Ann. Zool. Fennici 32: 429–433
Helsinki 8 December 1995
ISSN 0003-455X

Chromosome races and tissue heavy metals in free-living male common shrews (*Sorex araneus* L.)

Tadeusz Włostowski, Włodzimierz Chętnicki, Stanisław Fedyk, Alicja Krasowska & Agata Banaszek

Włostowski, T., Chętnicki, W., Fedyk, S., Krasowska, A. & Banaszek, A., Institute of Biology, Warsaw University Białystok Branch, Świerkowa 20 B, 15-950 Białystok, Poland

Received 28 April 1995, accepted 10 July 1995

The heavy metal concentrations of the liver and kidneys of free-living male common shrews of three chromosome races (Ulm, Stobnica (II), Łęgucki Młyn (IV)) and hybrids II/IV were determined. The concentrations of zinc, iron, manganese and cadmium as well as their distribution between liver and kidneys were not affected by the karyotype. Adult shrews of the Ulm race displayed a significantly lower liver copper concentration than that of the other shrews, whereas the kidney copper concentrations of the four groups did not differ significantly. These results suggest that the Ulm race is a discrepant one in terms of copper metabolism; the possible involvement of genetic basis in this phenomenon is discussed.

1. Introduction

Copper, zinc, iron and manganese — metals which are components or activators of many enzymes — play an important role in animal growth, development and reproduction (Underwood 1977). Deficiency or excess of any of these elements in animal tissues may lead to various diseases and even death (Rogers et al. 1985, Stemmer et al. 1985, Prohaska 1987). Therefore, the concentrations of these trace metals must be ever maintained at appropriate level.

So far, it has been demonstrated that the metal levels in mammalian tissues are affected by many factors, such as: the total content of a given metal in a diet and presence of components inhibiting its absorption, the source and level of protein in a diet, endocrine system, animal age and season (Hyvärinen 1972, Kirchgessner & Weigand 1983, Keen et al. 1985, Cossack 1986, Kennedy et al. 1986, Włostowski et al. 1988, Allain & Leblondel 1992). Beside that, recent studies indicate that the normal metabolism of these elements depends on many gene products (for review, see Prohaska 1987). This is evidenced especially by a number of animal mutants that exhibit abnormalities in the homeostasis of copper (mottled mouse — *brindled* and *blotchy*, toxic milk — *tx*, LEC rats), zinc (*acrodermatitis enteropatica* and lethal milk), and manganese (pallid – *pa*) (Hunt 1974, Chesters 1983, Rauch 1983, Prohaska 1987, Takeichi et al. 1988, Shiraishi et al. 1991). The idea is supported further by the fact that the individual differences in kidney copper concentration in rats and even the kidney and liver concentrations of cadmium in mice have a genetic basis

(Nederbragt & van Zutphen 1987, Shaikh et al. 1993).

In the present work we sought to extend the studies concerning a role of genetic basis in the trace element metabolism on common shrews populations of which in Europe display a great genetic differentiation (Hausser et al. 1994). We were especially interested whether or not the variability in autosome arm combinations affects the concentrations of copper, zinc, iron, manganese and cadmium in the liver and kidneys of these animals.

2. Material and methods

Male common shrews of the chromosome races: Ulm, Stobnica (II), Łęgucki Młyn (IV) and hybrids II/IV were studied in this work (Table 1). *S. araneus* were caught in the pitfalls at the places most suitable for shrews, i.e. wet grassland and shrubs. The adult shrews of the Ulm race were caught on Wolin island (53°57′N, 14°31′E) in June 1988, whereas individuals from the races II, IV and hybrids II/IV were caught in the hybrid zone (see Fedyk et al. 1991) at Mazurian region (53°40′N, 20°13′E) in June 1988 (adults) and September 1988, and 1989 (juveniles). Both the Wolin island and Mazurian region belong to relatively unpolluted areas of Poland.

Chromosome preparations were made from spleen by a standard *in vivo* method (Fedyk 1980) and G-banded after Seabright (1971) method. Shrew's liver and kidneys were excised and dried to a constant weight at 100°C. A portion of the liver (about 150 mg) and both kidneys were then placed in calibrated glass tubes with 1.5 ml of concentrated nitric acid. After 20 hours of sample digestion at room temperature, sulphuric (0.1 ml) and 72% perchloric (0.5 ml) acids were added and the mixture was heated at 100°C (1 hour), 150°C (1 hour) and finally at 200°C (1 hour). The residue, after digestion (about 0.3 ml), was made up with double distilled water to 5.0 ml (liver) or 3.0 ml (kidneys) (Włostowski et al. 1988).

