Effects of Cadmium and Zinc on Tissue Levels of Metallothionein

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Although the induced synthesis of metallothionein (MT) after exposure to certain metals has been known for some time, there is little information on the quantitation of MT in various tissues. In this study, tissue MT concentrations were measured by a modified Cd-saturation method in tissues of adult male rats after injection of different metal salts. There were differences in tissue levels of MT, depending on the injected metals. Of all the metals studied, Cd$^{2+}$ was the most effective element in increasing MT concentrations in liver, kidney, pancreas and small intestine. The highest increase in tissue MT concentration after CdCl$_2$ injection was found in the liver, while the pancreas contained the highest MT level after ZnSO$_4$ injection. Co and Ni salts increased MT levels in both liver and kidney, while Mn and Ca increased MT levels only in liver. A direct correlation between tissue MT levels and Cd or Zn concentration was observed in most of the tissues after injection of CdCl$_2$ or ZnSO$_4$. Although there was no positive relationship between tissue levels of MT and tissue Mn and Ni concentrations, the increase in hepatic Zn after injection of these metals was related to hepatic MT levels. The tissue distribution of injected Cd$^{2+}$ in control adult rats and Zn-deficient rats was similar. However, there was no increase in pancreatic MT levels in Zn-deficient rats after injection of CdCl$_2$. The high concentration of MT in pancreas after ZnSO$_4$ injection in adult rats and the inability of the pancreas to synthesize MT in Zn-deficient rats suggest that the induction of pancreatic MT synthesis is sensitive to Zn status. Thus, injection of different metals results in quantitative variations of tissue MT concentrations.

The induced synthesis of metallothionein (MT) or like proteins in different tissues of experimental animals on exposure to certain divalent metals has been known for some time (1-4). However, there are few data on the quantitation of MT in various tissues. Therefore, we have undertaken a detailed study on the estimation of tissue MT levels after injection of different metal salts in adult male rats. Since MT can bind with both essential and nonessential metals, it may play an important role in the pharmacokinetics and toxicity of these metals.

The different factors which influence the induced synthesis of MT in various tissues should be considered in order to understand the multiple biological functions of this metalloprotein. Although a number of environmental factors such as stress (5), starvation (6) and exposure to glucocorticoids (7) can induce the synthesis of MT-like proteins in liver, the most effective agents for their induction are certain divalent metal compounds (8). This report will summarize some of the most recent work from our laboratories on the induced synthesis of MT in various tissues by metals (9,10).

**Estimation of Tissue MT Concentrations**

During the last decade, several methods for the determination of MT in tissues were developed based on its metal binding properties and high thiol content (11-13). Although a radioimmunoassay for MT has also been developed (14,15), it has not been standardized to measure tissue concentrations of MT. In our recent studies, we have used a modified cadmium binding method to estimate MT in various tissues (11,16). This is a simple method which can be standardized to analyze a number of samples within a short time. In

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this method, the tissue samples are first saturated by addition of CdCl₂ in vitro. The excess Cd²⁺ and all the Cd-binding ligands other than MT in the samples are removed by addition of red blood cell hemolyzates and a subsequent heating step. Since it is known that 1 mole of thionein (molecular weight 6050) binds with 6 or 7 g-atoms of Cd²⁺, the actual concentration of MT in various tissues is calculated after estimation of Cd²⁺ in the heated supernatant.

The results obtained by this Cd-hem method was in good agreement when compared with G-75 method (11) and pulse polarographic analysis (16). The merits and limitations of the newly modified method are summarized in Table 1. This method is simple and rapid and has high reproducibility from tissue to tissue. Since it is based on the Cd²⁺-binding capacity of MT, any changes in metal binding may influence the estimation of MT in tissues. For example, if tissue samples contain MT with high copper and/or mercury content, it may result in underestimation of MT by the Cd-hem method. Also, this method is not sensitive enough to measure MT concentrations in blood or urine.

