Biochemical Pharmacology, Vol. 30, No. 15, pp. 2177–2178, 1981.
Printed in Great Britain.

0006-2952/81/152177–02 $02.00/0
Pergamon Press Ltd.

Content of mercury in chromatin and level of metallothionein proteins in kidneys and liver of rats*

(*Received* 19 *January* 1981; *accepted* 23 *February* 1981)

Binding of cadmium and mercury to components of cell nuclei [1–4] as well as induction of biosynthesis of MTP† [5] have been observed in animals exposed to these metals. The evident increase in the level of metallothionein mRNA in cells with elevation of the dose of administered metal [6–8] and the sensitivity of biosynthesis of metallothionein proteins to inhibitors of transcription [6, 9] seem to indicate a direct interaction of the inducing metal with chromatin. However till now experimental data are lacking which would confirm this suggestion.

In this study the relation between the mercury content in the chromatin and the dynamics of biosynthesis of metallothionein proteins in rat kidneys and liver was investigated.

Female Wistar rats weighing 180–200 g were used. Biosynthesis of metallothionein proteins was induced by intraperitoneal injection of a single dose of $HgCl_2$ (1 or 5 mg Hg/kg body weight). Rate of biosynthesis of MTP was monitored by measurements of incorporation of [^{35}S]cysteine into low mol. wt proteins. [^{35}S]Cysteine/ 80 μCi, sp. act. = 25 mCi·mmole^{-1}, Rotop, GDR/ was administered i.p. 1 hr prior to the decapitation. Radioactivity of the incorporated cysteine was measured in scintillation counter (Wallac 81000, LKB) after dissolving the sediment of low-molecular weight proteins (isolated according to Zelazowski and Piotrowski [11]) in NCS (Amersham, U.K.) and addition of 10 ml of Tritosol [10]. Estimations of MTP were performed by the radiochemical method with ^{203}Hg label [11] employing a metallothionein standard from horse kidney [12]. Chromatin was prepared according to Spelsberg and Hnilica [13] from nuclei isolated by the saccharose method including the step of Triton X-100 purification [4]. Mercury level in the chromatin was determined radiometrically with a gamma spectrometer (USB, Poland) after injection of $^{203}HgCl_2$ to animals. The content of chromatin protein was estimated by the method of Lowry *et al.* [14].

Injection of mercuric chloride at a dose of 1 mg Hg/kg resulted in an about 2-fold increase of the MTP level in kidneys (Table 1) preceded by a maximum of mercury content in the chromatin (*ca.* 0.08 μg Hg/mg protein) (Fig. 1a). After a dose of 5 mg Hg/kg incorporation of [^{35}S]cysteine was highest after about 24 hr. This increase in biosynthetic activity was preceded, too, by a peak of mercury content in the chromatin (Fig. 1b). However, the highest accumulation of metallothionein proteins was observed as late as on the third day after administration of the inducing metal. The lack of elevation in the level of these proteins in the first 2 days, in spite of their intensive biosynthesis and their relatively long (about 4-day) half-life [5] may be due to proteinuria occurring under conditions of high mercury level in the kidneys [15, 16].

There are equivocal data in the literature on the stimulation of biosynthesis of metallothionein-like proteins in the liver by mercury. Such proteins were isolated from rat liver by Winge and coworkers [17]. Piotrowski *et al.* [18] noted that after exposure of rats to mercuric chloride (sev-

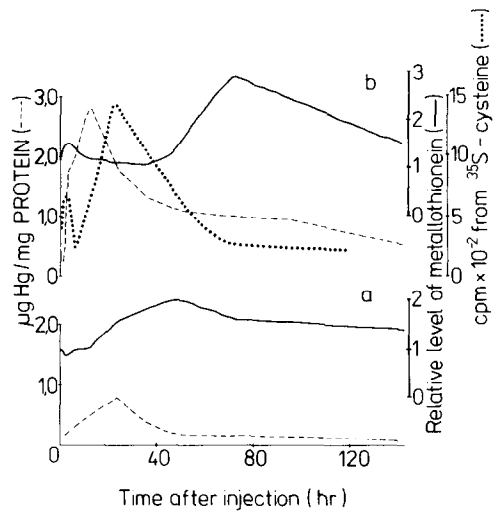

Fig. 1. Content of mercury in chromatin, incorporation of [^{35}S]cysteine (into low mol. wt proteins obtained from 0.2 g tissue) and level of metallothionein proteins (relative to physiological level of 176 ± 16 μg MTP/g tissue) in kidneys of rats exposed to $HgCl_2$ in doses of 1(a) and 5(b) mg Hg/kg body weight.

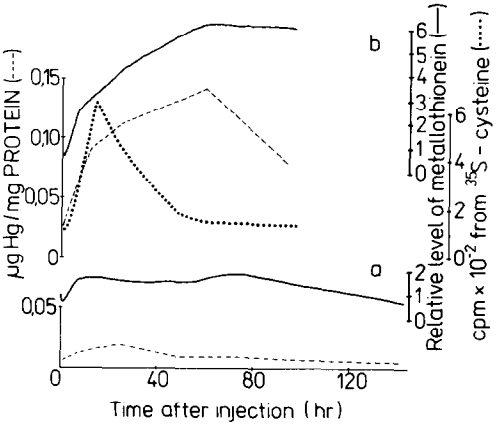

Fig. 2. Content of mercury in chromatin, incorporation of [^{35}S]cysteine (into low mol. weight proteins obtained from 0.2 g tissue) and level of metallothionein proteins (relative to physiological level of 90 ± 28 μg MTP/g tissue) in liver of rats exposed to $HgCl_2$ in doses of 1(a) and 5(b) mg Hg/kg body weight.