The concentrations of copper, zinc and iron were determined by atomic absorption spectrophotometry in an air-acetylene flame, whereas manganese and cadmium analyses were carried out by electrothermal atomic absorption spectrophotometry using AAS 3 Carl Zeiss Jena instrument with an EA 3 furnace attachment.

Results were expressed as µg/g dry wt ± *S.D.* To discriminate at least to some degree, the possible effects of different metal intakes on tissue heavy metals from those governed by genetic mechanisms involved e.g. in metal distribution between liver and kidneys after its absorption from the intestine (Nederbragt & van Zutphen 1987, Shaikh et al. 1993), the ratio between the metal concentration in liver and kidneys was also calculated. All data were analyzed by one-way analysis of variance. Differences between means were determined by the Duncan's multiple range test or the Student's *t*-test. The statistical analyses were performed on log- or arcsin-transformed data.

3. Results

The mean trace element concentrations of the liver and kidneys of each chromosome races of common shrews are presented in Table 2. As can be seen from Table 2, adult shrews of the Ulm race caught on Wolin island had a liver copper concentration that was significantly lower than that of the other shrews caught in Mazurian region. However, the kidney copper concentrations of the four groups of adult shrews were not significantly different; in effect the ratio (liver Cu/kidney Cu) occurred to be the lowest in the Ulm shrews. Regardless of the race, the copper concentrations of the liver (significant) and kidneys (not significant) of juvenile shrews caught in Mazurian region were lower than those of adults.

Table 1. Karyotypes and body weights of male common shrews of different chromosome races under study.

Chromosome races	Karyotypes (diagnostic metacentrics)	Body mass g ± *S.D.* (*n*) adult	Body mass g ± *S.D.* (*n*) juvenile
Ulm	*hi, gm*	11.5 ± 0.8 (15)	not studied
Stobnica (II)	*hi, ok, gm, np*	11.4 ± 1.1 (19)	7.7 ± 0.6 (19)
Łęgucki Młyn (IV)	*kh, oi, gr, mn*	11.2 ± 0.7 (20)	7.5 ± 0.8 (13)
Hybrids (II/IV)	*hi,ko,gr,mn* or *kh/hi/io/ok, r/rg/gm/mn/np/p*	11.3 ± 0.6 (20)	7.4 ± 0.6 (26)

In contrast to copper, the zinc, iron and manganese concentrations of the liver and kidneys of adult shrews of Ulm race were not significantly different from those of the races II, IV and hybrids II/IV (Table 2). Likewise, the concentrations of these metals in the liver and kidneys of juvenile shrews were not affected by the karyotype. However, the zinc concentrations in the liver and kidneys, and iron concentration in the kidneys of juvenile shrews were significantly lower than those of adult shrews. This implied that the relative tissue zinc distribution (liver Zn/kidney Zn) was similar to, but the distribution of iron (liver Fe/kidney Fe) was significantly different from that in the adults.

The liver and kidney cadmium concentrations of adult shrews of Ulm race caught on Wolin island were about twofold lower than those of the other shrews from Mazurian region (Table 2). It was found, however, that the ratios (liver Cd/ kidney Cd) of the four groups were not significantly different. This suggested that the differences in the liver and kidney cadmium concentrations were not attributed to genetic basis but rather to a dietary cadmium intake which might be different in Mazurian region from that on Wolin island.

4. Discussion

The present work demonstrated that the concentrations of zinc, iron, manganese and cadmium in the liver and kidneys of common shrews as well as the metal distribution between liver and kidneys are not affected by the karyotype. It cannot be ruled out, however, that the observed differences in liver copper concentration may be attributed to the vari-

Table 2. Heavy metal concentrations in the liver and kidneys of common shrews of different chromosome races (μg/g dry wt ± *S.D.*). There were no significant differences between the trace element concentration of juvenile shrews caught in 1988 and 1989 and therefore the results for both years have been combined to simplify presentation of the data. *- values are significantly different from the other three groups of adults at $p <$ 0.05 (Duncan's multiple range test); •- values are significantly different from race-matched adult at $p < 0.05$ (Student's *t*-test).