### Changes in Tissue MT Levels after Injection of Metal Salts

Although the recent findings using cDNA probe and genomic clones have clearly shown (17,18) specific inducible m-RNA for MT, the exact role of metals in the induced synthesis of MT is unclear. In addition to metals, MT synthesis can also be induced in rat liver by starvation and various other stress conditions (6). However, a comparative study (8) has shown that the injection of adrenocortical steroids and other stress conditions can increase the concentration of MT in hamster liver by only 50–80%, while injection of Zn²⁺ or Cd²⁺ salts can increase MT levels by 700–2000%. Thus, there is a definite difference in the magnitude of induction of MT synthesis by metals and other factors.

The induction of hepatic MT synthesis has been reported in a number of species on exposure to Cd²⁺ salts and also to high amounts of Zn²⁺ salts (1,19). However, data on the quantitation of MT levels in various other tissues from control experimental animals and those after exposure to different metals are limited. Such information is essential to compare the induction and deposition of MT in various organs in animals exposed to different metals.

We have recently measured MT concentration by the Cd-hem method in eleven different tissues of control male rats and those injected with various doses of Cd²⁺ (9) or Zn²⁺ salts (10). These studies show that, although the highest concentration of MT was present in the testes of control male rats, injection of both CdCl₂ and ZnSO₄ did not increase its concentration in our experimental conditions (Tables 2 and 3). However, it should be mentioned that the Cd-binding proteins in testes are not yet well characterized. The injection of CdCl₂ to rats resulted in a dose-dependent increase in MT accumulation in a number of tissues, the highest increase in concentration being in liver, kidney and pancreas (Table 2). There were also increases in MT levels in heart, stomach, small intestine and spleen after injection of CdCl₂. A different pattern of increase in tissue concentration of MT was observed after injection of ZnSO₄ (Table 3). When rats were injected with various doses of ZnSO₄, MT levels were increased only in pancreas, liver, kidney and small intestine. Unlike in CdCl₂-injected rats, the highest concentration of MT was found in the pancreas after ZnSO₄ injection at all dose levels studied. These results suggest that there are differences in organ-specific synthesis of MT after injection of CdCl₂ and ZnSO₄. This also provides additional evidence that MT is synthesized in response to injection of ZnSO₄ itself and not due to any trace contamination of Cd²⁺ in the ZnSO₄.

In a detailed systematic study undertaken at Joint Nuclear Research Centre at Ispra Establishment of Commission of the European Communities, Sabbioni and Marafante (20,21) have identified a number of metals which can both induce the synthesis of MT-like proteins and bind with them intracellularly in rat liver. Although certain other metals cannot induce its synthesis, they can bind with MT in vitro. A number of other metals which can neither induce MT synthesis nor bind with it in vitro also have been identified. Similar results have been reported from a number of other laboratories, although the induced proteins are not well characterized (22,23). In a recent study (24), the increase in MT levels in

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| Merits                                      | Problems               |
|---------------------------------------------|------------------------|
| Rapid and simple                            | Metal composition: 6 g-atoms of Cd per MT |
| Good agreement with G-75 method              | Metal binding affinity: Hg, Cu, Ag > Cd |
| Good reproducibility                        | Low sensitivity: urine, blood |

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**Table 1. Cd-hem method for metallothionein estimation.**
**Table 2. Metallothionein levels after injection of cadmium chloride.**

| Tissue       | Control (6.4 mg/kg) | Group I (12 mg/kg) | Group II (24 mg/kg) | Regression equation |
|--------------|---------------------|--------------------|---------------------|---------------------|
| Brain        | 4.8 ± 1.8           | 6.1 ± 0.3          | 5.5 ± 0.3           | 6.7 ± 1.5           |
| Lung         | 2.6 ± 1.2           | 4.8 ± 0.6          | 4.9 ± 0.7           | 6.6 ± 1.2           |
| Heart        | 0.7 ± 0.3           | 9.5 ± 0.6          | 11.8 ± 1.2          | 22.2 ± 5.4          |
| Liver        | 3.0 ± 1.2           | 238 ± 6.0          | 326 ± 1.8           | 699 ± 93.0          |
| Kidney       | 9.1 ± 2.1           | 80.0 ± 4.3         | 129 ± 5.2           | 213 ± 15.2          |
| Stomach      | 3.4 ± 0.9           | 7.1 ± 1.2          | 11.2 ± 0.3          | 15.2 ± 0.9          |
| Small intestine | 6.0 ± 0.6       | 8.8 ± 2.4          | 9.7 ± 2.4           | 16.7 ± 2.1          |
| Pancreas     | 5.8 ± 0.9           | 48.1 ± 9.7         | 78.8 ± 10.4         | 101 ± 7.4           |
| Spleen       | 1.2 ± 0.6           | 8.2 ± 0.3          | 10.9 ± 0.9          | 18.6 ± 0.9          |
| Testes       | 26.7 ± 1.2          | 25.5 ± 0.7         | 20.4 ± 3.3          | 8.9 ± 4.8           |
| Muscle       | 0.6 ± 0.0           | 0.7 ± 0.3          | 0.7 ± 0.3           | 1.8 ± 0.0           |

