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EFFECT OF PROTEIN QUALITY AND DIETARY LEVEL OF IRON, ZINC AND COPPER ON THE UTILIZATION OF THESE ELEMENTS BY GROWING RATS.* II. IRON, ZINC AND COPPER CONTENT IN TISSUES OF EXPERIMENTAL ANIMALS**

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Key words: tissue iron, tissue zinc, tissue copper, supplements, dietary protein.

Iron, zinc and copper distribution in rat tissues was studied in dependence on the dietary level of these elements and source of protein. All experimental diets had the same Fe:Zn:Cu ratio and as the amounts of these elements in the diets increased so did the Fe:Zn and Fe:Cu ratios in the liver, spleen and femur. The ratio of iron to other elements in those tissues was higher in animals fed the gluten diets than in animals on casein or casein-gluten diets.

Among factors affecting the utilization of iron, zinc and copper in the organism is the mutual proportion of these elements in the diet [8]. Interactions between these elements can occur not only during intestinal absorption but also in tissues where they can inhibit or promote reactions involving them [2], increase or limit accumulation [7], and stimulate or suppres excretion [8]. These interactions depend on many factors such as, age, sex, method of administration, nutritional status or diet composition [9].

In earlier studies [4] of iron, zinc and copper utilization in conditions of proportional increases of these elements content in diets containing casein, gluten and a mixture thereof it was found that their absorption increased with the increase of their content in the diet, regardless of protein quality, in spite of decreases of per cent coefficients of apparent absorption. The greatest amounts of these elements per 1 g of weight gain were absorbed by animals fed the gluten diet. No interaction between these elements was observed during intestinal absorption.

In this part of the study we investigated the distribution of iron, zinc and

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copper in selected tissues in conditions of proportional increases of the contents of these elements in the diet.

MATERIAL AND METHODS

Male Wistar rats initially weighing 102 ± 3 g were fed with experimental diets containing 20% protein supplied by casein (C) or wheat gluten (G) or a 1:1 mixture thereof (M). The diets contained different Fe, Zn and Cu level: 60% (DM — dificiency of minerals), 100% (SM — standard minerals) or 300% (HM — high minerals) of recommended amounts (35 mg Fe, 12 mg Zn and 5 mg Cu per 1 kg diet [11]. The composition of diets and determined contents of protein, Fe, Zn and Cu, as well as experimental conditions were listed in the first part of this report [4].

After six weeks of the experiment, the animals were fasted overnight, anesthetized with ether, blood samples were collected from ophthalmic arteries into test tubes with heparin, the animals were killed by dislocation, of cervizal vertebraue and livers, spleens, kidneys and femurs were removed for analyses. After rinsing with saline and blotted, the organs were weighed and deep frozen in polyethylene bags for later metal analysis.

Blood was centrifuged at 150 g for 15 min, and protein precipitated from the obtained plasma by adding an equal volume of 10% trichloroacetic acid [6].

Fe, Zn and Cu concentration in tissues and plasma were determined by flame atomic absoption spectrophotometry in a Perkin-Elmer No. 300 apparatus following dry ashing at 450°C and dissolution in hydrochloric acid.

Results were analyzed statistically using two-factor analysis of variance and Duncan's test [18]. Statements of singnificance were based on the 0.05 level of probability.

RESULTS

The Fe concentration in plasma and the investigated organs (except for kidneys) depended significantly on the mineral components content and source of protein in the diets (Table 1). Analysis of variance also demonstrated significant interactions of the two studied factors as regards Fe content in liver and kidneys.

Average Fe concentration in plasma increased with the increase of mineral components in diets, but Duncan's test revealed significant differences only between diets with the low and high mineral components content (DM and HM). Mean Fe concentration in plasma was highest for casein diets regardless of mineral components content, and lowest for gluten diets.

Fe concentrations in livers of rats fed the gluten (G) and casein-gluten (M) diets were significantly different for each level of mineral components; they were practically the same for CDM and CSM diets, and were significantly higher for the CHC diet.

