributed to the obtained ferric citrate:



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NUTRITIONAL IMPORTANCE OF ZINC IN PREGNANCY

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ABSTRACT

Zinc is an essential micronutrient with a special importance for human reproduction, both sexes including. In female, the role of zinc is more complex than in male and it is closely related to the levels of estrogen and progestative hormones and to the prostaglandins synthesis. During pregnancy, the mother organism requires about 100 mg zinc that is progressively accumulated, mainly in the last quarter. Owing to different reasons, the pregnant women are a population of probable risk of zinc deficiency. Its consequences for the mother and for the fetus are presented. The prevention or correction of zinc deficiency includes an appropriate food selection and/or zinc supplementation.

Key words: zinc, nutrition, pregnancy

INTRODUCTION

This year, after 50 years from the setting up of World Health Organization, the objective of the World Health Day was "Safe Motherhood" (Pregnancy is special. Let's make it safe), because, for the improvement of the health of world people in the future, it must give a particular attention to MOTHERS. This paper aimed to show that the adequate zinc nutrition during pregnancy might highly contribute to realize the objective of World Health Day of this year.

Pregnancy is a dynamic, ever-changing process influenced by many factors. Among the exogenous ones, the nutrition is very important. An adequate nutrition is achieved by consumption of a variety of foods that provide an appropriate mix of nutrients, so that deficiencies almost never occur. Zinc is an essential micronutrient with a special importance for human reproduction, both sexes including. We present in this paper the possible implications of dietary zinc deficiency in human pregnancy.

ROLE OF ZINC IN PREGNANCY

In female, the role of zinc is more complex than in males and it is closely related to the levels of estrogen and progestative hormones, and to the prostaglandins synthesis.

In animal experiments, zinc deficiency led to a very high number of morphologically or cytogenetically degenerated ovocytes, anomalies in the estrogen cycle or stopped ovulation. Pregnancy cannot occur in zinc deficient animals.

During pregnancy, some zinc-dependent hormonal mechanisms work (Favier, 1992a). In the first weeks, estrogens and the 17-OH progesterone of the corpus luteum play a prime role in maintaining nidation, and HCG hormone contributes to the maintaining of the corpus luteum. In the 10th week, the role of HCG decreases, and large quantity of estrogen and progesterone comes from the placenta. Other hormones play an important role, such as HPL, hormone closed to prolactine, of placental origin and endowed with somatotrophic properties. Zinc has a

role in HCG receptor sensitivity or in the action of estrogen and progesterone hormones on their respective receptors.

It has been proven that zinc is indispensable for the function of the mammary gland estrogenic receptor in mice and that the different estrogenic receptors have repetitive cysteine sequences, that make them function like zinc finger protein.

The triggering of labour is a complex process activated by prostaglandins, but inhibited by progesterone. A sudden drop in progesterone levels is normally determined by the induction of an ovarian enzyme, 20- α hydroxysteroid dehydrogenase, which transforms progesterone into an inactive derivative. Zinc deficiency in gestating rats - showed the drops in progesterone levels and led to a delay in the induction of 20 α -OH steroid dehydrogenase. PGF₂ prostaglandin may participate in the induction of this enzyme. The plasma level of PGF₂ was lower in zinc deficient rats. PGE₂ prostaglandins stimulate zinc absorption whereas PGF₂ brake its excretion at pharmacological doses. The prostaglandins most involved in delivery are PGE₂ which promote the secretion of relaxin by uterus, providing for dilatation of the cervix, and PGE₂ and PGF₂ which trigger the uterine contractions required for delivery.

Zinc influences the formation of prostaglandins by many mechanisms:

- it protects polyunsaturated acids from peroxydation,
- it intervenes on the absorption and metabolisation of linoleic acid, a main precursor for prostaglandins,
- it is a cofactor of Δ_6 -desaturase enzyme that plays a role in fatty acids metabolisation.
- zinc may act also on the already formed prostaglandins by its implications in the activity of some enzymes like prostaglandin isomerase and tromboxan sintetase;
- zinc influences the activity of some enzymes implicated in mobilization of prostaglandins precursors, like as A₂ phospholipase and C phospholipase.

Huang and his team (1982) found out that the transformation of the essential fatty acids in prostaglandins highly depends on the adequate availability of zinc. In this respect, zinc has opposite effects with copper.

Zinc implication in prostaglandins synthesis is proved by the fact that the sharp zinc toxicity in human is manifested by hypotension, edema, diarrhea, vomit, cardiac arrhythmia, fever, muscular pains and rigidity, symptoms which are owing to an excess of prostaglandins.

An appropriate zinc level in pregnant woman assures the normal evolution of pregnancy because this essential trace element is also implicated in (Papadopol, 1995):

- genetic message transmission by means of zinc finger protein,
- amino acids and protein synthesis,
- bones formation,
- cellular and humoral immunity,
- maintenance of the membranes structural integrity,
- the molecules protection from the oxidative injury.

ZINC REQUIREMENT OF PREGNANT WOMAN

Zinc requirement can be estimated according to the amount contained in tissues appearing during pregnancy (fetus, placenta) plus the fluids lost during delivery. The quantity of zinc required is 100 mg from which 66 mg will be contained by fetus (OMS, 1973). Zinc accumulates in the liver of the developing fetus and the

liver accounts for one quarter of the total body zinc at term. This accumulation of zinc by the pregnant women is progressive and very important in the last quarter. There is an estimated retention of 0,75 mg/day toward the end of pregnancy (Sandstead, 1973). In the light of a 20 % zinc bioavailability in food, WHO has estimated that the food requirement in women increases from 11 to 15 mg/day during pregnancy (OMS, 1973). The lack of menstruation spares only very little zinc (0,01 mg/day), whereas urinary losses increase by 0,4 mg/day at the end of the second quarter (Favier, 1992a).

Total physiological requirement of absorbed zinc during pregnancy was estimated to be 0.8, 1.0 and 1.4mg/day for the quarter 1,2 and 3 respectively (OMS, 1997).

ZINC DEFICIENCY AND PREGNANCY

Zinc deficiency is prevalent in human populations throughout the world, although its incidence is not well known. It is believed that zinc deficiency should be present in countries where the populations consume primarily cereal proteins. One would also expect to see a spectrum of zinc deficiency ranging from severe cases to marginally deficient examples in any given population (Prasad, 1983).

The pregnant women represent a population of probable risk of nutrition deficiencies and particularly zinc deficiency, because zinc stores in young female seem to be small and easily depletable. Adequate maternal zinc nutrition during pregnancy is essential for normal embryonic and fetal development in experimental animals. Factors contributing to the risk of low zinc level in pregnant women include inappropriate food selection, limited economic resources, low educational achievement and fractured home environment. Maternal age also may be a factor in the serum zinc concentrations during pregnancy, teenager women being more predisposed to deficiency (Cherry et al., 1989). Iron supplementation impairs zinc retention in human pregnancy because the increased ingestion of elemental iron depresses the zinc bioavailability by interelement competition in the bowel wall (Solomons, 1983).

It is very difficult to know whether a redistribution of zinc takes place during pregnancy, since the few studies of this subject diverge. Several authors found lower serum zinc at the end of pregnancy in women with a low social-economic status or vegetarian women who have low, poor intake.

Nearly all researches found a drop in serum or plasma zinc during pregnancy. This drop is very rapid until 35 day after ovulation; after a slight increase up to day 55, the reduction is regular until term (Favier, 1992a). Serum levels at term are 10-30% lower than levels before pregnancy, but in certain studies larger drops are described. There is no doubt that this drop is partly the result of hemodilution, but also of the drop in albumin levels which is the main vector in plasma. In pregnant women, serum zinc level drops under the influence of factors other than zinc intake, but insufficient intake does aggravate this drop. The experts consider that a level of about 50 µg/100 ml constitutes the threshold of a "normal" end of pregnancy drop.

Plasma or serum zinc level may be considered an enough good index of zinc nutritional status during pregnancy.

Erythrocyte zinc reflects the metal status for a longer period, because the average lifetime of red blood cells is about 120 days. The response of erythrocyte zinc to the dietary metal intake is weaker and slower comparing with serum zinc. Erythrocyte zinc is an important nutritional indicator for malnutrition.

Leukocyte zinc is considered as a very good index of zinc status, being very sensitive to the dietary intake variations. It may precociously detect the moderate zinc

deficiency but it is difficult to be determined. It indicates a drop in the zinc content of maternal tissues, confirmed by a larger reduction in serum zinc levels than expected as a result of hypervolemia and hypoalbuminemia, and by a reduction in zinc level in hair found by most authors. This drop is consistent with the additional requirement owing to the placental and fetal tissues (+5 mg/day toward the end of pregnancy) that are not covered by additional dietary intake or enhanced intestinal absorption rates. Thus, the additional requirements of fetal tissues are covered from the maternal tissues.

The analysis of amniotic fluid zinc can only serve to compare groups of pregnant women. Amniotic fluid contains peptides with broad-spectrum antimicrobial activity, which is zinc dependent (Sachs, 1979). Certain authors found a lower amniotic fluid level when the fetus was male (Favier, 1992a). It could not find associations between amniotic fluid zinc and pregnancy outcome (Mahomed et al., 1993; Tamura et al., 1994).

When considering zinc concentration in hair, some authors found that pregnancy does not affect it (Hambidge et al., 1983), while the other ones described a gradual reduction (Carbone et al., 1992).

CONSEQUENCES OF ZINC DEFICIENCY

a) For the mother

Given the complex biological role of zinc, it is not surprising that its deficiency can upset the gestation process. Zinc deficiency determines drop in corticosterone, the release and transport of estrogen, prostaglandin synthesis, increase in progesterone and it also lead to anorexia or a reduction in food intake, which itself has serious consequences (Favier and Favier, 1990). Taste disorders during pregnancy may be due to the decreased level of gustin, a zinc containing protein of parotid saliva (Hambidge et al., 1983).

Spontaneous abortion

During the first trimester, Breskin and his collaborators (1983) found an abnormally low level of zinc in subjects who had a spontaneous abortion. The experimental zinc deficiency triggers a drop in uterine fluid zinc, which is thought to be the early source of zinc intake for the ovum. Also, prostaglandin synthesis disorders resulting from zinc deficiency may play a role, since supplementing zinc-deficient pregnant rats with γ -linolenic acid reduced abortion and mortality (Dib and Carreau, 1987).

Hypertension and toxemia pregnancy

Some authors found a drop in maternal serum zinc or placental zinc in preeclampsia (Cherry et al., 1981). In other circumstances, zinc deficiency is associated with an increase in blood pressure (Lazebnik et al., 1989). Zinc action may entail a modification in hormonal secretions (estrogens, prostaglandins) but this trace element furthermore modulates the activity of the angiotensin-converting enzyme. Whatever the mechanism may be, there is a direct link with zinc status. This zinc-preeclampsia link should be related to the possible consequences of preeclampsia for the new born (reading or learning disorders, hyperactivity, epilepsy).

Other complications during pregnancy

Women with refractory anemia during pregnancy had lower serum zinc (Jameson, 1976). Other complications which can perhaps be linked to zinc deficiency are a large number of maternal infections, fetal distress and tissue fragility. The

frequency of *Candida* related vaginitis increased in pregnant women with lowered zincemia. Furthermore, experimental zinc deficiency reduced glucose tolerance both in male rats and in pregnant rats (Favier, 1992a).

Length of gestation

Zinc deficiency is associated with both prematurity and prolonged pregnancy (Jameson, 1976). The last one might be owing to an increase in the progesterone over estrogen ratio. Zinc supplements dropped number of cases of prematurity (Cherry et al., 1989) or postmaturity in pregnant women (Jameson, 1993).

Complications during delivery

Zinc deficiency upsets delivery in animals by excessive loss of blood and increased stress. Different authors found in pregnant women with low zincemia complications pertaining to length of labor or atonic bleeding during delivery (Jameson, 1976), a very significant increase in the prelabour phase and the period of dilatation.

Postpartum consequences

Zinc status slowly returns to normal after delivery. Serum zinc increases but 9 weeks after delivery remains below the initial level (Favier, 1992a). Moreover, breast-feeding is another condition in which the woman will dip into her reserves to provide zinc in breast milk. In rats, zinc deficiency reduces not only the total production of milk, but also its zinc content. This effect may be explained by the link between the zinc and prolactin and by the lower increase of the RNA level in mammary tissue where the casein is made.

b) For the foetus and new born

Foetal growth

The effect of experimental zinc deficiency in gestating animals is very clear in terms of fetal growth (Dămăceanu and Papadopol, 1997). In humans, several studies have sought a link between maternal zinc status or, in certain cases fetal tissue zinc, and the weight of the new born at birth. Although certain studies found no link between newborn weight and maternal zinc status (Tamura et al., 1992), most authors found a significant relationship between these parameters (Favier, 1992b; Scholl et al., 1993).

Malformations

In animal experiments, severe zinc deficiency cannot be brought before fertilization, since it stops the estrogen cycle and leads to absence of fertilization. Triggered the first day after fertilization, it leads to a very high percentage of bone, nervous, ocular, or pulmonary malformations. Even it was only triggered for 3 days, it leads to cerebral malformations (Favier, 1992a). The effect was very rapid and became apparent the third day on fertilized eggs. This can be explained by the ovum's dependence during the first few days on the uterine fluid as a source of zinc.