*Modified and adapted from a previous publication (9), Onosaka and Cherian, 1981.

**Table 3. Metallothionein levels after injection of zinc sulfate.**

| Tissue       | Control (40 mg/kg) | Group I (80 mg/kg) | Group II (140 mg/kg) | Regression equation |
|--------------|--------------------|--------------------|----------------------|---------------------|
| Brain        | 4.0 ± 0.7          | 5.8 ± 1.2          | 6.0 ± 1.0            | 6.1 ± 0.7           |
| Lung         | 1.6 ± 0.3          | 2.5 ± 0.3          | 2.2 ± 0.3            | 2.5 ± 0.9           |
| Heart        | 2.4 ± 0.6          | 2.5 ± 0.3          | 2.4 ± 0.0            | 1.9 ± 0.7           |
| Liver        | 2.5 ± 0.3          | 69.9 ± 14.3        | 110 ± 26.2           | 162 ± 18.2          |
| Kidney       | 7.5 ± 2.4          | 26.1 ± 6.6         | 38.0 ± 7.0           | 62.6 ± 14.1         |
| Stomach      | 5.7 ± 1.8          | 8.3 ± 1.2          | 6.0 ± 1.2            | 2.2 ± 0.3           |
| Small intestine | 3.1 ± 0.3       | 28.8 ± 9.7         | 61.4 ± 8.6           | 112 ± 16.7          |
| Pancreas     | 7.0 ± 1.3          | 107 ± 4.6          | 233 ± 29.8           | 274 ± 101           |
| Spleen       | 1.9 ± 0.3          | 4.0 ± 1.2          | 2.8 ± 0.4            | 2.2 ± 0.3           |
| Testes       | 26.5 ± 2.4         | 21.3 ± 1.8         | 24.0 ± 0.9           | 23.2 ± 2.1          |
| Muscle       | 1.0 ± 0.7          | 0.7 ± 0.9          | 0.7 ± 0.3            | trace               |

*Modified and adapted from a previous publication (10).

**Table 4. Relative deposition of metallothionein in tissues after injection of various metals.**

| Metal       | Liver | Kidney | Small intestine | Pancreas |
|-------------|-------|--------|-----------------|----------|
| Cd          | 3170  | 935    | 50              | 434      |
| Zn          | 130   | 56     | 25              | 152      |
| Co          | 127   | 16     |                 |          |
| Ni          | 117   | 103    |                 |          |
| Mn          | 47    |        |                 |          |
| Ca          | 29    |        |                 |          |

*Al, Cr, Fe, K, Mg, Na, Pb: no increase

different tissues were measured by the Cd-hem method after injection of various metals. The results on relative MT accumulation in liver, kidney, small intestine and pancreas are summarized in Table 4. The concentration of MT in four organs is expressed as nmole/g after injection of metal/kg. This has enabled us to compare directly the relative deposition of MT in different tissues after injection of various metals. The results showed that Cd$^{2+}$ was the most potent element in increasing MT in all the tissues studied and the maximum accumulation of MT was observed in liver. Zinc was also active in increasing MT in all the four organs but was less effective than Cd$^{2+}$. However, the highest increase in MT was in pancreas after injection of ZnSO$_4$. Both cobalt and nickel salts increased MT levels in liver and kidney while manganese and calcium salts increased MT levels only in liver. Injection of a number of other elements such as Al, Cr, Fe and Pb in rats did not increase MT levels in any tissues under these conditions when estimated by Cd-hem method. Although it is well known that both copper and mercury salts can also induce MT synthesis (25,26), these compounds were not examined in the present study because the Cd-hem method may not provide accurate measurement of MT containing a high concentration of copper or mercury.
Relationship between Tissue MT and Metal Levels

As shown in Tables 2 and 3, increased tissue levels of MT were observed in liver, kidney, stomach, small intestine, pancreas, spleen and heart after injection of CdCl₂, but an increase was found only in four tissues after ZnSO₄ injection. The highest concentration of MT was detected in the liver after CdCl₂ injection, whereas injection of ZnSO₄ resulted in the highest concentration of MT in the pancreas. These results were similar to metal injection and pancreas (5). Therefore, it is difficult to conclude that there is always a direct relationship between tissue MT levels and tissue deposition of the inducing metal.

Further analysis of the data showed a direct positive correlation between the deposition of Cd and MT in liver, kidney and pancreas (Fig. 1), even though there were differences in the levels of MT in various tissues. The slope of the regression equation between tissue Cd and MT levels indicated the apparent capacity of the tissue to induce MT synthesis in response to Cd deposition and was almost identical for liver and pancreas. Analysis of a number of other tissues also gave similar values (9). The slope obtained for kidney was slightly lower (Fig. 1), and it can be explained by the increased presence of copper in renal MT which gave lower estimations of MT by the Cd-hem method. Studies on induction of MT by Cd injection after injection of ZnSO₄ also showed a direct positive correlation between the tissue deposition of zinc and MT in pancreas, liver, kidney and small intestine, despite the differences in tissue levels of MT (10). Although these values were much lower on a molar basis than after CdCl₂ injection, they showed good agreement within tissues in ZnSO₄-injected rats. These results suggest that one of the major factors which affects the synthesis of MT in tissues after injection of Cd and Zn salts is the tissue-specific deposition of Cd and Zn.

In contrast to the results of ZnSO₄ and CdCl₂ injection studies, preliminary data on induction of hepatic MT synthesis by cobalt, nickel, manganese and calcium salts showed no direct relationship between tissue deposition of Ni and Mn and increase in tissue MT levels. The increase in hepatic MT after CaCl₂ injection was related to the deposition of calcium in the liver. Therefore it is difficult to conclude that there is always a direct relationship between tissue MT levels and tissue deposition of the inducing metal.

The Specific Role of Zinc in MT Synthesis

The effect of zinc deficiency (Zn-D) on the induction of MT synthesis was studied recently (10). In this study, we made rats Zn-deficient with a diet containing 1 ppm Zn for 18 days, and MT synthesis was induced in control and Zn-D rats by injection of CdCl₂ (1 mg Cd/kg), three times at 48-hr intervals. Cadmium salts were used to induce MT synthesis in this study because it was found to be the most effective agent for the induction of MT. When rats were sacrificed 24 hr after the last CdCl₂ injection, the distribution of Cd in different tissues was very similar in both groups (Table 5). There was no difference in the hepatic MT levels between the control and zinc-deficient groups. However, the MT levels in both kidney and pancreas of Zn-D rats were significantly lower than in the control group. These results raise a number of questions on the role of Zn in the synthesis of MT in certain tissues. The Zn status or the tissue levels of Zn may be an important factor in the regulation of induced MT synthesis by other metals. It should also be noted that a concurrent increase in hepatic Zn concentration was noted in rats injected with Cd salts (27).

The pancreas appears to be a critical organ in the metabolism of Zn because it accumulates a
significant amount of Zn on excessive exposure and also depletes Zn rapidly in Zn deficiency. A role for Zn in the stabilization of tissue MT has
been proposed (29). Zinc is also found in most of the isolated MT from various sources and a specific Zn binding site has been identified by structural
analysis of MT (29). Studies on increased tissue levels of MT after injection of metals such as Co, Ni, Mn and Ca also indicate a unique role for Zn
on the induced synthesis of MT.

