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Metallothioneins: Historical Development and Overview

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ABSTRACT: The history on research of metallothionein is reviewed. Various methods for isolation, characterization, and quantification are evaluated. The role of metallothionein in metal metabolism and toxicity is explained. Gender differences and polymorphism as well as possible relationships with diseases are discussed. The review is based on data from the literature and on own original experimental and epidemiological data. Aspects on future research within the metallothionein field are indicated.

KEYWORDS: disease · metabolism · metallothionein · metals · toxicity · transport

1. INTRODUCTION

Metallothioneins (MT) are sulfur-rich low molecular mass (6–7 kDa) proteins with 7 metal ions that constitute a natural part in forming the three-dimensional structure. Research on MT has been going on for more than 50 years and this chapter displays the progress made; it reviews the biochemical and experimental methods used in this research since the beginning.

Many metal binding proteins are known with a specific function of the metal ion(s), e.g., hemoglobin with Fe as a central metal ion is in charge of supplying oxygen to the cell. Metallothionein is a protein as well and importantly, it also serves functions in the cell; sometimes it is mentioned in relation to chelators because of its capacity to bind metal ions. Already in the late 1970's it has been proposed to administer to humans the amino acid sequence, i.e., the thionein part of the protein, or even the MT rich in zinc, to treat renal effects caused by Cd in the kidney; however, this is likely to be harmful because an exchange of Zn^{2+} by Cd^{2+} may take place. When cadmium-metallothionein (CdMT) is given to laboratory animals renal damage is seen already at a Cd level of 10 μg/g wet tissue because MT goes straight to the kidney which has a much lower capacity for the synthesis of MT than liver. So far no positive role of MT has been shown in the treatment of cadmium poisoning that would be similar to that seen with EDTA in lead poisoning, or BAL and DMSA in mercury poisoning.

Below we give an overview on the historical development of MT research. We concentrate mainly on mammalian metallothioneins and indicate also several of the aspects discussed in other chapters. Since the early days, both isolation and characterization as well as the role of MT in Cd toxicology [1,2] have contributed to the understanding of MT and its role as a general sequestering protein for toxic metals also reducing cellular occurrence of reactive oxygen species. Future developments in the application of molecular "omics" technologies (genomics, proteomics, and metabolomics) will undoubtedly lead to a further understanding of the role of MT in biology and help in biomonitoring of environmental exposures.

2. HISTORY OF METALLOTHIONEINS

The first publication in 1957 on a cadmium-binding protein in equine tissue [3] was initiated by a report in the form of an abstract [4] dealing with cadmium in human organs. Small amounts of cadmium had been shown to be present in tissues and body fluids in several animal species. Various hypotheses were postulated to explain this unexpected finding: Either would cadmium be coordinated to a macromolecule and then have a natural function in biological systems or else cadmium could just be a contaminant. In 1960 the first detailed report on metallothionein was published [5,6]. The cadmium-containing protein, isolated from equine renal tissue, was described and named "metallothionein" because of its extremely high sulfur content of 4.1%/g dry weight and 2.9% of Cd and 0.6% of Zn. Isolation was performed from five frozen horse kidneys with for that time conventional methods.

Later studies reported data on physical properties and the molecular weight was estimated to be 10000 ± 260. The specific absorption at 250 nm was explained by cadmium mercaptide charge transfer bonds. Metallothionein was assumed to lack aromatic amino acids as indicated by the absence of an absorption at 280 nm.This was later verified by amino acid analyses [7,8] which also showed that the high sulfur content was due to cysteine. At that time reactive mercapto groups in proteins were determined by titration with silver ions, CMB, and N-ethylmaleimide. Amino acids were identified by two-dimensional paper chromatography and ion exchange chromatography. Cysteine residues were quantified as cysteic acid after oxidation of metallothionein with performic acid and as derivates of N-ethylmaleimide. The sedimentation constant was determined via a Schlieren diagram by sedimentation in an ultracentrifuge at 1.75 s (s020w). The diffusion constant, i.e., the partial specific volume, and the friction ratio were also reported. The estimated molecular weight of the protein was still varying from 9790–10500. This was in part explained by the formation of various artefacts during preparation. Metal analyses gave 5.2 g atoms/mol or 5.9% of MT weight for Cd and 3.3 g atoms/mol or 2.2% by weight for zinc. Some exchange between zinc and cadmium was obviously taking place. It was suggested that bonding with three deprotonated SH-groups and one atom of either cadmium or zinc occurred.

As part of the research of the Swedish group on the health effects caused by cadmium, a study on rabbits [9] showed that cadmium-metallothionein could be induced by repeated injections of small doses of cadmium. A single high dose of cadmium [1] was found to be more toxic to the organism giving rise to liver damage and lethality, while the same dose administered as several small doses during a prolonged exposure time gave no such effects. In fact, animals with induced metallothionein synthesis by pretreatment with smaller doses of cadmium developed resistance to acute toxicity to the liver [1] and the testes

[10]. Isolation of the cadmium-binding protein from livers of cadmium exposed rabbits showed an increase of metallothionein in relation to the administered dose or amount of cadmium present [7]. In animals protected by pretreatment, cadmium in the target tissues, liver and testis, was bound to a low molecular weight protein corresponding to metallothionein.