In human studies, zinc deficiency was associated with abnormal central nervous system development (Favier and Favier, 1990; Jameson, 1976, Jameson, 1983), but some authors did not find such associations (Hambridge et al., 1993). The main teratogenic manifestation of zinc deficiency seems to be a defective closing of the neural tube (spina bifida or anencephaly). The hypothesis of Sever and Emanuel regarding the origin of spina bifida was based on the coincidence between regions of the world where this anomaly was most frequent and regions with a high prevalence of zinc deficiency. Many studies have shown there is a disturbed zinc status of

mothers of children with neural tube defects in comparison with the mothers of healthy children. This effect may be explained (Favier, 1992a) by the fact that zinc deficiency reduces the activity of numerous enzymes in the brain: thymidine kinase, 2'3' cyclic nucleotidyl phosphorylase, lactate dehydrogenase, L-glutamate dehydrogenase. It may lead to a stop in cell multiplication at a crucial period in morphogenesis. Zinc is involved in the expression of certain genes through the zinc finger protein mechanism. Zinc is also necessary in many cell differentiation factors, Nerve Growth Factor and gustin. Zinc deficiency also upsets the cytoskeletal assembly of brain cells. The speed at which cells travel is also partly conditioned by this phenomenon and plays a considerable role in tissue movements during embryogenesis. Finally, some authors have presumed that the teratogenic effect of zinc was owing to a hyperproduction of free radicals that are liable to damage DNA. Since decreased mother serum zinc was associated with different fetal malformations, it was suggested that this indicator be considered a marker for these anomalies (Sharma et al., 1994).

Zinc deficiency *potentates* the effect of many *teratogenic drugs* in animals (Favier, 1992a), either compounds that can chelate zinc, such as EDTA or penicillamine, or drugs not known as chelators, such as 6 mercaptopurine, thalidomide, salicylate, acetazolamide or valproate. The teratogenic effect of alcohol is also enhanced by zinc deficiency in rats. Alcoholism leads not only to zinc deficiency, but also an inhibition of the placental transport of zinc (Beer et al., 1990). Zincemia is abnormally low in newborn with fetal alcoholic syndrome. Diabetes malformations may be the result of secondary zinc deficiency.

Newborn affectation

Gestational zinc deficiency in animals can upset the functioning of several organs after birth. Test learning and memorization difficulties have been discovered in babies born of zinc-deficient animals and these findings were linked with a drop in neurons during the first week of life. The immunity of mice born of mothers fed 5 ppm of zinc was abnormal for 5 months even when the baby mice were fed normal diets. In the case of greater deficiency, the animals were not able to form rosettes after immunization (Favier, 1992a). Also, transient hypogamaglobulinemia in the newborn may be owing to gestational zinc deficiency. Indeed, this anomaly is very often associated with mental retardation or malformations that zinc deficiency also creates in animals.

Zinc deficiency can also lead to stillbirths, a low birth weight of infants (Bedval and Bahuguna, 1994), prematurity (Sandstead, 1973), reduced Apgar score (Favier and Favier, 1990).

During the first few months after birth, newborns depend on milk for their zinc intake. A clear difference in zinc bioavailability in breast milk and infant formula has been shown. This difference can be attribute to the existence of a citrate rich fraction, the presence of lactoferrin and the lower casein or phosphorus content of breast milk. Whatever the type of feeding, zincemia in the newborn experiences a drop during the first two months after birth. This drop lasts longer in premature babies born with lower zinc reserves, since liver zinc mainly accumulates during the last weeks of gestation. In premature babies, the zinc balance remains negative two months or more owing to an immature digestive tract that excretes more zinc than it absorbs (Favier, 1992b).

ZINC INTAKE IMPROVEMENT DURING PREGNANCY

In order to prevent or correct zinc deficiency during pregnancy, the best sources of trace element are lean red meat (40-50 mg zinc/kg wet weight), cheese (30-40 mg zinc/kg wet weight), cereal produces (30-50 mg zinc/kg wet weight), lean pork (20-30 mg zinc/kg wet weight) (OMS, 1997). The bioavailability of zinc from vegetables is highly reduced by the fiber and phytat content.

As to therapeutically zinc supplementation, certain studies found no beneficial effects, whereas the other ones recorded an increase in zincemia, a drop in the number of pregnancy-related toxemia, an increase of new born weight (Goldenberg et al., 1995), a reduction in the length of bleeding, extended labor or prematurity (Jameson, 1993). The effective dose is between 20-40 mg/d, rather associated with iron and folate. Supplementation only with iron or folate determines a decrease of serum zinc (Jameson, 1993; Massol and Hanonik, 1994).

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STUDY OF THE RELATIONSHIPS BETWEEN MAGNESIUM AND SOME TRACE ELEMENTS IN A GROUP OF NORMAL PREGNANT WOMEN

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ABSTRACT

It is well known that iron, zinc and copper vary usually significantly during pregnancy. Magnesium necessity is also increased in pregnant women, determining low levels of the element. The aim of the study was to find out possible relationships between magnesium and the mentioned essential trace elements. 68 apparently healthy pregnant women, 18-40 years aged, at the beginning of pregnancy (13.2 ± 3.8 weeks) and at the end of pregnancy (34.4 ± 2.6 weeks) were investigated. Serum levels of magnesium, iron, zinc and copper and erythrocyte levels of magnesium and zinc were assessed. Relationships between magnesium levels and trace element levels were determined using correlation coefficient. Our results showed, with one exception, no significant correlations between magnesium levels and iron, zinc or copper levels ($p > 0.05$) in both stages of pregnancy, meaning that magnesium varied during normal pregnancy generally without connection with the studied trace elements.

Key words: magnesium, relationships, iron, zinc copper, pregnancy.

INTRODUCTION

It is well known that iron, zinc and copper are trace elements very important for pregnant women which usually significantly vary during pregnancy.

Magnesium, element occurring in larger amount in organism, has also an especial importance in pregnant women. The recommended dietary allowance for magnesium is 450 mg/day for pregnant women (Halpern, 1995). It was found that early magnesium intake in pregnancy could decrease the percentage of spontaneous abortions and could protect the patients from premature labour. Magnesium supplement is widely accepted for treatment eclamptic convulsions and it has been found to be a safe treatment in pregnancy-induced hypertension (Molnar, 1998). The administration of magnesium and human chorionic gonadotropin together reduces early pregnancy loss (Toth et al., 1998).

Pregnancy can affect magnesium homeostasis (Halpern, 1995), mainly if the social-economic status and, consequently, magnesium intake is not good enough. The increased magnesium needs of mother and foetus explain the high metal retention, especially in the last quarter of pregnancy (Mărgineanu and Miu, 1984). An increased magnesium excretion through the kidneys occurs, determining also low levels of the element in woman organism (Molnar, 1998). This levels was associated in newborn with a neuromuscular hyperexcitability syndrome (Mărgineanu and Miu, 1984), prematurely, mortality (Zeană, 1994).

On the other hand, magnesium has considerable influence on the bioavailability and activity of trace elements (Szilagyi et al., 1998). So, magnesium deficient diet significantly increases intestinal absorption of iron and zinc. The metabolisms of these elements interfere with that of copper.

The aim of our study was to find out possible relationships between magnesium and the trace elements iron, zinc or copper, in normal pregnant women.

MATERIALS AND METHODS

At 68 apparently healthy pregnant women, 25.4 ± 4.3 years (ranging between 18 and 40 years) at the beginning of pregnancy (13.2 ± 3.8 weeks) and at the end of pregnancy (34.4 ± 2.6 weeks) were investigated. Serum levels of magnesium and iron and erythrocyte magnesium levels by spectrophotometric method were assessed. Serum and erythrocyte zinc levels and serum copper levels by atomic absorption spectrophotometric method were determined.

The obtained data were compared with a control group of 65 apparently healthy nonpregnant women, 26.9 ± 7.8 years (range: 18 - 41 years).

Statistical analysis was performed by the Student t test with significance at $p < 0.05$. The relation between magnesium and the trace elements was determined using correlation coefficient (r).

RESULTS AND DISCUSSION

As one can see (Table I, Figures 1, 2 and 3) mean values of serum magnesium, serum zinc and erythrocyte zinc were significantly lower comparing with controls beginning with the first stage of pregnancy. As concern serum iron, the difference was significant only in the second stage. Serum copper in pregnant women was significantly higher than in controls even in the first stage of pregnancy.

Erythrocyte magnesium did not significantly differ versus controls.

If iron, zinc and copper varied during pregnancy, serum magnesium, after a significant decrease in the early pregnancy, presented almost constant levels. Erythrocyte magnesium, a store form of the element, was not disturbed by pregnancy.

Table 1. Mean values of the assessed indicators.

Specification	Controls	Pregnant women	
		First stage	Second stage
Serum Magnesium (mg/dl)	1.94 ± 0.34	1.82 ± 0.15 $p < 0.01$	1.80 ± 0.18 $p < 0.01$
Serum iron ($\mu\text{g/dl}$)	88.0 ± 19.0	86.6 ± 15.8 NS	60.3 ± 17.4 $p < 0.001$
Serum zinc ($\mu\text{g/dl}$)	82.3 ± 18.6	72.9 ± 14.5 $p < 0.01$	64.9 ± 12.9 $p < 0.001$
Serum copper ($\mu\text{g/dl}$)	84.3 ± 23.0	120.7 ± 30.2 $p < 0.001$	133.0 ± 26.1 $p < 0.001$
Erythrocyte magnesium (mg/dl)	4.74 ± 0.61	4.65 ± 0.28 NS	4.61 ± 0.49 NS
Erythrocyte zinc ($\mu\text{g/ml}$)	13.0 ± 2.05	12.2 ± 1.69 $p < 0.01$	11.3 ± 1.4 $p < 0.001$

NS: nonsignificant ($p > 0.05$)

Figure 1. Serum mean values of iron, zinc, copper

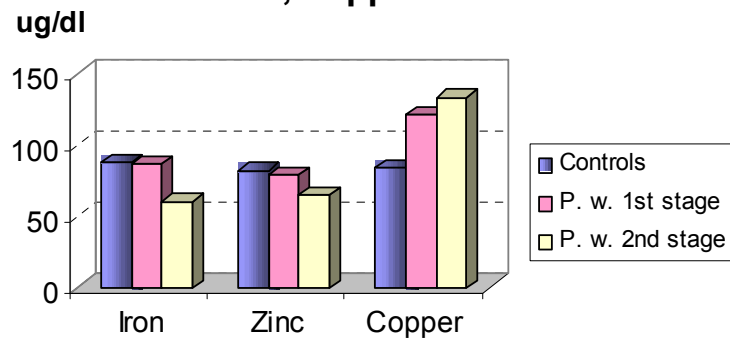


Figure 2. Mean values of serum and erythrocyte magnesium

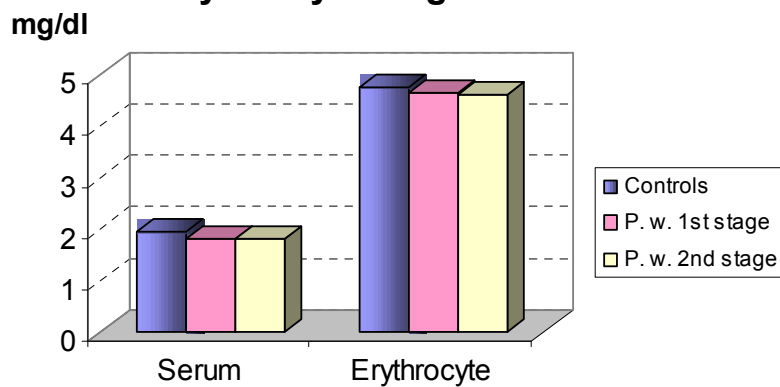
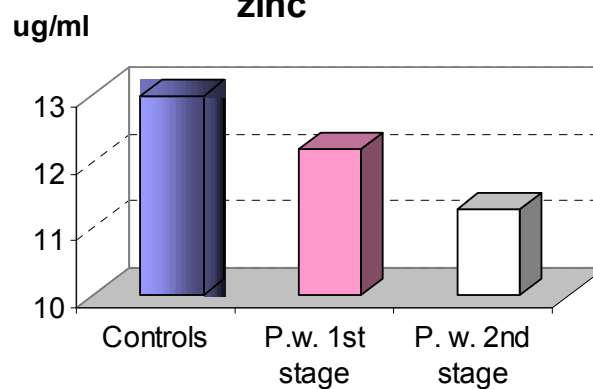


Figure 3. Mean values of erythrocyte zinc



Serum magnesium values did not correlate with the serum values of the studied trace elements, and erythrocyte magnesium values did not correlate with serum or erythrocyte zinc values and with serum iron values ($p > 0.05$ for correlation index) in both stage of pregnancy. There was found a single significant correlation between erythrocyte magnesium and serum copper in the second stage of pregnancy (Table 2).