It is well known that metals such as Ni, Mn, Co and Ca have very little affinity for MT as compared to the binding of MT with Cd, Zn, Cu and Hg.
Recent results (Table 4) suggested that injection of these metals can increase the hepatic MT and Zn levels in rats. Although these proteins are not well characterized, the preliminary results suggest a direct relationship between increased MT and Zn levels in liver after injection of Ni and Mn, but no correlation between tissue MT and tissue levels of these metals themselves. Thus it is possible that the increase in hepatic MT following injection of these metals may be due to an indirect effect of increased hepatic zinc concentration which may induce MT synthesis. It can also be speculated from these results that Zn may be the primary inducer of MT, at least in certain tissues and that other metals such as Cd and Hg are simply transferred to MT according to their binding affinity with subsequent displacement of Zn. In the case of Co, Ni, Mn and Ca, they have low affinity for MT and therefore are not transferred to newly synthesized MT. However, this hypothesis cannot completely explain the differences in inducibility of MT in various tissues by different metals and needs further investigation.

Recent studies (30) from our laboratory have shown the presence of high concentrations of hepatic MT and Zn in fetal and newborn rat livers and their dynamic state during postnatal development. These results strongly support the role of MT as a temporary reservoir for Zn or as an effective storage protein for Zn during development and growth in mammalian neonates. Thus the biological role of MT in Zn metabolism is somewhat analogous to that of ferritin in iron metabolism. The role of MT during growth and development may be unique because of the universal requirement for zinc in essential processes such as DNA, RNA and protein synthesis.

Table 5. Tissue distribution of zinc, cadmium and metallothionein in control and zinc-deficient rats.*

| Tissue    | Zn, μmole/g tissue | Cd, μmole/g tissue | MT, μmole/g tissue |
|-----------|--------------------|--------------------|--------------------|
|           | Control            | Zn-D               | Control            | Zn-D               |
| Liver     | 1147 ± 118         | 887 ± 109*         | 527 ± 80           | 589 ± 54           | 191 ± 21         | 179 ± 18         |
| Kidney    | 504 ± 18           | 398 ± 23**         | 205 ± 12           | 205 ± 9            | 69 ± 3           | 55 ± 3*          |
| Pancreas  | 504 ± 6            | 244 ± 14**         | 62 ± 5             | 56 ± 13            | 28 ± 4           | 4 ± 1**          |
| Plasma    | 15.3 ± 4.4         | 5.4 ± 0.2*         |                    |                    |                 |                 |

*Modified and adapted from a previous publication (10).

*p < 0.05.

**p < 0.01.

REFERENCES
1. Shaikh, Z. A., and Lucis, O. J. Induction of cadmium binding protein (abstract). Fed. Proc. 29: 298 (1970).
2. Piscator, M. On cadmium in normal human kidneys together with a report on the isolation of metallothionein from livers of cadmium-exposed rabbits. Nor. Hyd. Tidskr. 45: 76–82 (1964).
3. Piotrowski, J. K., Trojanowska, B., Wianiewska-Knyp, J. M., and Bolanowska, W. Mercury binding in the kidney and liver of rats repeatedly exposed to mercury chloride: induction of metallothionein by mercury and cadmium. Toxicol. Appl. Pharmacol. 27: 11–19 (1974).
4. Kägi, J. H. R., and Nordberg, M. Metallothionein. Birkhäuser Verlag, Basel, 1979, pp. 71–74.
5. Oh, S. H., Deagen, J. T., Whanger, P. D., and Weswig, P. H. Biological function of metallothionein. V. Its induction in rat by various stresses. Am. J. Physiol. 234: E282–285 (1978).
6. Bremner, I., Davies, N. T., and Mills, C. F. The effect of zinc deficiency and food restriction on hepatic zinc protein in the rat. Biochem. Soc. Trans. 1: 982–985 (1973).
7. Etzel, K. R., Shapiro, S. G., and Cousins, R. J. Regulation of liver metallothionein and plasma zinc by the glucocorticoid dexamethasone. Biochem. Biophys. Res. Commun. 89: 1120–1126 (1979).
8. Klaassen, C. D. Induction of metallothionein by adrenocortical steroids. Toxicology 20: 275–279 (1981).
9. Onosaka, S., and Cheria, M. G. The induced synthesis of metallothionein in various tissues of rat in response to metals. I. Effect of repeated injection of cadmium salts. Toxicology 22: 91–101 (1981).
10. Onosaka, S., and Cheria, M. G. The induced synthesis of metallothionein in various tissues of rat in response to metals. II. Influence of zinc status and specific effect on pancreatic metallothionein. Toxicology 23: 11–20 (1982).
11. Onosaka, S., Tanaka, K., Doi, M., and Okahara, K. A simplified procedure for determination of metallothionein in animal tissues. Eisei Kagaku 24: 128–133 (1978).
12. Zelazowski, A. J., and Piotrowski, J. K. A modified procedure for determination of metallothionein like protein in animal tissues. Acta Biochem. Pol. 24: 97–103 (1977).
13. Olafson, R. W., and Sim, R. G. An electrochemical approach to quantitation and characterization of metallothionein. Anal. Biochem. 100: 343–351 (1979).
14. Vander Malloie, R. J., and Garvey, J. S. Radioimmunoasay of metallothioneins. J. Biol. Chem. 254: 8416–8421 (1979).
15. Tokyama, C., Shaikh, Z. A., Ellis, K. J., and Cohn, S. H. Metallothionein excretion in urine upon cadmium-exposure: its relationship with liver and kidney cadmium. Toxicology 22: 181–191 (1981).