*Supported by the Polish Academy of Sciences (538/VI).
†Abbreviation: MTP, metallothionein proteins.

Table 1. Content of metallothionein proteins (μg/g tissue) in kidneys and liver of rats exposed to mercuric chloride (single dose, i.p.). Mean values from 2 to 6 experiments ± S.D.

Physiological level of metallothionein proteins	Dose mg Hg/kg body wt	Hours after administration									
		1	3	6	12	24	48	60	72	96	144
Kidneys 176 ± 16	1	167 ± 74	150 ± 42	167 ± 38	181 ± 67	282 ± 70	352 ± 104	317	282	264	238
	5	246 ± 73	264 ± 59	238 ± 62	211 ± 31	194 ± 62	246 ± 38	405	510	422	264
Liver 90 ± 28	1	63 ± 14	90 ± 13	135 ± 14	153 ± 32	144 ± 34	130 ± 25	144 ± 41	166 ± 44	130 ± 20	74 ± 17
	5	67 ± 12	121 ± 31	215 ± 56	287 ± 34	377 ± 41	502 ± 14	556	552	–	–

eral s.c. injections of 0.25 and 0.5 mg Hg/kg) metallothionein is synthesized only in the kidneys. In our experiments at a dose of 1 mg Hg/kg and at a relatively low mercury content in the hepatic chromatin (below 0.02 μg Hg/mg protein) the amount of synthesized MTP only slightly exceeded the physiological level (Fig. 2a). On the other hand a high dose of $HgCl_2$ (5 mg Hg/kg) induced a considerable rise in the content of this metal in the hepatic chromatin (over 0.1 μg Hg/mg protein) accompanied by a 6-fold increase in the level of inducible metallothionein-like proteins in this organ (Fig. 2b).

The data presented suggest that mercury induces MTP biosynthesis by direct action on the chromatin and by binding to its components. Lower doses of $HgCl_2$ bring about a significant induction of MTP only in the kidneys where under these conditions a relatively high mercury content in the chromatin is attained. On the other hand, at higher doses of mercury a severalfold increase of MTP over the physiological level is observed in both kidneys and liver. The relative increase of the MTP content is even greater in the latter organ.

Acknowledgement—We would like to thank Dr. A. Zelazowski for the kind gift of a metallothionein standard.

Institute of Environmental Research and Bioanalysis
Medical Academy of Łódź
Narutowicza 120*a*
91-145 *Łódź*
Poland

MAREK RÓŻALSKI
EWA KUZIEMSKA
RYSZARD WIERZBICKI

REFERENCES

1. S. K. Chanda and M. G. Cherian, *Biochem. biophys. Res. Commum.* **50**, 1013 (1973).
2. S. E. Bryan, A. L. Guy and K. J. Hardy, *Biochemistry* **13**, 313 (1974).
3. H. A. Hidalgo and S. E. Bryan, *Toxicol. Appl. Pharmacol.* **42**, 319 (1977).
4. M. Różalski and R. Wierzbicki, *Environ. Res.* **20**, 465 (1979).
5. J. Kägi and M. Piscator, *Experientia Suppl.* **34** (1979).
6. S. G. Shapiro, K. S. Squibb, L. A. Markowitz and R. J. Cousins, *Biochem. J.* **175**, 833 (1978).
7. R. D. Andersen and U. Weser, *Biochem. J.* **175**, 841 (1978).
8. M. D. Enger, L. B. Rall and C. E. Hildebrand, *Nucleic Acids Res.* **7**, 271 (1979).
9. K. S. Squibb, R. J. Cousins and S. L. Feldman, *Biochem. J.* **164**, 223 (1977).
10. U. Fricke, *Anal. Biochem.* **63**, 555 (1975).
11. A. J. Zelazowski and J. K. Piotrowski, *Acta Biochem. Polon.* **24**, 97 (1977).
12. A. J. Zelazowski, J. K. Piotrowski, E. M. Mogilnicka, J. A. Szymańska and B. W. Kaszper, *Bromat. Chem. Toksykol.* **11**, 151 (1978).
13. T. C. Spelsberg and L. S. Hnilica, *Biochim. biophys. Acta* **228** 202 (1971).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. J. Piotrowski, W. Bolanowska, B. Trojanowska and S. Szendzikowski, *Med. Pr.* **20**, 6 (1969).
16. S. A. Kempson, B. G. Ellis and R. G. Price, *Chem.-biol. Interact.* **18**, 217 (1977).
17. D. R. Winge, R. Premakumar and K. V. Rajagopalan, *Archs Biochem. Biophys.* **170**, 242 (1975).
18. J. K. Piotrowski, B. Trojanowska and A. Sapota, *Archs Toxicol.* **32**, 351 (1974).