Table 2. Correlation of magnesium levels with iron, zinc and copper levels

Specification		Serum magnesium			Erythrocyte magnesium			Eryth. Magnesium - Eryth. zinc
		Serum iron	Serum zinc	Serum copper	Serum iron	Serum zinc	Serum copper	
Controls	n r p	57-0.20 >0.1	64+0.10 >0.1	64+0.05 >0.1	54+0.16 >0.1	59-0.07 >0.1	59+0.15 >0.1	57-0.10 >0.1
P.w.1st stage	n r p	67+0.12 >0.1	67+0.07 >0.1	67-0.15 >0.1	57+0.03 >0.1	57-0.03 >0.1	57-0.12 >0.1	57-0.17 >0.1
P.w.2nd stage	n r p	66+0.06 >0.1	66+0.08 >0.1	66+0.18 >0.1	60+0.03 >0.1	60+0.08 >0.01	60-0.27 <0.05	60-0.09 >0.1

CONCLUSIONS

In our group of normal pregnant women, serum magnesium level decreased significantly versus controls at the beginning of the pregnancy, and than it remained relatively constant, while erythrocyte magnesium did not significantly varied versus controls.

Serum or erythrocyte levels of magnesium generally did not correlate with serum iron, zinc or copper levels, showing no connection with these trace elements.

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THE STUDY OF METALLOPHTHALOCYANINES THIN LAYERS EXPOSES AT CHEMICAL POLLUTANTS

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ABSTRACT

Metallophthalocyanines (MPc) include a number of compound with growing interest toward their applications in different areas such as: nonlinear optics, optoelectronics, semiconductor devices, chemical catalysis, electrochemistry, photochemistry, photodynamic therapy of cancer.

Due to their use as chemical sensors for several gaseous substances (potential pollutants like: NH_3 , NO, NO_2 , CO, H_2 , H_2S , SO_2 , Cl_2 , volatile amines), advanced fundamental and also applied investigations were sensitized worldwide. It was shown that molecular organic based on phthalocyanines can be described in order to exhibit p- or n- conducting behaviour.

The observation that the semiconducting properties of phthalocyanines are modulated by the absorption and desorption of gases has led to significant efforts toward their incorporation in chemical sensors. Phthalocyanines possess other properties that are also favorable for sensing application. These properties include manipulation as microelectronic device compatible thin films, good chemical and thermal stability toward many environments and good potential for development of gas specificity.

It is hoped that the gas specificity may be developed by making appropriate substitution of metals in the cavity and organic substituents of metals in the cavity and organic substituents at the periphery of the phthalocyanines structure.

Key words: phthalocyanines, chemical sensors

INTRODUCTION

The observation that the semiconducting properties of phthalocyanines are influenced by the absorption and desorption of gases has led to significant efforts toward their incorporation in chemical sensors. Phthalocyanines possess other properties that are also favorable for sensing application. These properties include manipulation as microelectronic device compatible thin films, good chemical and thermal stability in many environments and good potential for improvement of gas specificity determination. It is hoped that the gas specificity may be developed by making appropriate substitution of metals in the cavity and organic substituents of metals and organic substituents at the periphery of the phthalocyanines structure.

MATERIALS AND METHODS

Phthalocyanines (Pcs) containing central metal (M) atoms, Cu, Co, Ni, Pd, Pt, and gases and vapours (NO_2 , I_2 , NH_3) were synthesised in our laboratory according to

known procedures (Leznoff, 1992). For purification, repeated sublimations at low pressure were carried out. The thin layer, deposited in high vacuum on an optical glass surface, has $\approx 1\mu\text{m}$ thickness. Before the deposition of MPC, a thin layer of gold, with proper geometry, was deposited on the glass surface, which constitute an electrode pair. The gold contacts are accessible through the terminals. The structure of the semiconductor sensor is presented in fig.1.

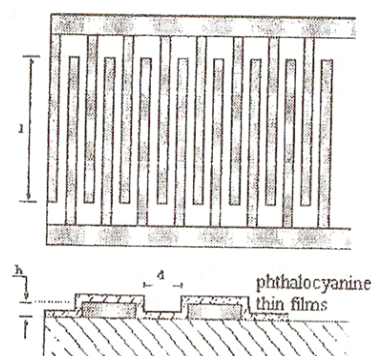


Fig. 1. The structure of the semiconductor sensor

A phthalocyanines sensor consists of two components: a planar interdigital electrode and a chemically sensitive coating. The substrate electronically monitors the coating for a chemically stimulated property change in response to the presence of a particular gas. It is the function of the phthalocyanine coating to absorb or form a weak bond with that particular gas and to undergo a large change in conductivity as a result of this interaction. This is thought to occur as a consequence of donor-acceptor complex formation with the equilibrium driven by partial pressure of the gaseous component.

RESULTS AND DISCUSSIONS

The interaction between MPC thin layer and various chemical agents of the electron acceptor type (NO_2 , I_2 vapours, etc) leads to a decrease of the electrons density on the d orbitals of the central M^{2+} ion coordination and therefore increases the concentration of the holes and the conductivity of the thin layer (fig.2, fig. 4, fig. 5, fig. 6, fig.7, fig.8, fig.9, with NO_2 and I_2 vapours).

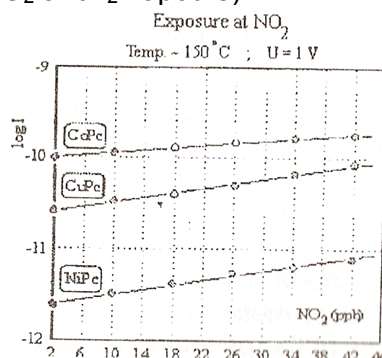


Fig. 2. The interaction between MPC thin layer and NO_2 vapours

This phenomenon is in contrast with the effect induced by exposure to chemical agents with density of electrons donor (NH_3): interaction with with electron

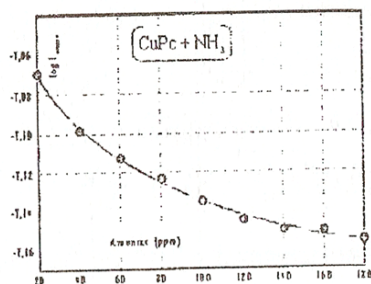


Fig.3. The interaction CuPc –NH₃

If one compares the conductivity versus I₂ vapour concentration curves for cases: CoPc, NiPc and CuPc (order that corresponds to the increase of the metal M²⁺ ion electrons number), was found that the same electron acceptor (I₂ vapours) produce a variation more abrupt of the curve in case that the metallic ion electron number is low (CoPc) and a slower variation in case that the metallic ion contains more electrons (CuPc). The above observation have been confirmed (in concordance with our suppositions), by shifting the metal ion electrons number towards higher periods from the same sugroup of the periodical system.

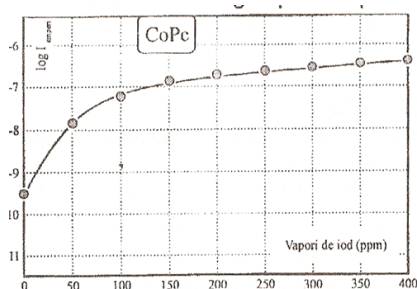


Fig.4. The interaction CoPc-I₂ vapours

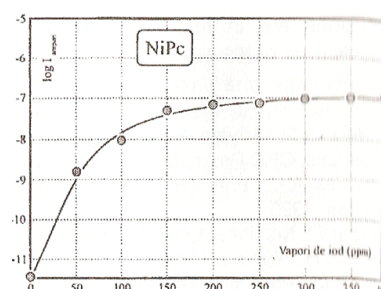


Fig.5. The interaction NiPc-I₂ vapours

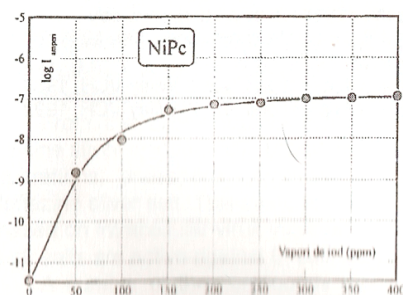


Fig.6. The interaction NiPc-I₂ vapours

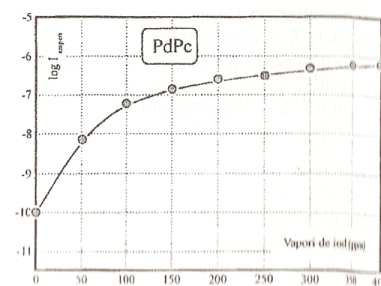


Fig.7. The interaction PdPc-I₂ vapours

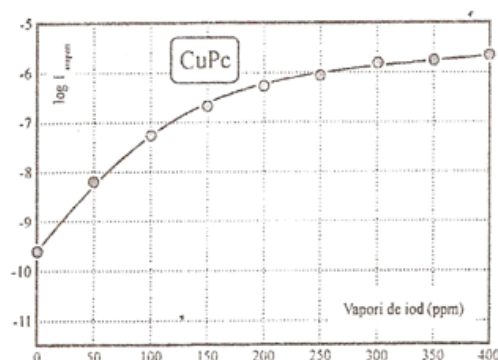


Fig.8. The interaction CuPc-I₂ vapours

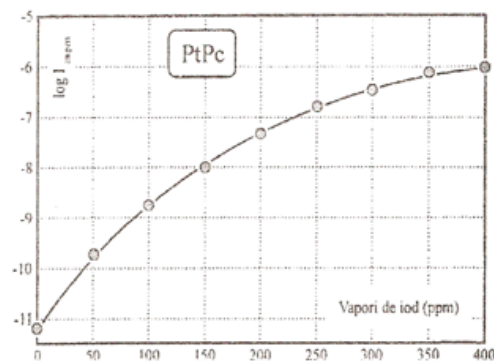


Fig.9. The interaction PtPc-I₂ vapours

The number of the electrons of the central metallic ion increase in the order: Ni, Pd, Pt. Therefore, as a consequence of the chemical agents action as electron acceptors, each of the MPc (M= Ni, Pd, Pt) increase their conductivity.

CONCLUSIONS

Comparing the tangent of the conductivity versus I₂ vapour concentration in the same domain of the I₂ vapour concentrations, was showed that the conductivity sensitivity at I₂ vapour concentration variations is higher in the case NiPc (where the smaller number of the electrons are for Ni²⁺) and lower for Pd and Pt. This conclusions are in accordance with the data in the literature showing that the conductivity is of the p-type.

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SULPHANYLAMIDE COORDINATIONAL COMPOUNDS SYNTHESIS AND STUDYING WITH THE GOAL OF THEIR USING IN MEDICINE, VETERINARY MEDICINE AND PLANT GROWING

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ABSTRACT

During last few years we have studied a group of Co(III) dioximates with sulphanylamides. These compounds were shown to have antibacterial and anticoccide properties and to help against chemical poisonings. Some of cobalt complexes (CODIMEZ, CODINOR) are effective against animal poisoning by agricultural pesticides or can be effectively used against some parasites of domestic animals.

Key words: sulphanilamide, chemical poisoning.

INTRODUCTION

Sulphanilamides $p\text{-NH}_2\text{C}_6\text{H}_4\text{SO}_2\text{NHR}$ (SAM) is known to have anti-microbe properties and are widely used in medicine, veterinary medicine and agriculture. Of special interest are their molecular complexes with biologically active substances (amines, acids, amino-acids etc.), which either enhance the activity of the sulpanilamides or promote their faster penetration to the thick organism's tissues. It must be emphasised that not all of the isomeric or tautomeric modifications are highly active, therefor not any molecular compound can be used as a drug. Hence, both the properties and space structure of every compound proposed for practical use, must be studied thoroughly.

In living organisms the sulphamides interact with protein substances and metal ions, forming coordination compounds, which in their turn act on the sources of bacterial infections. We synthesised and studied the complexes with biologically active metals. This complexes can be divided into the following groups: $M(\text{SAM-H})_n$, $\text{MX}_n \cdot m\text{SAM}$, $\{\text{SAM+H}\}_n\{\text{MX}_m\}$. Also the complexes with two different sulphanilamides were synthesised, as well as the compounds, including other physiologically active preparations.

From the above named complex compounds in medicine and veterinary medicine silver, copper, zinc, cobalt, iron, manganese salts are used as antibacterial preparations, antiseptics, sterilising agents, titagenes etc. The most active is sulphazine silver salt. This salt prevents conjunctivitis of domestic animals. Its action is based on intracellular virus inactivation. Sulphathiosole and sulphaphormilthiasole cobalt salts are active against *E. coli*, *S.pyogenosus*, *S.aureus* etc.

A number of the compounds are used to cure protozoosmosis of domestic animals, mostly of pigs. Silver or zinc salts with sulphazene are used to prevent tooth

cavity of animals. Sulphomethazine sodium salt is added to drinking water of cattle for some microbial disease prophylaxis.

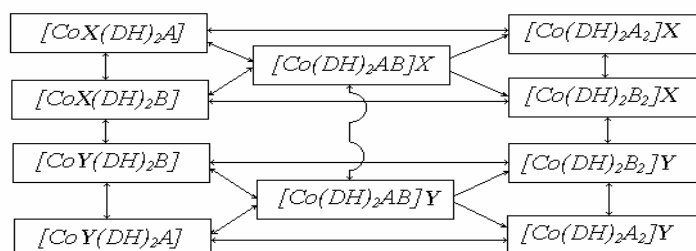
The known preparation Ceresam-M (para-toluensulphanilid salt) have plant growth regulating properties and is effective for alfalfa, wheat, French kidney beans, peas etc.

A lot of other complex compounds of sulphanilamides with amino-acids, alcohols, amines have also been tested. Some of them were shown to be anti-carcinogenic.

MATERIALS AND METHODS

As a continuation of this research Co(III) coordination compounds with α -dioximes and sulphanilamides synthesis methods were elaborated. The properties of the synthesised compounds both in solid state and in solution have been studied. Table shows their chemical transformation:

The indicated complexes contain microelement cobalt, coordinated to four nitrogen atoms, the same way as in vitamin B₁₂ molecule. The most important thing



here is that cobalt dioximates are able to conduct the same chemical transformations as vitamin B₁₂. All these made us look for biologically active preparations among Co(III) dioximates, especially if in their inside sphere were introduced sulphanilamide molecules (one, two, or two molecules of different sulphanilamides).

It has been shown as well that acido-ligands nature (Cl^- , Br^- , I^- , NCO^- , NCS^- , N_3^- , NO_2^- etc.) influences considerably the complexes $[Co^*(DH)_2(SAM)]$ and $[Co(DH)_2(SAM)_2]^*(DH - \text{dimethylglyoxime monoanion})$ physiologic action. The complexes with the following sulphanilamides $p-NH_2C_6H_4SO_2NHR$: $R=H$, C_2H_5 , C_6H_5 , $C_6H_4NH_2$, $C_6H_4NO_2$, C_5H_4N , $C_4N_2H_3$, C_3H_2NO , C_3H_2NS , $NHC(NH)NH_2$, $NHC(O)NH_2$, $NHC(S)NH_2$ etc. were studied.

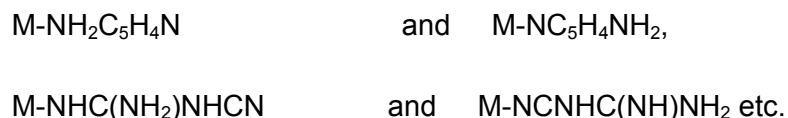
The first experiments showed that the complexes with dimethylglyoxime are biologically active, while the complexes with diphenylglyoxime ($DifH_2$), though having analogical structure, are practically not active. Proceeding from these data, further

on were synthesised and studied the complexes, containing dimethylglyoxime, cobalt salt and corresponding sulphanilamide.

The synthesised complexes physic-chemical properties and structure were studied by UV-VIS, IR, RMN, Raman spectroscopy methods, thermoanalytical methods, as well as by X-ray structural analysis.

RESULTS AND DISCUSSIONS

The complexes are referred to as Co(III) trans-dioximates. Some of them exist as two or three isomers. NCS⁻ ions form three types of bond: M-NCS, M-SCN, M-SCN-M. Some organic polyfunctional ligands act analogously:



Studying of the synthesised complexes therapeutic activity against animal poisonings by chemicals applied in agriculture of Moldova revealed a group of the compounds proposed as antidotes for pesticides GRANOZAN, BI-58, Vofatox. Besides therapeutic action, the complexes contribute to considerable increase of livestock production (meat, milk, etc.).

"CODICLOGU" preparation is effective against rabbit coccidia (Shofranksy et al., 1987). Preparations of the anagocic composition are highly effective against bacterial disease (Popa et al., 1990; Shofranksy and Abramova, 1992).

Some of these compounds are active for plant growth regulators. Here first of all should be mentioned nitro group containing complexes, and guanidine derivatives (Shtefirtza et al., 1996).

CONCLUSIONS

Trans-dioximates biological activity is due to Co(DH)₂ group stability in wide pH range. This stability is explained by intramolecular hydrogen bonds O-H...O. In similar systems cobalt oxidation degree changes from +2 to +4. Besides, it should be mentioned that A and B ligands in Co(DH)₂AB molecules can be substituted for other molecules (or ions), especially if they contain donor atoms O, S, N, P, As, Sb etc. In some cases studied preparations therapeutic activity was due to the fact that as a result of substitution reaction in an organism (e.g. for a pesticide molecule), a sulphanilamide molecule is introduced, while the pesticide is removed from the organism.

Anyway, Cobalt(III) dioximates activity is not a sum of cobalt salt, dimethylglyoxime and sulphanilamide activities, but the whole complex's individual property, which approximates it to vitamin B₁₂.

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MAGNESIUM MODULATES HUMAN SAPHENOUS VEIN SMOOTH MUSCLE CONTRACTILITY

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ABSTRACT

The aim of the present study was to investigate the in vitro responses of human saphenous vein smooth muscle to graded reduction of magnesium extracellular concentration. Segments of human saphenous veins from patients undergoing stripping for varicose veins (n=8) were used. The preparations were introduced in a thermostated and oxygenated organ chamber and connected to a force transducer, the isometric force being registered on a polygraph. The contractile responses induced by endothelin-1, norepinephrine and epinephrine in normal (1.2mM Mg) and modified Krebs-Henseleit solutions (containing 0.8, 0.4 and 0 mM Mg), were registered. Stepwise magnesium reduction until complete removal augmented endothelin-1 induced contractions. In case of norepinephrine and epinephrine, 0.8 and 0.4 mM Mg solutions reduced their contractile effects (abolished by L-NNa preincubation), while complete absence of magnesium increased them.

Key words: magnesium, human saphenous vein, contractility

INTRODUCTION

Many studies have provided solid evidence for an important role of intracellular magnesium concentration in vascular smooth muscle contraction and in blood pressure regulation (Altura and Altura, 1990). Although the basis for the role of Mg^{2+} in vasoconstriction and vasorelaxation was not elucidated, it has been postulated that Mg^{2+} may act extracellularly by inhibiting Ca^{2+} entry and contractile action of vasoconstrictor agents or internally as an intracellular Ca^{2+} antagonist. In fact, various Mg^{2+} - Ca^{2+} interactions have been described (Vormann and Günther, 1993; Howard et al., 1995; Laurant and Berthelot, 1993; Yoshimura et al., 1997;):

in vascular smooth muscle cells:

- Mg^{2+} inhibits Na^+ / Ca^{2+} exchange competitively to Ca^{2+} , without being transported by this membranary exchanger;
- high/low extracellular Mg^{2+} levels reduced/increased the contractile effect of various substances, such as catecholamines, angiotensin, vasoactive peptides, prostaglandins, probably by inhibiting Ca^{2+} influx in the smooth muscle cell;
- Mg^{2+} inhibits the Ca^{2+} -induced Ca^{2+} release from the sarcoplasmic reticulum and the IP_3 -induced Ca^{2+} -release from mitochondria;

in endothelial cells:

- Mg^{2+} presents an indirect effect on vascular smooth muscle tone by competing with Ca^{2+} at the endothelial cell membrane, thereby changing the synthesis of some endothelium-derived vasoactive factors (nitric oxide).

- Moreover, Mg^{2+} deficit is quite frequent, being induced by a variety of causes. Two major forms of magnesium deficiency are described (Speich and Bousquet, 1991):
- primary magnesium deficits with different forms: neuromuscular (spasmophilia), endocrine, cardiovascular (Mg^{2+} deficiency is apparently involved in atherosclerosis, ischemia, arterial hypertension, vascular spasms, phlebotrombosis), allergic or pseudoallergic forms
- secondary magnesium deficits caused by inadequate magnesium intake, decreased magnesium resorption, increased renal elimination or other causes of magnesium loss.

Magnesium overloading occurs just in rare cases (the main causes are acute and chronic renal failure with magnesium intake).

Taking into account the previous statements, we tried to establish the influence of magnesium withdrawal on human saphenous vein smooth muscle contractility. Saphenous veins are an important source of autologous grafts for revascularization procedures and the graft vasospasm, which is important for postoperative morbidity, could be related to changes in extracellular magnesium concentration. Furthermore, a very common pathology of these veins is represented by varicosities; the etiology of this disease is still not well understood, recent theories take into consideration a possible disfunction of the venous smooth muscle. The aim of our study was not only to investigate the response of isolated human saphenous vein segments to changes of magnesium concentration, but also to observe the role of endothelium in these responses.

MATERIALS AND METHODS

Segments of human saphenous veins (HSV), macroscopically normal, were obtained from patients undergoing stripping operations for varicose veins (n=8, 5 females and 3 males, mean age = 51 years). The veins were immediately placed into ice-cold Krebs-Henseleit solution and transported to the laboratory. The interval between surgical removal of the segment and its use was maximum one hour. After dissecting the connective tissue, the venous segments, 8-10 mm length, were cut up in spirals and introduced in isolated organ chambers filled with 25 ml Krebs-Henseleit solution, bubbled with a mixture of 95% O_2 and 5% CO_2 , and kept at 37° Celsius. The Krebs-Henseleit fluid had the following composition in mM/l: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.55. The venous spirals were suspended in the chamber between a fixed base and a force transducer (K30, type 351, Hugo Sachs Elektronik) for the measurement of isometric force. All the results were registered on a Graphtec WR-3320 (Hugo Sachs Elektronik) polygraph. Each preparation was allowed to equilibrate for at least one hour before testing and then placed to its optimal point on a length-tension curve.

To study contractions, the following agents were used: norepinephrine (Arterenol), epinephrine, and endothelin-1. To evaluate relaxation, preparations were first contracted, then acetylcholine was added. The role of the endothelial layer was studied using intact venous segments incubated without and with a nitric oxide-synthase inhibitor N^G -nitro-L-arginine (L-NNA). The drugs were dissolved in distilled water, and L-NNA was sonicated before use; their concentrations are expressed as final molar organ chamber concentrations. All the chemicals were purchased from Sigma Chemicals Co. (St. Louis, Mo.)

In order to obtain magnesium deficiency in the Krebs-Henseleit fluid, the Mg^{2+} concentration was gradually decreased to 0.8 (physiological level), 0.4 and 0 mM (pathophysiological ranges); the isotonicity of the solution was maintained by appropriate changes of the NaCl concentration.

After the addition of the pharmacological agents, the organ chamber was rinsed with Krebs-Henseleit solution and the next experiment began after 10-15 minutes, when vessels returned to baseline tension.

Data analysis was performed with Student t-test and all values are presented as mean $\pm$ standard error of the mean (SEM).

RESULTS AND DISCUSSIONS

The results of our organ chamber experiments confirmed that HSV preparations contracts to norepinephrine (NE)(10^{-8} - 10^{-5} M), epinephrine (E)(10^{-8} - 10^{-5} M) and endothelin-1(ET-1)(10^{-11} - 10^{-7} M) in a dose-dependent manner, the relative magnitude of control constrictor response was: endothelin-1 $\gg$ norepinephrine $>$ epinephrine (fig.1).

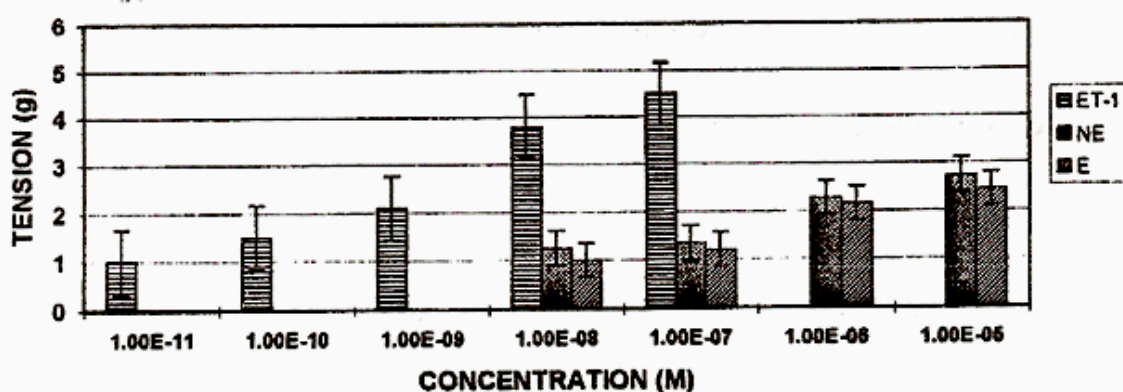


Fig. 1. The contractile response of human saphenous vein to ET-1, NE, E.

Preparations are also additionally tested for endothelium-dependent relaxation following submaximal NE contraction. When contracted with NE (10^{-7} M), endothelium-dependent relaxations to acetylcholine (10^{-7} - 10^{-5} M) were weak; the maximum relaxation was 32% from the maximal contraction induced by norepinephrine. L-NNA (10^{-5} M) prevented the relaxations to acetylcholine and unmasked endothelium-dependent contractions (fig.2).

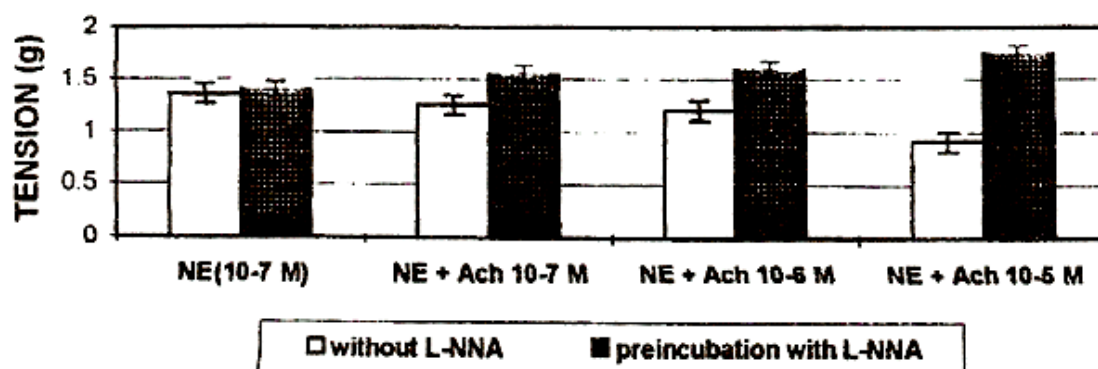


Fig. 2. The effects of acetylcholine on NE-induced contraction.

We also tested the influence of nitric oxide production on the contractile responses induced by ET-1, NE and E. The inhibitor of nitric oxide formation, L-NNA, in dose of 10^{-5} M, induced an enhancement of these effects, which was not statistically significant ($p > 0.05$) - fig.3, 4, 5.

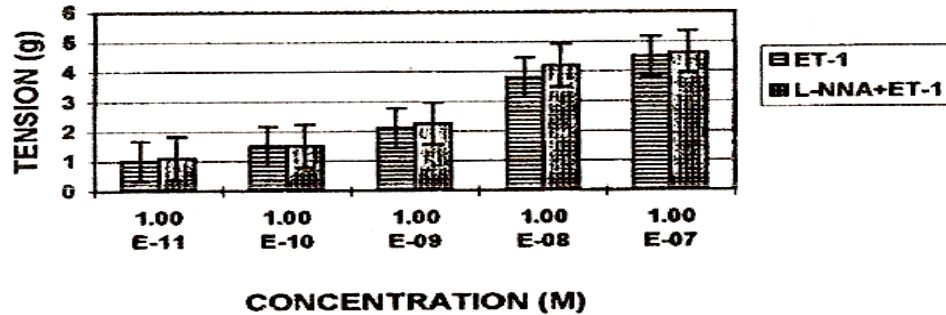


Fig. 3. The effect of L-NNA preincubation on ET-1 induced contraction

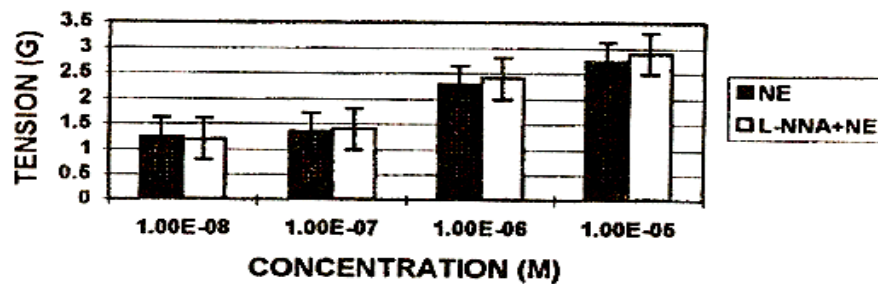


Fig. 4. The influence of L-NNA preincubation on NE-induced contraction

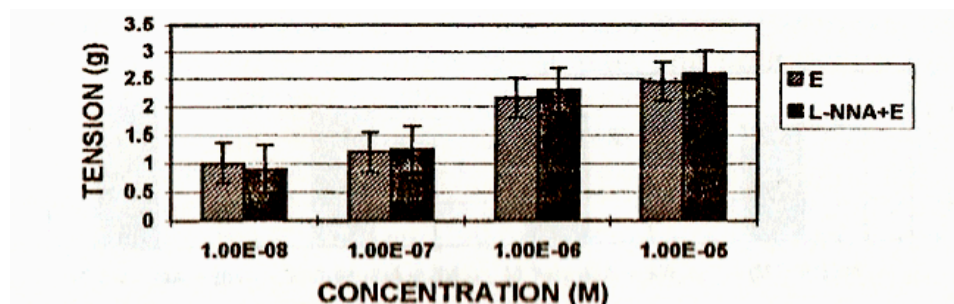


Fig. 5. The action of L-NNA preincubation on E-induced contraction

In order to study the influence of magnesium deficiency on saphenous vein responses to the three vasoconstrictor agents, we used them in a dose equal to EC_{50} (the concentration which induces 50% from the maximal contraction). EC_{50} was 10^{-9} M for ET-1, and 10^{-8} M for NE and E (fig.6).

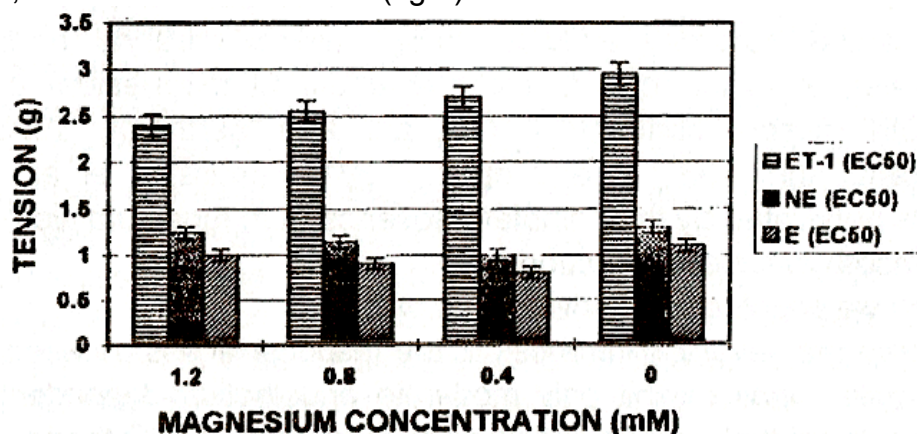


Fig. 6. The effects of magnesium withdrawal on the contractile responses induced by ET-1, NE and E

As presented in figure 6, stepwise lowering the magnesium concentration in the organ chamber fluid had different consequences on the contractile actions of ET-1, NE and E:

- both decreased magnesium levels and its absence induced a significant enhancement of ET-1 induced contraction;
- lowering magnesium concentration to 0.8 and 0.4 mM was followed by a reduction of NE and E contractile responses ($p < 0.05$), while the complete removal of Mg^{2+} caused return of the venous tone to a level similar to the initial one (the difference was not statistically significant, $p > 0.05$).

In the presence of L-NNA (10^{-5} M), stepwise reduction of the magnesium concentration generated a sequentially elevation of the contractile responses produced by ET-1, NE and E (fig.7).

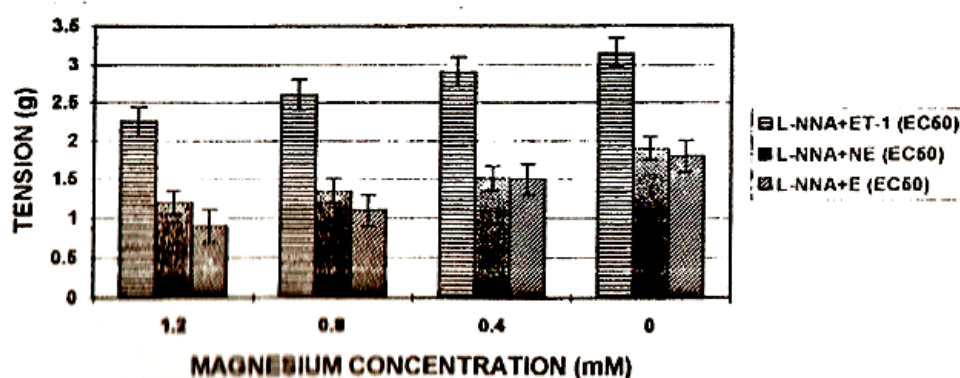


Fig. 7. The effects of magnesium withdrawal on the contractile responses produced by ET-1, NE and E in the presence of L-NNA

The responses of the preparations to NE in case of magnesium withdrawal and L-NNA incubation were similar to those obtained using venous preparations with disrupted endothelium (Siska et al., 1995).

Many experiments were performed on arterial preparations (isolated rat aorta, rabbit aorta, canine coronary arteries, feline middle cerebral arteries, human coronary arteries), in order to establish the effect of magnesium deficiency on vascular smooth muscle contractility (Laurant and Berthelot, 1993; Feletou et al., 1994; Ku and Ann, 1987; Szabo et al., 1991; Kimura T. et al., 1989). Two opposite effects generated by the complete absence of magnesium were described:

- direct smooth muscle contraction, and
- relaxation via endothelium-derived nitric oxide.

However, there are several differences in the responsiveness between arteries and veins. For example, veins exhibit only moderate endothelium-dependent relaxations, probably due to the fact that venous endothelium produces less NO than the arterial one (Moncada and Higgs, 1991). There are just a few studies concerning the effect of magnesium on venous tone. For example, Gold et.al (1990) showed that complete removal of magnesium from the extracellular medium increases NO release from bovine pulmonary vein and artery. Szabo et al. (1992) noted that reduction of magnesium concentration caused feline femoral vein relaxation (after precontraction with norepinephrine), while its total absence returned the tone to the initial level induced by norepinephrine; in denuded vein rings and in the presence of haemoglobin, magnesium reduction was followed by a dose-dependent elevation of the norepinephrine-induced response.

The results presented here show that reducing magnesium concentration in the organ bath produced both endothelium-dependent relaxations and venous smooth muscle contractions. Lowering magnesium concentration to 0.8 and 0.4 mM caused a decrease of NE and E-induced contractions and an enhancement of ET-1 induced contractile response. In all cases, the absence of magnesium produced an increase of the contractile action, similar to the initial response in case of NE and E, and significantly greater in case of ET-1 ($p < 0.05$). The fact that L-NNA abolishes the relaxing effects noted in case 0.8 and 0.4 mM Mg, suggest an involvement of NO.

Comparative to NE and E, graded reduction of magnesium concentration produced just an augmentation of ET-1 induced contractions; this finding agrees to other experimental studies which showed that in saphenous veins contracted with endothelin-1 but not with norepinephrine, the relaxations induced by nitrovasodilators were blunted, suggesting a specific interaction of the peptide with the cGMP-relaxation (Yang et al., 1989; Lüscher et al., 1990).

Both effects, vasodilation and vasoconstriction, induced by magnesium withdrawal seem to be the result from an enhancement calcium entry into the endothelial and venous smooth muscle cells, respectively. Moreover, extracellular magnesium could have a direct effect on nitric oxide synthase (NOS) activity. There are studies which suggested that an elevated magnesium concentration inhibits NO release by inhibiting NOS activity and antagonizing intracellular calcium, the precise mechanism and the location of this antagonism being unclear (Howard et al., 1995).

The clinical importance of magnesium deficiency is revealed by the study of Weglicki et al. (1992), who reported that in rodents fed with a Mg^{2+} -deficient diet, defined cardiac lesions can be induced within two to three weeks; during this time, the rodents

exhibit high circulating macrophage-derived cytokine levels (IL-1, IL-6 and $\text{TNF}\alpha$) together with significantly elevated plasmatic levels of endothelin. In this case the increased production of endothelin could be due to the activation of endothelial cells by the inflammatory cytokines, especially IL-1 and is probably involved in the development of the vascular spasm accompanying magnesium-deficiency. Moreover, preproendothelin mRNA quantitated by RT-PCR tended to be higher in varicose veins compared to normal saphenous veins (Barber et al., 1997).

The relevance of magnesium involvement in NO release is also suggested by the studies of De Caterina et al. (1995), who reported that, in a concentration-dependent manner, NO inhibited interleukin 1 (IL-1) stimulated VCAM-1 expression on human saphenous vein endothelial cells by 35-55% as determined by cell surface enzyme immunoassays and flow cytometry. This inhibition was paralleled by reduced monocyte adhesion to endothelial monolayers; NO also decreased the endothelial expression of other leukocyte adhesion molecules (E-selectin and to a lesser extent, intercellular adhesion molecule-1) and secreted cytokines (IL-6 and IL-8). The authors suggest that NO's ability to limit endothelial activation and inhibit monocyte adhesion may contribute to some of its antiatherogenic and antiinflammatory properties within the vessel wall. This is an interesting observation because in case of varicose veins, due to blood stagnation and reduced shear stress a decrease of NO production may occur; beside this, venous stasis and subsequent hypoxia induce the activation of endothelial cells: they release inflammatory mediators and become adhesive for leukocytes, especially neutrophils which are then activated and release free radicals and proteases which are able to degrade the extracellular matrix.

CONCLUSIONS

The present findings support the idea that graded magnesium withdrawal modulates the effects of vasoconstrictor agents such as endothelin-1, norepinephrine and epinephrine, emphasizing the importance of this element in the regulation of venous tone. Venous endothelium is involved in these responses probably because of magnesium active participation in the regulation of NOS activity and intracellular calcium. The augmentation of the contractile responses of ET-1, NE and E could be due to the reciprocal relationship between calcium and magnesium ions in the modulation of vascular smooth muscle tone and reactivity.