Techniques newly developed in the 1960's were used for isolation of the protein. After homogenization of the tissues rivanol was applied to precipitate high molecular weight proteins and cell fragments. Several steps of precipitation, dialyses, and various gel chromatography steps were carried out, as Sephadex gel had previously been introduced into protein chemistry. The initial assumptions by Piscator in 1964 [9] were later confirmed in these animal experiments which demonstrated indeed that exposure to cadmium increased the concentration of metallothionein in the liver. These findings gave further support to the original ideas of metallothionein induction as a mechanism of making tissue less sensitive to cadmium toxicity. In this group [11–13], working with the toxicity and kinetics of cadmium, it was known that cadmium gave rise to adverse health effects upon increasing exposure, particularly to renal damage. Metallothionein research now continued or developed into two tracks – one in protein chemistry and another one focusing on kinetics and toxicity of cadmium and other metal ions.

However, all studies demanded pure and well characterized metallothionein and this was prepared with techniques modern at that time. Tissue was homogenized in a buffer system, mostly of Tris-HCl in sodium chloride with a pH of 8.1. This step was followed by ultracentrifugation at 105000gav and the supernatant was taken for gel chromatography (Sephadex gel G-75). If the absorption ratio at 250 and 280 nm was low, improvement could be achieved in one step by Sephadex G-50 used for preparative purposes. When the fractions eluted as MT on G-75 Sephadex were separated on G-50, a protein was isolated with a high absorption at 280 nm and no metal content [11]. Further separation by isoelectric focusing or ion exchange chromatography after concentrating and desalting by ultrafiltration on UM-2 filters with a cut off level for a molecular weight of 1000 [7] showed different fractions containing MT. Further separation by isoelectric focusing of rabbit liver revealed at least three major forms of MT with pI 3.9, 4.5, and 6.0. Two of these were characterized by amino acid analyses [7] and identified as form I and II of MT. To be successful with the preparation of metallothionein from tissue it became obvious quite early that avoidance of oxidation of the protein by rapid preparation and working at a cool temperature was crucial. Mercaptoethanol could, however, restore oxidized metallothionein [12,13] as shown by gel chromatograpy on Sephadex G-75 where metallothionein showed up at the ordinary position after treatment with mercaptoethanol. An important contribution to the tertiary structure was made [14] when two metal clusters were described, i.e., an α- and a

β-domain with four and three metals, respectively, as part of the structure. The α-domain constitutes the C-terminal and the β-domain the N-terminal end of the protein.

The other track of research already indicated above expanded to the importance of MT for different metal ions, in particular for copper [15] and mercury [16–18]. Pioneering work [10] showed that MT could protect against testicular damage caused by cadmium. Knowledge on MT and its involvement in the transport of cadmium and that cadmium is partly present in blood [2] bound to MT led to a metabolic model for cadmium toxicity. Further work focused on the speciation of cadmium and it was found that CdMT is taken up in the renal tubules and causes renal damage at cadmium concentrations as low as 10 μg/g renal tissue [19].

The identification of a cadmium-binding protein in mammals, which was first believed to have a high molecular mass, turned out to be a low molecular mass protein (see also Chapter 10). As part of the research on cadmium and adverse health effects in Sweden, a project on MT was developed and it was shown that MT is a most important protein in the metabolism and kinetics of cadmium in animals and humans. Methods for isolation and characterization of MT were developed. To study the history of MT also means to consider available analytical techniques and methods. In the late 1960's and early 1970's only three full length articles were available, two in English and one in Swedish. The combination of available knowledge about protein separation and radioactive techniques made it possible to isolate, characterize, and study the role of MT [1].

In the 1970's and early 1980's only a limited number of groups performed research related to metallothionein. However, an increasing number of publications in which a different nomenclature for MT was used made it clear that an evaluation of the knowledge available at that time would be of importance. Hence, a workshop with approximately 25 invited participants, who had submitted background manuscripts, was arranged and a tentative report [20] was prepared and distributed in advance to each participant. A consensus report was agreed during the meeting held in Zürich in 1978 [21].

Agreement on the nomenclature of MT in the mentioned first workshop [20,21] stimulated interest in MT research. For a long time after the first workshop Roman numbers, like MT-I and MT-II, were used to identify different MTs. This is no longer consistent with the nowadays accepted terminology, developed by Kägi and coworkers [21], which uses Arabic numbers. The official designation in the SwissProt (proteins) data base and the MCBI data base (Medical Center for Biotechnology Information), which deals with the genome and also gives proteins, uses Arabic numbers, e.g., MT-1 and MT-2. However, in all these data bases the older designations like MT-I or MT-II as well as names given during the discovery of a protein are given as synonyms (SwissProt) or aliases in MCBI. