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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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Table 1. Cd-hem method for metallothionein estimation.

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

this method, the tissue samples are first saturated by addition of $CdCl_2$ *in vitro*. The excess Cd^{2+} 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 Cd^{2+}, the actual concentration of MT in various tissues is calculated after estimation of Cd^{2+} 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 Cd^{2+}-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 Zn^{2+} or Cd^{2+} 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 Cd^{2+} salts and also to high amounts of Zn^{2+} 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 Cd^{2+} (*9*) or Zn^{2+} salts (*10*). These studies show that, although the highest concentration of MT was present in the testes of control male rats, injection of both $CdCl_2$ and $ZnSO_4$ 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 $CdCl_2$ 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 $CdCl_2$. A different pattern of increase in tissue concentration of MT was observed after injection of $ZnSO_4$ (Table 3). When rats were injected with various doses of $ZnSO_4$, MT levels were increased only in pancreas, liver, kidney and small intestine. Unlike in $CdCl_2$-injected rats, the highest concentration of MT was found in the pancreas after $ZnSO_4$ injection at all dose levels studied. These results suggest that there are differences in organ-specific synthesis of MT after injection of $CdCl_2$ and $ZnSO_4$. This also provides additional evidence that MT is synthesized in response to injection of $ZnSO_4$ itself and not due to any trace contamination of Cd^{2+} in the $ZnSO_4$.

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

Table 2. Metallothionein levels after injection of cadmium chloride.[a]

Tissue	Metallothionein, μmole/g tissue[b]				Regression equation[c]
	Control	Group I (6.4 mg/kg)	Group II (12 mg/kg)	Group III (24 mg/kg)	
Brain	4.8 ± 1.8	6.1 ± 0.3	5.5 ± 0.3	6.7 ± 1.5	
Lung	2.8 ± 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	MT = 0.86 Cd + 2.0
Liver	3.0 ± 1.2	238 ± 6.0	326 ± 1.8	699 ± 93.0	MT = 28.3 Cd + 17.1
Kidney	9.1 ± 2.1	80.0 ± 4.3	129 ± 5.2	213 ± 15.2	MT = 8.35 Cd + 19.2
Stomach	3.4 ± 0.9	7.1 ± 1.2	11.2 ± 0.3	15.2 ± 0.9	MT = 0.49 Cd + 4.0
Small intestine	6.0 ± 0.6	8.8 ± 2.4	9.7 ± 2.4	16.7 ± 2.1	MT = 0.44 Cd + 5.6
Pancreas	5.8 ± 0.9	48.1 ± 9.7	78.8 ± 10.4	101 ± 7.4	MT = 3.81 Cd + 17.8
Spleen	1.2 ± 0.6	8.2 ± 0.3	10.9 ± 0.9	18.6 ± 0.9	MT = 0.70 Cd + 2.3
Testes	26.7 ± 1.2	25.5 ± 0.7	20.4 ± 3.3	8.9 ± 4.8	
Muscle	0.6 ± 0	0.7 ± 0.3	0.7 ± 0.3	1.8 ± 0	

[a]Modified and adapted from a previous publication (9), Onosaka and Cherian, 1981.
[b]Mean ± SD from three rats.
[c]MT (μmole/g tissue) = a [Cd] (mg/kg) + b.

Table 3. Metallothionein levels after injection of zinc sulfate.[a]

Tissue	Metallothionein, μmole/g tissue[b]				Regression equation[c]
	Control	Group I (40 mg/kg)	Group II (80 mg/kg)	Group III (140 mg/kg)	
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	1.9 ± 0.7	
Liver	2.5 ± 0.3	69.9 ± 14.3	110 ± 26.2	162 ± 18.2	MT = 1.11 Zn + 13.8
Kidney	7.5 ± 2.4	26.1 ± 6.6	38.0 ± 7.0	62.6 ± 14.1	MT = 0.39 Zn + 8.5
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	MT = 0.79 Zn + 0.2
Pancreas	7.0 ± 1.3	107 ± 4.6	233 ± 29.8	274 ± 101	MT = 2.66 Zn + 7.8
Spleen	1.9 ± 0.3	4.0 ± 1.2	2.8 ± 0.4	2.2 ± 0.3	
Testes	25.5 ± 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	

[a]Modified and adapted from a previous publication *(10)*.
[b]Mean ± SD from three rats.
[c]MT (μmole/g tissue) = a [Zn] (mg/kg) + b.

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 nmole 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.

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

Metal[a]	Metallothionein, μmole/g tissue/μmole metal/kg			
	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			

[a]Al, Cr, Fe, K, Mg, Na, Pb: no increase

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 $CdCl_2$ but an increase was found only in four tissues after $ZnSO_4$ injection. The highest concentration of MT was detected in the liver after $CdCl_2$ injection, whereas injection of $ZnSO_4$ resulted in the highest concentration of MT in the pancreas. There are similar differences after injection of other metals (Table 4). Thus, injection of different metals may result in quantitative variation in the tissue distribution of both metals and MT. However, the differences in accumulation of MT in tissues cannot be directly considered as either a measure of the capacity of the tissue or the ability of the metal to induce the synthesis of MT. Such interpretations may result in misleading conclusions regarding both the relative induction capacity of tissues and the relative potency of inducing agents on the synthesis of MT.

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 after injection of $ZnSO_4$ 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 $CdCl_2$ injection, they showed good agreement within tissues in $ZnSO_4$-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 $ZnSO_4$ and $CdCl_2$ 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 $CaCl_2$ 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.

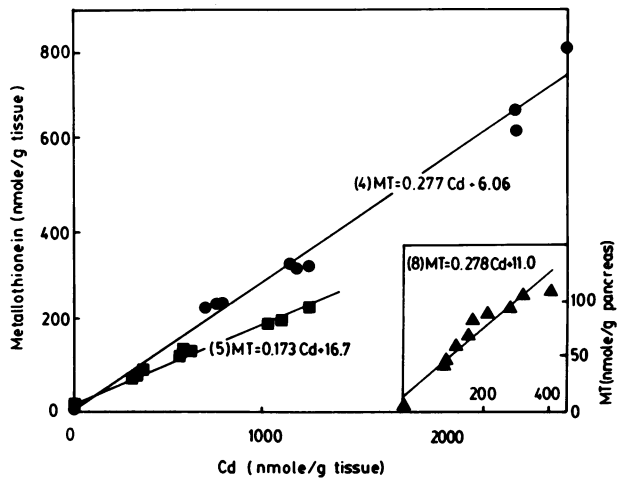

FIGURE 1. Relationship between cadmium and metallothionein concentrations in (●) liver (*4*), kidney (*5*) and (▲) pancreas (*8*) of rats injected with $CdCl_2$. Modified and adapted from a previous publication (*9*).

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 $CdCl_2$ (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 $CdCl_2$ 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

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

	Zn, μmole/g tissue		Cd, μmole/g tissue		MT, μmole/g tissue	
Tissue	Control	Zn-D	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*				

[a]Modified and adapted from a previous publication *(10)*.
*$p < 0.05$.
**$p < 0.01$.

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 (*28*). 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 the 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.

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