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INTRAVENOUS MAGNESIUM APPLICATION – NEW THERAPEUTIC STRATEGIES IN ACUTE MYOCARDIAL INFARCTION AND PREVENTION OF REPERFUSION INJURY

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ABSTRACT

Although therapeutic strategies for the treatment of acute coronary syndromes have drastically improved during the last decade, the prognosis of these patients is still determined by malignant postinfarction events, such as lifethreatening arrhythmia, left ventricular failure and reperfusion injury. As necrosis is continuing in the time course of 3 to 6 hours from the endocardium towards the subepicardium, early vessel patency is one of the major concerns of treatment. The paradox reperfusion injury occurs after vessel patency has been established, assumingly caused by the reoxygenation of the ischemic myocardium and is followed by myocardial stunning, malignant arrhythmia and often fatal outcome. The application of intravenous magnesium in acute coronary syndromes has shown to reduce postinfarction events if established before reperfusion, thus probably inhibiting the so-called oxygen paradox.

Key words: intravenous magnesium, acute coronary syndromes, reperfusion injury

INTRODUCTION

The application of intravenous magnesium in acute coronary syndromes has been discussed controversially during the last decade. Although new pharmacological, interventional and surgical strategies have improved the outcome of these patients essentially (de Vreede et al., 1991; Glogar et al., 1996), there is still the determining factor of lifethreatening events limiting the prognosis (Krikorian et al., 1995). Reperfusion injury followed by malignant arrhythmia, myocardial stunning and left ventricular failure is one of the main concerns post establishment of vessel patency and reperfusion of ischemic myocardium. Although the mechanisms are not fully understood until now, the application of intravenous magnesium seems to be of essential benefit (Antman, 1996; Seelig and Elin, 1996).

POSSIBLE CELLULAR MECHANISM OF MAGNESIUM IN ACUTE CORONARY SYNDROMES

Magnesium acts as cofactor for over 350 enzymes and metabolic processes in the human body. Its prevalence as a cofactor of the cellular energy metabolism as magnesium-ATP is of obvious importance (Classen, 1986; Francis, 1990; Smetana

et al., 1991;). During ischemia caused by impaired coronary blood flow the need for magnesium augments, leading to increased utilization of intracellular reserves. As the equilibrium of the electrolyte-fluxes is very sensitive to changes of the ionic stability, an increased influx of Calcium is taking place (Polmeni and Plage; 1974; Tongyai et al., 1989). This disturbance of cellular homeostasis is followed by growing instability of the cell membrane, finally leading to membrane leakage and loss of magnesium into the extracellular compartment. These incidents are followed by increasing loss of function of the myocyte, leading to development of systemic stress and to a further rise in catecholamine output. A vicious cycle consisting of increased catecholamine stimulation, extended activation of the energy metabolism of the cell and therefore augmented need for magnesium may develop, finally leading to the total loss of cell function and cell lysis. Magnesium may therefore play a pivotal role in the prevention of reperfusion injury by membrane stabilization and reduction of calcium overload in the myocardial mitochondria and by conservation of ATP as magnesium ATP, combined with the mechanism of improved coronary blood flow by vasodilatation (Altura and Altura, 1987; Vigorito et al., 1991; Woods, 1991).

Besides these elementary effects magnesium acts as a physiological calcium antagonist, leading to vasodilatation and in consequence to a reduction of the ventricular afterload. An inhibition of platelet aggregation is also described.

MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

Coronary reperfusion within a critical period is essential for survival of ischemic myocardium. Under experimental conditions coronary occlusion followed by reperfusion revealed progression of irreversible injury of the ischemic myocardium from the endocardium towards the subepicardium with potentially salvageable myocardium for up to 3 to 6 hours (Hearse, 1977; Reimer et al., 1977). The course of events is depending on degree of coronary stenosis, size of the ischemic area, size and number of pre-existing collateral vessels, and hemodynamic complications. The observation of myocardial stunning, ventricular arrhythmia, or even lethal cell injury in the sequel of successful reperfusion therapy confirmed the clinical relevance of reperfusion injury in acute myocardial ischemia (Kusama et al., 1990; Bolli, 1992; Black et al., 1994).

THE OXYGEN PARADOX

Four main mechanisms have been discussed for the appearance of reperfusion injury: 1) activation of the complement system (Black et al., 1994; Lucchesi, 1994), 2) oxygen-derived free radicals (Hillis and Braunwald, 1977; Lucchesi, 1990), 3) intracellular calcium accumulation (Kusoka et al., 1990; du Toit and Opie, 1992; Smetana et al., 1997) and 4) Apoptosis (Kajstura et al., 1996; Olivetti et al., 1996; Veinot et al., 1997;).

Ischemia and reperfusion cause inflammation in the affected myocardium, leading to the activation of the complement system and initiating a cascade of factors bringing further threat to the already jeopardized myocardium. Neutrophils migrate in and around the ischemic area, releasing free oxygen-derived radicals and lysozyme enzymes. It has also been suggested, that neutrophils contribute to reperfusion injury by intravascular aggregation in the myocardial capillary system causing inadequate flow phenomena. Those neutrophil cells start their migration into

the ischemic areas shortly after the onset of ischemia and increase progressively in number during the following hours, so that there is a direct relationship between number of neutrophils in the injured area and length of ischemia. In the mean time the mediator of complement-system-related cytotoxicity, the membrane attack complex is formed. This complex leads to the lysis of the attacked cell by inserting a pore into the cell membrane, though enabling the cell to control homeostasis and finally leading to rupture of the membrane and cell lysis.

Finally edema occurs as a result of inflammation, causing mechanical inhibition of diffusion. A disturbance of the calcium and magnesium homeostasis might contribute to reperfusion injury and myocardial stunning as well. Coronary artery disease and myocardial ischemia trigger an increased concentration of catecholamines in the serum. This increase is directly related to the severity of coronary artery disease and can be considered as a compensatory mechanism of the body to increase perfusion pressure of the vital organs and to improve myocardial contractility.

As described above, this inadequate rise in catecholamines might initiate a vicious cycle, leading to intracellular calcium accumulation and finally loss of cellular function.

Activated by myocardial ischemia apoptosis occurs, known as the programmed death of the cell which is following coronary vessel occlusion due to pathophysiological or iatrogenic causes. The process of apoptosis might contribute independently from necrosis to myocyte cell death, and is continuing even during and after coronary reperfusion. Extensive apoptotic cardiomyocytes were observed especially at the borders of infarcted myocardium, whereas very few apoptic cells were found in the rest of vital, non-infarcted myocardium. These findings implicate a second, independent factor for myocardial death besides normal cell necrosis due to ischemia. Apoptosis is seemingly not influenced by the restoration of coronary bloodflow.

The production of heat shock proteins (Ambrosio et al., 1986; Hendrick and Hartl, 1993; Dillman and Mestril, 1995) is triggered by myocardial ischemia as well and is believed to be a rescue mechanism of the stressed cell. They are chaperones associating with malformed proteins preventing their aggregation and seem to protect the myocardial muscle from recurrent ischemia.

THE ROLE OF MAGNESIUM IN ACUTE CORONARY SYNDROMES AND REPERFUSION INJURY - STUDY RESULTS

The wellknown discrepancies in the outcome of LIMIT-2 and ISIS-4 have been discussed in extenso elsewhere (Woods et al., 1992; 1994; ISIS-4, 1995; Antman, 1996; Seelig and Elin, 1996). If the possible benefit of intravenous magnesium in reperfusion is reviewed, the beneficial effect in LIMIT-2 could be easily explained: In contrast to the treatment strategy applied in ISIS-4 where magnesium was only infused after an average time window of 8 hours, well after the establishment of reperfusion therapy, magnesium infusion was started earlier (average 3-5 hours post onset of symptoms) and before the application of revascularization strategies in LIMIT-2. As described above, the main effect of magnesium is believed to be reached by cell stabilization, thus preventing loss of cellular function and occurrence of arrhythmia. Vasodilatation, the second main effect of magnesium leads to a

reduction of the ventricular afterload and of left ventricular failure. In LIMIT- 2 the better outcome of left ventricular function compared to the control group was also, possibly related to the inhibition of myocardial stunning and cell death post myocardial infarction. It seems save to speculate that the beneficial outcome of patients with early application of magnesium therapy is due to the prevention of intracellular calcium overload and membrane stabilization, making the cell less vulnerable for mediators of reperfusion injury. We therefore conclude, that magnesium plays a decisive role in the prevention of ischemic heart failure and reperfusion injury, and that the application of intravenous magnesium in a well defined time window, but definitely before the onset of reperfusion is of essential benefit.

FUTURE STRATEGIES – NEW STUDIES

In a new protocol developed at our clinic we analyze blood obtained from patients with high-risk multivessel disease undergoing retroperfusion – supported PTCA (Percutaneous transluminal coronary angioplasty) in the catheterization laboratory for ionized magnesium, total magnesium and lactate changes during ischemia. Retroperfusion is applied via a balloon-tipped catheter placed in the great cardiac vein for selective protection of the myocardium supplied by the left coronary artery. By active injection of arterial, oxygenated blood in the left cardiac vein nutrition of the ischemic myocardium during coronary intervention (balloon inflation and stent placement) is conserved, thus preventing the occurrence of ischemia-triggered arrhythmia and pump-failure. Blood is taken from the great cardiac vein before the beginning of the intervention, during balloon inflation on the arterial side, and post ischemia. First analyses show an increase in lactate and a trend towards a decrease in ionized magnesium during ischemia, total magnesium remains unchanged, possibly due to the limited extent of ischemia. Further investigations in this field are to be continued.

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CO₂ – ASSIMILATION AND PRODUCTIVITY OF SOYBEAN PLANTS UNDER LOW TEMPERATURE STRESS AND Fe³⁺-Co²⁺ TRINUCLEAR CLUSTER TREATMENT

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ABSTRACT

This study was undertaken to determine the role of new coordinate compound – hexa-μ-acetato (0.0')-μ₃-oxo-tris(diethyl-nicotinamid) diFe(III)Co(II) monohidrate (“difecoden”) in regulation of productivity and resistance of soybean plants subjected to low temperature action. Inoculated with Rhizobium japonicum soybean (Glicine max.L.Merr.) seeds were treated with water, IAA or difecoden. The plants were grown in controlled condition of greenhouse. At the 3-leaf stage part of the plants were transferred to 4°C for 48 h. After exposition the plants were returned to 25°C (control). Data were obtained for plant growth and photosynthesis. It was demonstrated the positive effect of difecoden on CO₂ assimilation, biomass production, leaf area, net photosynthetic productivity, as well as yield and dry weight of 1000 seeds of soybean plants subjected to temperature stress. There are the evidences that difecoden application promote the stabilization of yield and can be used as a method for plant protection to low temperature action.

Key words: low temperature action, difecoden application, soybean productivity, growth, photosynthesis and resistance.

INTRODUCTION

During the early vegetative period the day- and night-temperature variation tends to be extreme for plant productivity. Low temperature caused the alterations in the activity of different physiological processes, in particular, photosynthesis, leading to reduced crop growth and yield (Taylor and Rowley, 1971; Oquist, 1983; Baker et al., 1968). In this context one of the actual problems in agriculture is the examination of new and perspective biologically active compounds (BAC), the application of which can increase the plant productivity and resistance to unfavorable conditions.

Usage of the coordinate compounds on the base of metabolites derivatives, with certain metal-trace elements is considered effective due to its more rapidly penetration in the cells and lower toxicity in comparison with metal ions from non organic compounds (Wallage et al., 1964).

The aim of this paper is to study the consequences of low nonfreezing temperature action on the growth and productivity of soybean plants following the treatment of seeds with the BAC. In the experiments was used a new coordinate compound – hexa-μ-acetato(0.0')-μ₃-oxo-tris(diethylnicotinamid)diFe(III)Co(II) monohydrate (conventional “difecoden”).

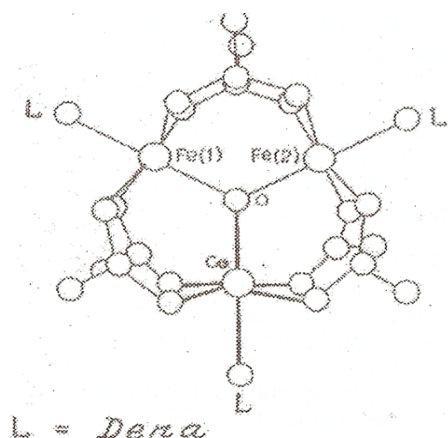
MATERIALS AND METHODS

Inoculated with *Rhizobium japonicum* soybean (*Glycine max.* L. Merr.) seeds were treated with water, indol-3-acetylacid (IAA), known as simulator of adaptive initial processes to cold (Volkova and Titov, 1993) or difecoden. The plants were grown in controlled environment conditions of greenhouse. Temperature treatments in experiments were imposed when plants had 3 levels. At this time, half of the plants were subjected to low temperature (4°C for 48 h). The plants without temperature treatment were used as control plants. After the exposition all plants were returned to 25°C.