Table 1. Fe concentration in plasma and tissues (mean ± standard error of mean)

Diet	Fe concentration				
	plasma (μg/100 cm^3)	liver	spleen	kidneys	femur
		μg/g dry weight			
CDM (7)*	190 ± 19	187.8 ± 16.3	553.7 ± 58.3	149.0 ± 9.7	76.9 ± 6.4
CSM (16)	200 ± 11	194.3 ± 8.9	627.6 ± 22.5	186.5 ± 6.1	81.8 ± 5.6
CHM (6)	216 ± 46	346.2 ± 19.3	984.4 ± 44.7	259.4 ± 14.2	101.3 ± 6.2
GDM (6)	80 ± 11	233.5 ± 20.4	719.9 ± 29.7	186.9 ± 13.9	138.3 ± 12.3
GSM (15)	119 ± 14	314.9 ± 17.8	897.6 ± 57.0	219.3 ± 6.5	144.9 ± 16.8
GHM (6)	188 ± 29	465.9 ± 12.0	1127.5 ± 47.9	226.7 ± 14.5	151.1 ± 12.5
MDM (10)	141 ± 32	127.9 ± 6.4	615.2 ± 55.5	167.3 ± 6.1	51.7 ± 3.2
MSM (27)	159 ± 14	182.8 ± 6.0	611.8 ± 19.0	203.0 ± 5.1	67.0 ± 3.5
MHM (10)	233 ± 34	281.7 ± 9.4	974.0 ± 50.3	263.0 ± 14.7	71.3 ± 3.9
	analysis of variance (p values)				
protein	0.0033	0.0001	0.0001	NS	0.0001
mineral components	0.0034	0.0001	0.0001	0.0001	0.0200
P x M	SI**	0.0206	SI	0.0099	SI

* number of animals in group

** not significant ($p > 0.05$)

As regards protein quality there were no significant differences in mean Fe concentrations in spleen for the DM and SM diets, while in HM diets with the greatest contents of the investigated elements these concentrations were significantly higher.

Moreover, Duncan's test did not reveal differences in Fe concentration in kidneys and femurs of rats fed gluten diets, but indicated them in kidneys of animals fed the casein and mixed DM and HM diets.

Mean Fe concentration in bones was lowest for the M and highest for the G diets; Duncan's test showed there were no differences resulting from the amounts of mineral components in the case of gluten diets.

The source of protein in the diets significantly affected zinc concentration (Table 2) in plasma, livers, kidneys and femurs. Regardless of the level of the studied elements in the diets, the livers and bones of rats fed G diets contained significantly higher Zn concentrations than the corresponding organs of animals on C and M diets. In plasma the Zn concentrations were highest for C diets.

Mean Zn concentration in kidneys and bones was significantly higher in rats fed the HM diets than in animals receiving the SM and DM ones. It was also observed that in the case of G and M diets, the greater the content of mineral components in the diet the lower splenic Zn concentration, although the differences were not statistically significant.

The mean Cu concentration (Table 3) in plasma, liver, and in particular in kidneys of rats fed the G diets was higher than for the other diets; no significant differences as regards this characteristic were found in tissues of animals fed the C and M diets.

Plasma Cu levels in rats fed Hn diets were significantly higher as compared with DM diets, this being true for all protein sources. On the other hand, the livers of rats fed the SM diets accumulated more Cu than livers of rats eating the DM and HM diets.

Mean Cu concentrations in kidneys of rats fed HM diets was significantly higher than in the case of SM and DM diets; figures for the latter two did not differ.

The Cu concentration in bones decreased with the increase of the content of elements in the diet. Cu concentrations in the spleen exhibited the same tendencies although no statistically significant differences were demonstrated among dietary treatments.

DISCUSSION

As reported in the first part of the report [4], we found that the greater the content of elements in the diet, the greater the amount of these elements absorbed from the gastrointestinal tract, this being reflected in the concentrations of elements in tissues. However, this effect differed depending on the element as well as tissue, and being most pronounced in the case of Fe in liver, Zn in bones, and Cu in kidneys, organs where the particular elements accumulate most [11]. It

Table 2. Zn concentration in plasma and tissues (mean ± standard error of mean) *

Diet	Zn concentration				
	plasma (μg/100 cm^3)	liver	spleen	kidneys	femur
		μg/g dry weight			
CDM	200 ± 15	75.3 ± 3.0	85.4 ± 1.3	77.4 ± 1.8	142.0 ± 17.3
CSM	191 ± 15	77.6 ± 2.4	78.1 ± 3.4	85.3 ± 2.8	169.5 ± 5.9
CHM	200 ± 17	83.3 ± 3.6	84.3 ± 4.2	99.8 ± 4.9	175.0 ± 11.8
GDM	181 ± 16	85.8 ± 2.3	86.5 ± 4.7	86.4 ± 4.3	241.5 ± 14.6
GSM	174 ± 11	91.5 ± 3.3	84.1 ± 2.2	90.9 ± 3.1	256.3 ± 14.3
GHM	197 ± 13	97.1 ± 3.9	82.8 ± 3.6	95.4 ± 1.4	284.1 ± 15.1
MDM	156 ± 19	80.3 ± 3.2	105.5 ± 6.8	78.2 ± 2.7	172.5 ± 4.6
MSM	160 ± 14	84.2 ± 2.5	87.2 ± 4.3	80.5 ± 0.4	198.9 ± 7.2
MHM	163 ± 10	84.4 ± 2.6	82.0 ± 6.5	84.6 ± 3.1	256.5 ± 5.4
		analysis of variance (p values)			
protein	0.0438	0.0002	SI	0.0010	0.0001
mineral components	SI	SI*	SI	0.0002	0.0001
P x M	SI	SI	SI	SI	SI