16. Onosaka, S., and Cherian, M. G. Comparison of metallothionein determination by polarographic and cadmium-saturation methods. Toxicol. Appl. Pharmacol. 63: 270–274 (1982).

17. Hager, L. J., and Palmiter, R. D. Transcriptional regulation of mouse liver metallothionein—I gene by glucocorticoids. Nature 291: 340–342 (1981).

18. Ohl, S., Cardenosa, G., Pine, R., and Huang, P. C. Cadmium induced accumulation of metallothionein m-RNA from rat liver. J. Biol. Chem. 256: 2180–2184 (1981).

19. Webb, M. Binding of cadmium ions by rat liver and kidney. Biochem. Pharmacol. 21: 2751–2765 (1972).

20. Sabbioni, E., and Marafante, E. Heavy metals in rat liver cadmium binding protein. Environ. Physiol. Biochem. 5: 132–141 (1975).

21. Sabbioni, E., and Marafante, E. Accumulation of cadmium in rat liver cadmium binding protein following single and repeated cadmium administration. Environ. Physiol. Biochem. 5: 465–473 (1975).

22. Zelazowski, A. J., and Piotrowski, J. K. The levels of metallothionein like proteins in animal tissues. Experientia 33: 1624–1625 (1977).

23. Eaton, D. L., Stacey, N. H., Wong, K. L., and Klaassen, C. D. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450. Toxicol. Appl. Pharmacol. 55: 393–402 (1980).

24. Onosaka, S., Yoshiya, S., Min, K., Fukuhara, T., and Tanaka, K. The induced synthesis of metallothionein in various tissues of rat after injection of various metals. Eisei Kagaku, in press.

25. Bremner, I., and Marshall, R. B. Hepatic copper and zinc binding proteins in ruminants. 2. Relationship between Cu and Zn concentrations and the occurrence of a metallothionein-like fraction. Brit. J. Nutr. 32: 293–300 (1974).

26. Piotrowski, J. K., Trojanowska, B., and Sapota, A. Binding of cadmium and mercury by metallothionein in the kidneys and liver of rats following repeated administration. Arch. Toxicol. 32: 351–360 (1974).

27. Nordberg, G. F., Nordberg, M., Piscator, M., and Vesterberg, O. Separation of two forms of rabbit metallothionein by isoelectric focusing. Biochem. J. 126: 491–498 (1972).

28. Bremner, I., and Young, B. W. Isolation of copper, zincthioneins from livers of copper injected rats. Biochem. J. 157: 517–520 (1976).

29. Briggs, R. W., and Armitage, I. M. Evidence for site selective metal binding in calf liver metallothionein. J. Biol. Chem. 257: 1259–1262 (1982).

30. Panemangalore, M., Banerjee, D., Onosaka, S., and Cherian, M. G. Changes in the intracellular accumulation and distribution of metallothionein in rat liver and kidney during postnatal development. Dev. Biol. 97: 95–102 (1983).