The biomass accumulation, leaf area, net photosynthetic productivity before and after temperature action were determined (Mokronosov and Kovaleva, 1989). CO₂ assimilation was measured in an open gas exchange system using an infrared gas analyzer for registration of CO₂ levels. The degrees of modification of recorded values, as well as the index of plant resistance were calculated (Methody matematicheskoi biologii, 1983). After effect of treatment with the BAC and low temperature action upon plant yield was determined.

RESULTS AND DISCUSSION

The compound [Fe₂^{III} Co^{II}(CH₃COO)₆(DENA)₃]H₂O (DENA is N, N' diethylnicotinamide) was obtained by substitution of a water molecule in [Fe₂^{III} Co^{II}(CH₃COO)₆(H₂O)₃] with molecule of N, N'-diethylnicotinamide. It is a brown crystalline powder, easily soluble in acetone, alcohol, and chloroform, more soluble in water and diethyl ether.



Its structure allows classifying it as a trinuclear cluster. Metal atoms have an octahedral environment and a common oxygen atom, forming an almost equilateral triangle. In each octahedron there are an N, N'-diethylnicotinamide molecule in trans position coordinated to heterocycle by nitrogen donor atom. Six anions of acetic acid are bridged to pairs of metal atoms their oxygen donor atoms forming octahedron's equatorial part. The characteristic frequencies in IR spectrum are within the range of ν (COO⁻) = 1638, 1610 and 1420 cm⁻¹; ν (CO(L)) = 1632 cm⁻¹; ν (Fe₂Co) = 620 cm⁻¹.

Nicotinamide, iron and cobalt are the essential components of difecoden and plays a remarkable role in plant development. Nicotinamide is the functional part of pyridinnucleotides which may serve as oxidative agent (NAD⁺) in catabolic processes or as reductive agent (NADPH) in anabolic pathways, in particular, in photosynthesis (Goodwin and Mercer, 1983). Iron is known as stimulator of chlorophyll biosynthesis and photosynthetic electron transport (Abadia, 1995). Cobalt influenced upon structural and functional composition of photosynthetic apparatus, its presence result in increasing of chloroplast amount due to stimulation of growing processes because of inhibition of ethylene synthesis and altering of hormonal balance in favour of auxines (Lipskaya, 1990).

These position serves as base for our hypothesis suggesting that difecoden may play a regulatory role in photosynthesis of soybean plants, growing in different conditions of environment.

In previous studies it was demonstrated that application of physiologically effective concentration of difecoden (0.001%) is accompanied by an increase of germinating energy, biomass accumulation and growth of seedling (Stefirta et al., 1998). The length of soybean roots after difecoden treatment rise by 70.7% in relation to control, and by 37.5% - to IAA.

Temperature stress alters the physiological and metabolic pathways in soybean plants. The photosynthetic apparatus was the primary site of damage caused by low temperature action.

Low temperature action during the growing season resulted in a depression of CO_2 -assimilation. The inhibition was more pronounced for the untreated plants.

A further effect of the low temperature action is the decline in dry matter production.

Figure 1 gives an example of the changes is CO_2 fixation capacity of soybean plants in relation to treatment with the BAC and temperature effect. It was shown that BAC application result to increasing of CO_2 assimilation at 25 °C, more effective in the case of difecoden treatment.

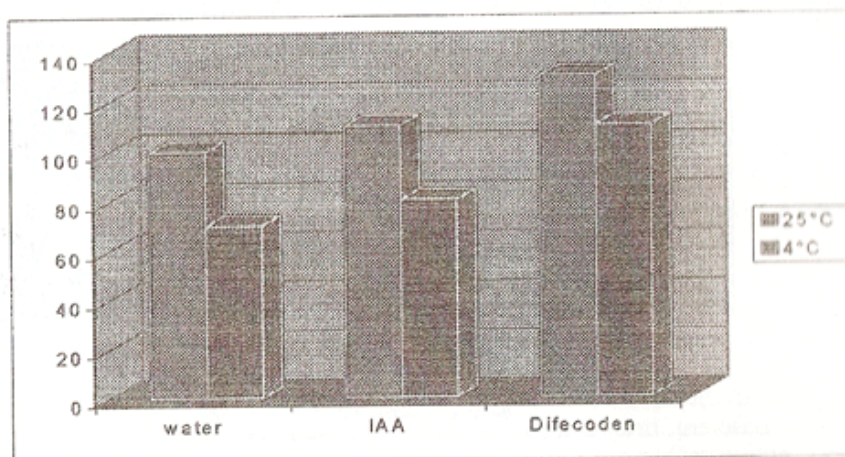


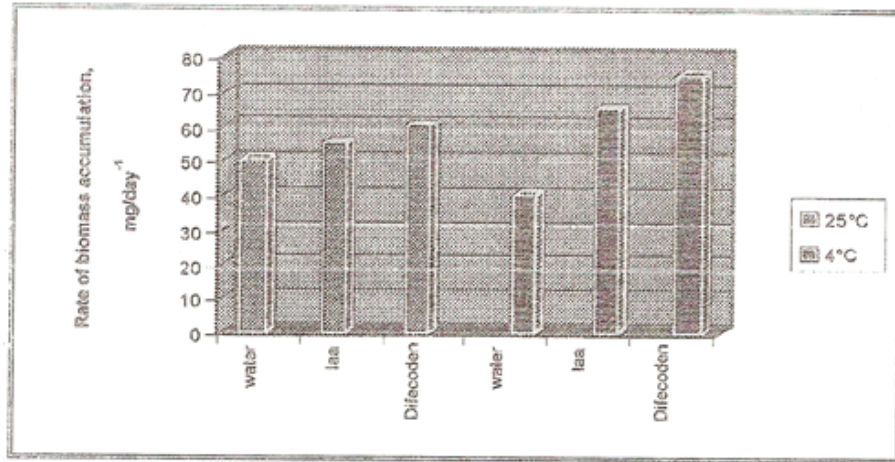
Fig. 1. CO_2 fixation capacity of soybeen plants treated with BAC and subjected to low temperature action (% in relation to control)

Distribution of dry matter production among plant parts as well as total biomass was altered during vegetation under low temperature action. Growth diagrams for soybean plants subjected to 48 h at 4°C and then returned to 25 °C are shown in figure 2. The rate of dry weight accumulation during the stress was inhibited by 25% while for plants treated with IAA or difecoden was shown the increase by 4.5% and 22.4%, respectively. In this case the degree of modification in stress conditions varied from -13.67 for untreated plants to + 13.85 for plants with difecoden application.

The similar changes were observed in relation to leaf area. Its decline in response to temperature effect was lower for difecoden treated plants and the

degrees of modification alter from +0.324 for untreated plants to +0.991 for plants treated with difecoden (fig.2 a and b).

a)



b)

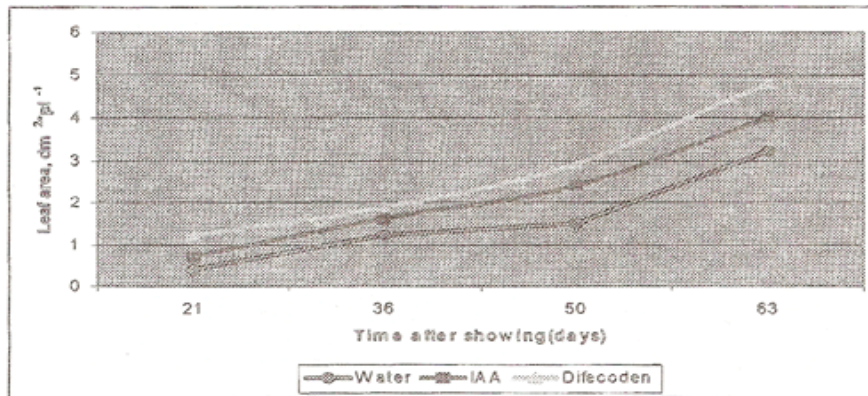


Fig. 2 (a and b) Growth in dry weight and leaf area of soybean plants treated with BAC and subjected to low temperature action

These differences in growth characteristics and CO₂ fixation capacity are likely to be reflected in changes of net photosynthetic productivity. Low temperature action reduced net photosynthetic productivity of soybean plants, but the degree of modification in the case of difecoden application was superior (-39.45), whereas in the control the transfer to 4 °C lead to a decrease up to -52.45.

It was demonstrated that difecoden application promote the stabilization of plant yield in stress conditions and more higher dry weight of 1000 seeds in comparison with other variants (table 1). Index of resistance to unfavorable conditions during active vegetative period was placed in the following consequence: difecoden > IAA > water.

Table 1. Effect of treatments with BAC upon yield of soybean plants subjected to low temperature action

Variant	-	Yield, G per plant	Degree of modification	Mass of 1000 seeds g.d.w.	Degree of modification	Index of resistance
Water	*	3.51 + 0.09	- 0.36	212.58 + 2.68	- 25.06	1.575
	**	3.15 ± 0.24		187.52 ± 1.43		
IAA	*	4.86 + 0.06	- 0.05	219.02 + 1.55	- 12.14	2.405
	**	4.81 ± 0.06		206.88 ± 0.78		
Difecoden	*	5.34 + 0.09	- 0.02	232.98 + 0.91	- 3.22	2.660
	**	5.32 ± 0.08		229.76 ± 0.37		

* control plants

** plants subjected to low temperature

The data presented in this study provide evidence that difecoden can be used as a method for plant protection to unfavorable temperature action.

CONCLUSIONS

1. The application of new coordinate compound – hexa- μ -acetato(00')- μ_3 -oxo-tris(diethyl-nicotinamide)diFe(III)Co(II) monohydrate (conventional “difecoden”) conducted to the increase of growing processes and photosynthesis. These positive alterations are connected probably to the presence in difecoden composition of a very important for plant metabolism nicotinamide as well as iron and cobalt ions.

2. Low temperature stress led to inhibition of plant productivity and resistance, that are reflected in the decline of net assimilation rate biomass production, leaf development, dry weight of seeds and total yield. Treatment with difecoden promotes the stabilization of yield and may act a protector under unfavorable temperature conditions.

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LEAD AND CADMIUM CONTENT IN DIFFERENT FOOD PRODUCTS IN POLAND

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ABSTRACT

The purpose of the investigation was an assessment of the degree of contamination of Polish food products: bread, products for infants and children, meat and fish products, confectionery, ice-cream, soft drinks and wines with lead and cadmium. The evaluation was based on the results of determinations carried out by the Voivodship Sanitary-Epidemiological Stations (VSES) during the routine hygienic control of food in years 1992-1996, as well as within monitoring research coordinated by the Department of Food Research of the National Institute of Hygiene. The content of metals was determined using AAS flame method after dry mineralization of samples at 400 °C and extraction of APDC complexes to methyl isobutyl ketone. The laboratories in the VSES which performed analyses participate in proficiency testing programmes organised regularly since 1991 by the National Institute of Hygiene, 2-3 times each year. In the statistical analysis of results the median value, arithmetic mean and the 90th percentile value were calculated. The analysis of these data showed that most food products contained Pb and Cd in quantities well below the permissible limits accepted in Poland.

Key words: food products, contamination, lead, cadmium

INTRODUCTION

Lead and cadmium are food contaminants considered as most hazardous to human health. Due to their toxic action on the human organism, particularly to infants and children, way of their accumulation and distribution in the environment, values of Provisional Tolerable Weekly Intakes (PTWI) as well as maximum limits for food products has been established internationally (WHO Techn. Report Series 1989 and 1993, Alinorm 97/12A 1996). In Poland the content of heavy metals in foods has long been subject to restrictions. Maximum limits currently in force are defined in the Ordinance of the Minister of Health and Human Welfare of March 31, 1993.

MATERIALS AND METHODS

Following foods were investigated:

- 1) *bread*
- 2) *products for infants and children*
- 3) *meat canned*
- 4) *fish canned*
- 5) *confectionery*
- 6) *ice-cream*

7) *soft drinks*

8) *wines*

Samples were taken from the retail network by Voivodship Sanitary-Epidemiological Stations which are responsible for food safety.

Results for bread and products for infants and children were obtained from monitoring research coordinated by Food Research Department of the National Institute of Hygiene and during routine hygienic control of food.

Metal contents were determined using AAS flame method after dry ashing of samples at 400°C and extraction of metal complexes with ammonium-1-pyrrolidine-dithiocarbamate to methyl isobutyl ketone (Zawadzka, Wojciechowska-Mazurek, 1984).

The laboratories in the VSES participate in proficiency testing programs organised regularly since 1991 by the National Institute of Hygiene, 2-3 times each year. Such programs are conducted in accordance with recommendations of the Joint UNEP/FAO/WHO Food Contamination Monitoring Program (GEMS/Food).

RESULTS AND DISCUSSIONS

Results on the Pb and Cd contents in different foods are presented on charts. Below fig. 1 presents Pb content in selected groups of food products, 1992-1996, (mg/kg).