	Adults				Juveniles		
Organ	Ulm race $n = 15$	Race II $n = 19$	Race IV $n = 20$	Hybrids II/IV $n = 20$	Race II $n = 19$	Race IV $n = 13$	Hybrids II/IV $n = 26$
Copper (Cu)							
Liver	27.4 ± 3.0*	37.1 ± 5.0	37.4 ± 5.3	36.1 ± 4.9	25.0 ± 4.8•	27.1 ± 3.3•	27.6 ± 3.2•
Kidneys	46.3 ± 5.0	43.2 ± 5.8	43.9 ± 6.7	42.8 ± 5.9	36.1 ± 5.8	37.2 ± 5.0	37.7 ± 4.0
Liver/Kidneys	0.60 ± 0.08*	0.84 ± 0.10	0.86 ± 0.09	0.83 ± 0.11	0.70 ± 0.10	0.74 ± 0.11	0.73 ± 0.10
Zinc (Zn)							
Liver	125 ± 16	130 ± 18	120 ± 10	137 ± 14	90 ± 16•	89 ± 15•	94 ± 10•
Kidneys	153 ± 20	145 ± 20	140 ± 11	152 ± 15	93 ± 15•	95 ± 14•	100 ± 16•
Liver/Kidneys	0.84 ± 0.11	0.89 ± 0.16	0.90 ± 0.17	0.94 ± 0.11	0.95 ± 0.15	0.93 ± 0.11	0.94 ± 0.10
Iron (Fe)							
Liver	1180 ± 170	1200 ± 180	1150 ± 180	1240 ± 250	1100 ± 170	1200 ± 180	1000 ± 200
Kidneys	600 ± 96	600 ± 80	521 ± 90	627 ± 96	380 ± 80•	360 ± 85•	350 ± 90•
Liver/Kidneys	1.97 ± 0.20	2.00 ± 0.20	2.26 ± 0.18	2.10 ± 0.20	2.80 ± 0.30•	3.10 ± 0.35•	2.80 ± 0.32•
Manganese (Mn)							
Liver	11.8 ± 2.6	11.4 ± 2.6	11.5 ± 2.8	13.1 ± 2.7	12.0 ± 2.6	11.9 ± 2.7	12.9 ± 2.6
Kidneys	5.8 ± 1.3	5.6 ± 1.5	5.3 ± 1.7	5.8 ± 1.8	5.3 ± 1.6	5.2 ± 1.5	5.6 ± 1.7
Liver/Kidneys	2.10 ± 0.17	2.10 ± 0.17	2.24 ± 0.25	2.27 ± 0.21	2.26 ± 0.20	2.25 ± 0.24	2.30 ± 0.20
Cadmium (Cd)							
Liver	0.93 ± 0.50*	1.85 ± 0.55	1.73 ± 0.50	2.06 ± 0.85			
Kidneys	2.00 ± 0.75*	4.00 ± 1.00	3.57 ± 0.87	4.30 ± 1.60			
Liver/Kidneys	0.49 ± 0.08	0.47 ± 0.10	0.50 ± 0.08	0.47 ± 0.11			

ability in autosome arm combinations. The assumption is supported by the fact that the karyotype of Ulm race from Wolin differs from that of the other races (Table 1) and concurrently the liver copper concentrations of the Ulm shrews are significantly lower than those of the other races inhabiting Mazurian region (Table 2). One may conclude, however, that this difference may have been due to a variation in copper content of shrews' food in Mazurian region and on Wolin island. But even if the latter possibility is true, a question arises as to why the kidney copper concentration of the Ulm shrews tended to increase above the level of the Mazurian shrews (Table 2). In addition, it has been found, at least in bank voles, that any changes in both liver and kidney copper concentrations follow closely those of dietary copper (Włostowski et al. 1988). Therefore, it may be supposed that the distinct distribution of copper between liver and kidneys of the Ulm shrews may have been owing to another cause. It is worth noting that the situation we observed in the Ulm race bears a resemblance to that of the X-chromosome-linked mouse mutations of copper metabolism, namely *brindled* and *blotchy* (Prohaska 1986, 1988, Shiraishi et al. 1991). In both cases copper leaves certain organs, such as liver where it should stay and accumulates excessively in others such as kidneys and intestine. However, the hemizygote mice (br/y) die at about two weeks of age and do not attain an adult age as do our male shrews of the Ulm race. This strongly suggests that other locus (loci), if any, may be involved in the changes of copper metabolism in the Ulm race of adult common shrews.

Nederbragt et al. (1987) pointed out that, in addition to animal mutants, a comparison of strain differences in tissue copper concentration may be a suitable animal model for elucidating various aspects of copper metabolism. In this context the Ulm race of common shrews is worth studying in the future, especially in laboratory conditions.

The common shrews appeared to be a useful bioindicator of heavy metal pollution (Pankakoski et al. 1994). Our results indicate that regardless of chromosome race these animals can be used for studies on environmental pollution with cadmium, zinc, iron and manganese, but a care should be taken into consideration when comparing copper levels.

Acknowledgements. This study was sponsored under Grant CPBP 04.03.II/6 from the Polish Academy of Sciences and Grant 0237/P2/93/05 from the Committee of Scientific Research.

References

Allain, P. & Leblondel, G. 1992: Endocrine regulation of trace element homeostasis in the rat. — Biol. Trace Element Res. 32: 187–199.

Chesters, J. K. 1983: Zinc metabolism in animal: pathology, immunology and genetics. — J. Inher. Metab. Dis. 6 (Suppl. 1): 34–38.