* not significant ($p > 0.5$)

Table 3. Cu concentration in plasma and tissues (mean ± standard error of mean)

Diet		Cu concentration			
	plasma μg/100 cm^3	liver	spleen	kidneys	ferum
		μg/g dry weight			
CDM	79 ± 7	10.7 ± 0.7	ND*	25.7 ± 4.5	4.78 ± 0.67
CSM	94 ± 5	15.2 ± 1.2	ND	36.4 ± 2.0	3.99 ± 0.41
CHM	97 ± 4	12.2 ± 0.5	ND	63.5 ± 8.1	ND
GDM	97 ± 6	14.4 ± 1.2	12.3 ± 3.6	62.4 ± 10.6	6.05 ± 1.18
GSM	98 ± 3	15.3 ± 0.8	10.9 ± 1.1	69.8 ± 6.7	4.61 ± 0.55
GHM	117 ± 10	15.8 ± 1.2	9.3 ± 1.3	109.5 ± 4.6	3.95 ± 0.33
MDM	84 ± 3	12.4 ± 0.8	11.9 ± 4.1	23.8 ± 1.9	4.95 ± 0.60
MSM	97 ± 3	14.4 ± 0.5	11.4 ± 1.9	42.1 ± 2.6	3.87 ± 0.21
MHM	105 ± 6	12.5 ± 1.0	9.3 ± 3.1	62.5 ± 8.1	3.15 ± 0.17
		analysis of variance (p values)			
protein	0.0173	0.0041	SI	0.0001	SI
mineral components	0.0008	0.0031	SI	0.0001	0.0015
P x M	SI**	SI	SI	SI	SI

* no data

** not significant ($p < 0.05$)

must be noted that in our experiments the differences between the applied doses of elements were not great, especially in the case of DM and SM diets. Other authors also failed to observe differences in the elements' concentrations in tissues when the amounts of components consumed in the diet varied slightly [11, 16, 22]. When the wide range of doses was used concentrations in tissues differed significantly [12, 13, 19, 20], especially when deficient level was taken as reference points [12, 13, 16].

In our experiments the Zn concentrations in plasma, liver and spleen remained the same regardless of the content of this element in the diet. The concentration of Cu in liver and femur decreased with the increase of the elemenets' concentration in the diets. Despite the practically constant Fe:Zn and Fe:Cu ratios in the diets, Fe was found to accumulate in the tissues in relatively higher quantities than the other two elements as the diets had more mineral components. Evidence of this is the higher Fe:Cu ratio in liver, spleen and femur for HM diets regardles of protein source (25.4, 118.8 and 30.5, respectively) as compared to figures for SM and especially DM diets (14.7, 55.1 and 16.5, respectively), and higher Fe:Zn ratios in liver, spleen and kidneys (HM diets: 3.8, 12.5 and 2.7; DM diets: 2.3, 6.9 and 2.1, respectively). Our findings could be explained by possible negative interactions between Fe, Zn and Cu in tissues [9].

Our results conform the metabolic interdependence of mineral components and protein. Rats supplied increased doses of Fe, Zn and Cu in casein and casein-gluten diets attained higher body weights [4], and the weight of their internal organs increased accordingly but the level of minerals was lower than on gluten diets. To obtain identical concentrations of the elements in tissues as on gluten diets, there would had to be greater concentrations of these elements in the diets. Rats fed gluten diets absorbed greater amounts of elements per 1 q of weight gain. Thid finding can explain the higher concentrations of Fe, Zn and Cu in most of their tissues.

Similar results were obtained by Motsinger and Magee [14] who compared concentrations of the three elements considered here in livers of rats fed gluten, soy and casein diets. Rutkowska et al. [16] also observed higher Zn concentrations in the liver and spleen in the case of gluten diet, but failed to detect differences in Fe concentration in tissues; they also found that Cu concentrations in the liver were higher for casein than for gluten diets. The reason for lack of agreement of these observations with our findings may be that the Rutkowska et al. [16] applied higher level of iron in the experimental diets than we did.