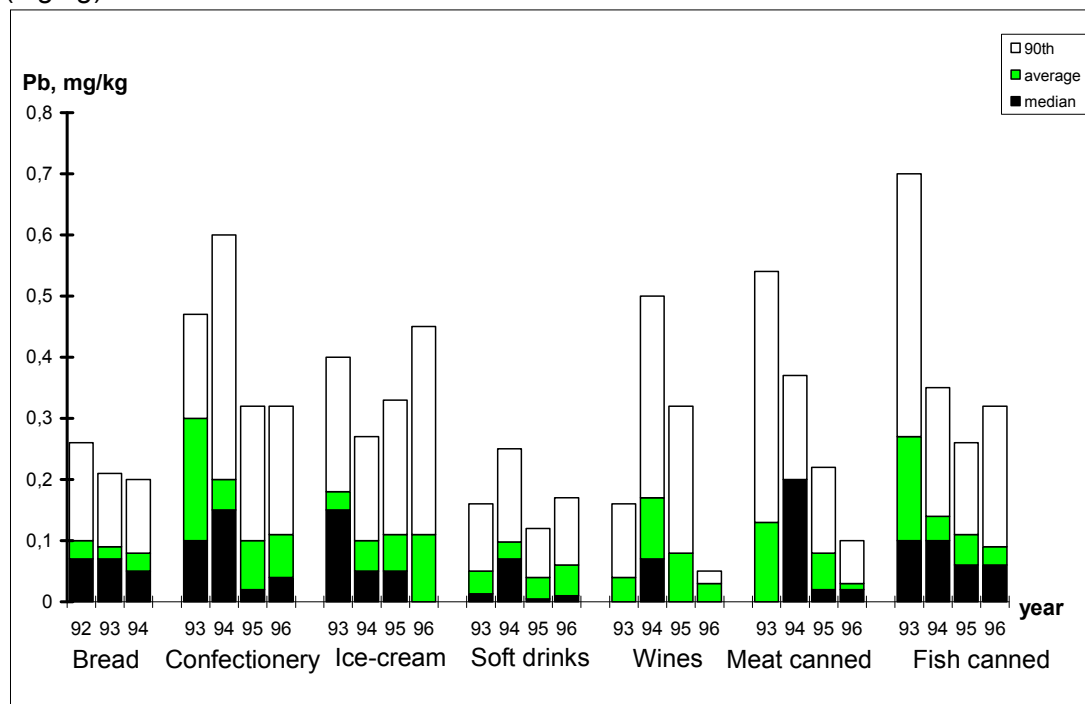


Fig. 1. Quantum of Pb in some food products (mg/kg).

Lead content in bread was far below the existing limit 0.4 mg/kg. Mean and median values did not exceed 0.07 and 0.10 mg/kg respectively while 90th percentile was less than 0.26 mg/kg. Respective values for cadmium were as follows: 0.023; 0.020 and 0.047 mg/kg. Similar figures have been reported in Finland (Tahvonnen R., Kumpulainen J.T., 1994 and 1996).

Cadmium content in canned meat and fish products was in agreement with currently being in force value 0.05 mg/kg. Contamination with lead, as in previous investigations, (Karłowski et al, 1993) was low: mean and median figures in tested samples was under 0.3 mg/kg, pointing to a possibility of lowering existing limit.

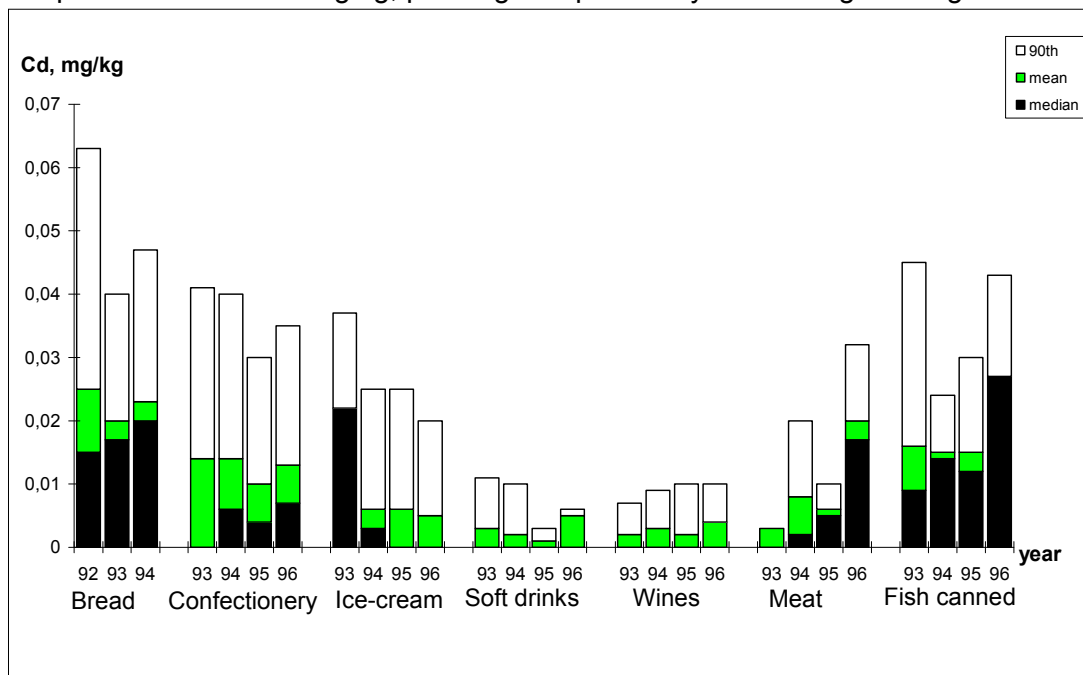


Fig. 2. Cadmium content in selected groups of food products, 1992-1996 (mg/kg)

Mean lead content in investigated samples of confectionery, ice-cream and wines did not reach 0.2 mg/kg (except of 93 year's samples of confectionery), and in drinks was lower than 0.10 mg/l. 90th percentile values calculated for results obtained for samples of soft drinks and wines were ≤ 0.01 mg/l. The contamination of confectionery and ice-cream with this metal was slightly higher but still low: mean values for these foods were less than 0.020 mg/kg.

The levels reported by the laboratories of VSES for heavy metals contents in products for infants and children were much below the maximum permissible levels (Ordinance of the Ministry of Health). The lowest lead and cadmium contents were reported for fruit/vegetable juices and milk products, where the average content of lead in these groups of products did not exceed 0.03 mg/kg and the median was 0.02 mg/kg. The respective values for cadmium are 0.004 and 0.002 mg/kg. Low lead contents were also often reported in vegetable/meat products. However, the 90th percentile content of Pb and Cd in cereal, cereal/fruit, milk/cereal/fruit products and pomaces slightly exceeded permitted levels, though in all years of investigations the median value was much lower. The highest average content of cadmium (0.013mg/kg) was reported in the group of cereal and cereal/fruit products in 1992.

Results for lead (median of <0.01-0.05 mg/kg, average 0.01-0.07 mg/kg) allow to reduce the permitted content of this metal in all products for infants and children circulated on the Polish market to 0.1 mg/kg. Figure 3 and 4 show the quantum of lead and cadmium in some products for infants and children.

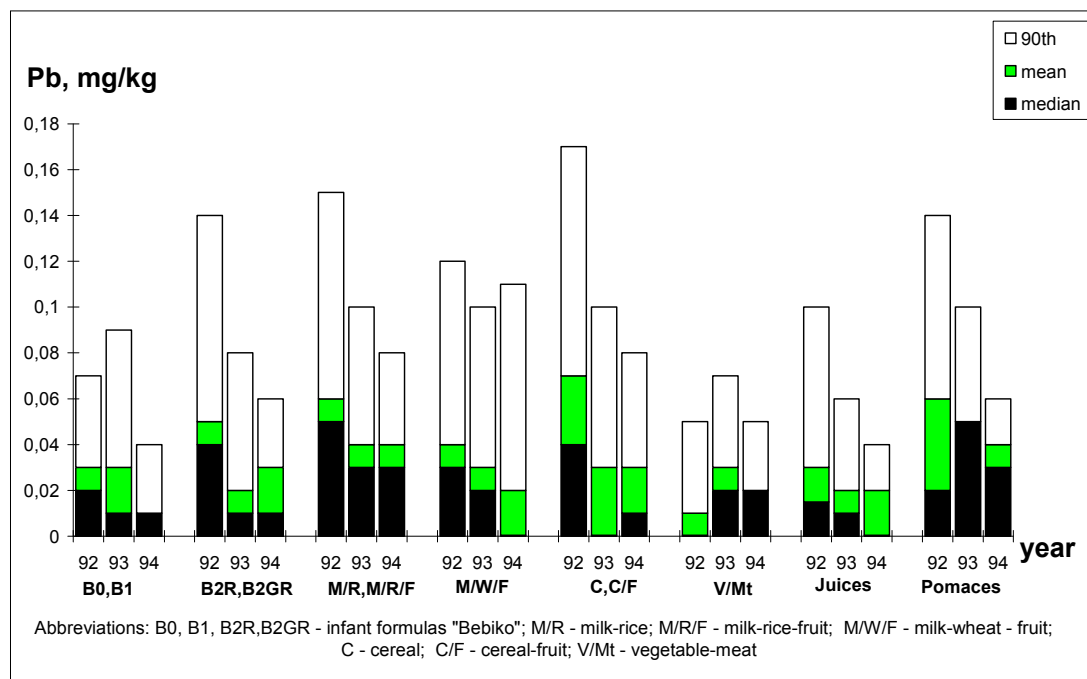


Fig 3. Content of lead in Polish products for infants and children (mg/kg)

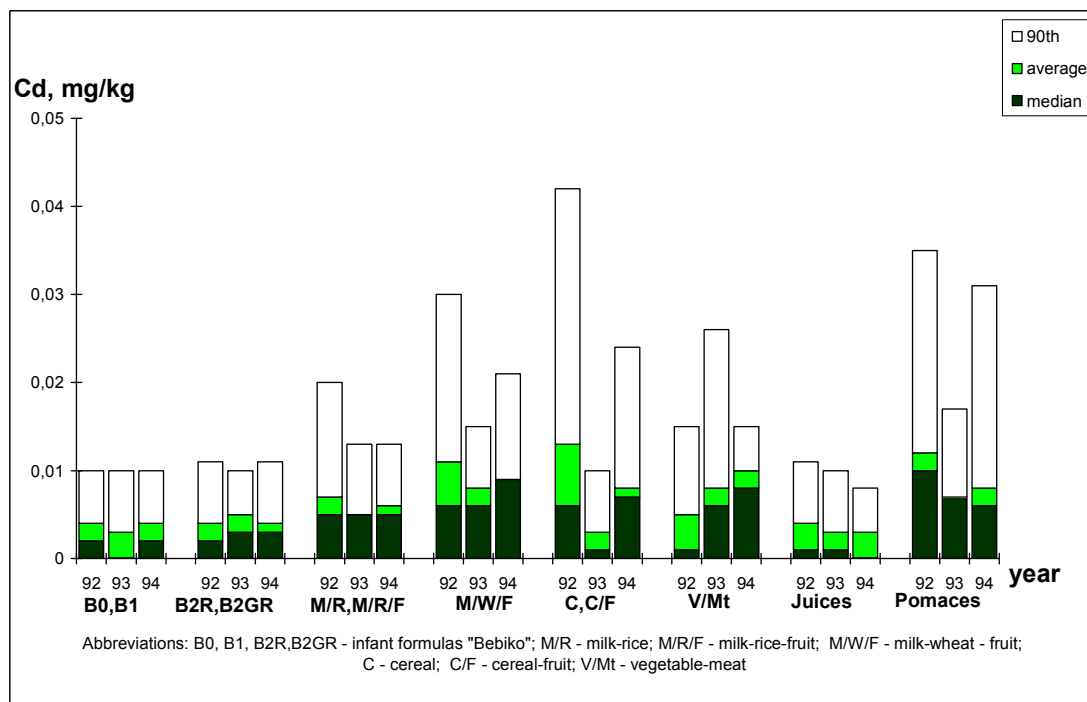


Fig 4. Content of cadmium in Polish products for infants and children (mg/kg)

Results obtained over the few last years point to a possibility of lowering of existing limits of Pb and Cd for some groups of foods and introducing such limits for other groups. Propositions for some revised limits are given in table 1 (Karłowski et al., 1997).

Table 1. The quantitative data for lead and cadmium in some food products.

Specification	Pb (mg/kg)	Cd (mg/kg)
Products for infants and children:		
– milk, milk-cereal, milk-cereal-fruit and meat, meat-vegetable	0,10	0,01
– cereal, cereal-fruit	0,10	0,02
Bread	0,30	0,05
Meat preserves	0,30	0,05
Fish preserves	0,50	0,05
Confectionery (except of chocolate and chewing-gum)	0,30	0,05
Ice-cream	0,30	0,03
Soft drinks	0,10	0,01
Wines	0,20	0,01

CONCLUSIONS

1. The charts show the statistical analysis regarding lead and cadmium in different food products, reported to the period 1992-1996
2. The content in heavy metals (lead and cadmium) of infant and children products was much below the maximum admitted levels

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The Editorial Board expresses its gratitude to all personalities who honoured with their presence and/or papers the Symposium and its regrets of not receiving all the papers in extenso until now for publishing.

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