Cossack, Z. T. 1986: Somatomedin-C and zinc status in rats as affected by Zn, protein and food intake. — Br. J. Nutr. 56: 163–169.

Fedyk, S. 1980: Chromosome polymorphism in a population of Sorex araneus L. at Białowieża. — Folia Biol. (Kraków) 28: 83–120.

Fedyk, S., Chętnicki, W. & Banaszek, A. 1991: Genetic differentiation of Polish populations of Sorex araneus L. III. Interchromosomal recombination in a hybrid zone. — Evolution 45: 1384–1392.

Hausser, J., Fedyk, S., Fredga, K., Searle, J. B., Volobuev, V., Wójcik, J. M., & Zima, J. 1994: Definition and nomenclature of the chromosome races of S. araneus. — Folia Zool. 43 (Suppl. 1): 1–9.

Hunt, D. M. 1974: Defect in copper transport underlies mottled mutation in mice. — Nature 249: 852–854.

Hyvärinen, H. 1972: Seasonal changes in the copper content of the liver of the common shrew, Sorex araneus over a two-year period. — J. Zool. (Lond.) 166: 411–416.

Keen, C. L., Reinstein, N. H., Gondey-Lefevre, J., Lonnerdal, B., Schneeman, B. O. & Hurley, L. S. 1985: Effect of dietary copper and zinc levels on tissue copper, zinc, and iron in male rats. — Biol. Trace Element Res. 8: 123–136.

Kennedy, M. L., Failla, M. L. & Smith, J. C. 1986: Influence of genetic obesity on tissue concentrations of zinc, copper, manganese and iron in mice. — J. Nutr. 116: 1432–1441.

Kirchgessner, M. & Weigand, E. 1983: Zinc absorption and excretion in relation to nutrition — In: Siegel, H. (ed.), Metal ions in biological systems, vol. 15. Mercel Dekker, New York, Basel: 319–361.

Nederbragt, H. & van Zutphen, L. F. M. 1987: Strain differences in kidney copper concentrations of male rats. — Biol. Trace Element Res. 14: 217–223.

Pankakoski, E., Koivisto, I., Hyvärinen, H. & Terhivuo, J. 1994: Shrews as indicators of heavy metal pollution. — In: Merrit, J. F., Kirkland, G. L., Jr. & Rose, R. K. (eds.), Advances in the Biology of Shrews. Carnegie Mus. Natur. Hist., Spec. Publ. 18: 137–149.

Prohaska, J. R. 1986: Genetic diseases of copper metabolism. — Clin. Physiol. Biochem. 4: 87–93.

— 1987: Functions of trace elements in brain metabolism. — Physiol. Rev. 67: 858 — 901.

— 1988: Effect of dietary copper deficiency on heterozygous female brindled mice. — Nutr. Res. 8: 1079–1084.

Rauch, H. 1983: Toxic milk, a new mutation affecting copper metabolism in the mouse. — J. Hered. 74: 141–144.

Rogers, J. M., Keen, C. L. & Hurley, L. S. 1985: Zinc deficiency in pregnant Long-Evans hooded rats: teratogenicity and tissue trace elements. — Teratology 31: 89–100.

Seabright, M. 1971: A rapid banding technique for human chromosomes. — Lancet 2: 971–972.

Stemmer, K. L., Petering, H. S., Murthy, L., Finelli, V. N. & Menden, E. E. 1985: Coper deficiency effects on cardiovascular system and lipid metabolism in the rat: the role of dietary proteins and excessive zinc. — Ann. Nutr. Metab. 29: 332–347.

Shaikh, Z. A., Jordan, S. A. & Tewari, P. C. 1993: Cadmium disposition and metallothionein induction in mice: strain-, sex-, and dose-dependent differences. — Toxicology 80: 51–70.

Shiraishi, N., Taguchi, T. & Kinebuchi, H. 1991: Metallothionein messenger RNA levels in the macular mutant mouse: an animal model of Menkes' Kinky-Hair disease. — Biol. Neonate 60: 52 — 61.

Takeichi, N., Kabayashi, H., Yoshida, M. C., Sasaki, M., Dempo, K & Mori, M. 1988: Spontaneous hepatitis in Long-Evans rats. A potential animal model for fulminant hepatitis in man. — Acta Pathol. Jpn. 38: 1369–1375.

Underwood, E. J. 1977: Trace elements in human and animal nutrition. — Academic Press, New York: 545 pp.

Włostowski, T., Chętnicki, W., Gierłachowska-Bałdyga, W. & Chycak, B. 1988: Zinc, iron, copper, manganese, calcium and magnesium supply status of free-living bank voles. — Acta Theriol. 33: 555–573.