No clear-cut dependence between concentration of elements in tissues and protein quality was found comparing the C (casein) and (M) casein-gluten diets. Mineral components from the M diet were absorbed to a slighly lesser extent than from the C diet [4] but the amino acid composition of the former was more suited to the animals needs and this could have affected transport processes in blood and accumulation of the elements in other tissues. Similar results were obtained by other authors [10, 15-17] who compared highly-nutritive proteins which did not inhibit the growth of rats.

The distribution of mineral elements in tissues also depended on the source of protein. In rats fed the gluten diet the Fe concentration in plasma was lower while in tissues it was higher, this possibly being due to the lower level of transferin in these rats [3] as compared to animals fed the diets with two other source of protein.

The fact that casein contains less cystein than wheat gluten can be the reason why Zn concentration was lower in bones, at the same time being higher in plasma of animals fed the casein diet, and why the Cu concentration in kidneys was higher in the case of gluten diets. Greger and Mulvaney [10] demonstrated that supplementation of soy with cystein significantly increased Zn content in bones and Cu concentration in kidneys.

In spite of the practically constant Fe:Zn and Fe:Cu ratios in the diets, the accumulation of Fe in relation to Zn and Cu, in all the studied tissues (except kidneys) in rats fed G diets was higher than in rats eating the other proteins. The average Fe:Cu ratio in liver, spleen and femur of rats fed G diets was 20.6, 90.7 and 30.9, respectively, whereas in the case of M diets the respective figures were much lower: 15.1, 70.7 and 16.8. Similarly, the average Fe:Zn ratio for gluten diets was 3.4, 11.3 and 0.56 in liver, spleen and femur, respectively, while the figures for M diets being 2.3, 8.0 and 0.31.

The available literature does not quote optimal proportions between the investigated elements in tissues of laboratory animals. The amounts of mineral components in diets and the protein quality supplied by them no doubt significantly affect the proportions between these elements.

In view of recent evidence that excess quantities of Fe in tissues lead to increased production of free radicals [21] and that the activity of copper- and zinc-dependent superoxide dismutase decreeases [5], it is advisable to carry out more detailed biochemical studies that would confirm the significance of these observations and explain the role these phenomena may play in the metabolism.

CONCLUSIONS

1. Tissues saturation with Fe, Zn and Cu as the result of simultaneous increasing of mineral components intake depends on the organ as well as the level of elements and protein quality in the diets.

2. Despite the constant Fe:Zn:Cu ration in diets and after absorption from the gastrointestinal tract, increased supplies of these elements led to greater accumulation of Fe compared to Zn and Cu in liver, spleen and femur, greater accumulation of Cu than Fe in kidneys, and greater accumulation of Zn than Cu in bones.

3. In the case of the growth-inhibiting gluten diets, the accumulation of Fe compared to Zn and Cu in liver, spleen and bones was greater and in kidneys lower than in the case of casein, and especially casein-gluten diets.

4. The significanse of changes in the proportions of Fe, Zn and Cu in tissues for the functioning of the organism must be clarified in further studies.

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WPŁYW JAKOŚCI BIAŁKA ORAZ ZAWARTOŚCI ŻELAZA, CYNKU I MIEDZI W DIECIE NA ICH WYKORZYSTANIE PRZEZ ROSNĄCE SZCZURY. II. ZAWARTOŚĆ ŻELAZA, CYNKU I MIEDZI W TKANKACH ZWIERZĄT

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Streszczenie

Rosnące szczury samce były karmione przez 6 tygodni dietami zawierającymi 20% białka, którego źródłem była kazeina, gluten pszenny lub ich mieszanina (1:1). Zawartość żelaza, cynku i miedzi była zróżnicowana następująco: 50, 100 lub 300% zaleceń dla szczurów. Mimo stałego stosunku Fe:Zn:Cu w dietach i po absorbcji z przewodu pokarmowego wzrost podaży badanych składników powodował proporcjonalnie większe gromadzenie żelaza w stosunku do cynku i miedzi w wątrobie, śledzionie i kościach, miedzi w stosunku do żelaza w nerkach i cynku w stosunku do miedzi w kościach. Na diecie glutenowej, ograniczającej wzrost zwierząt, gromadzenie żelaza w wątrobie, śledzionie i kościach było proporcjonalnie większe, a w nerkach proporcjonalnie mniejsze niż w diecie kazeinowej, a w szczególności kazeinowo-glutenowej.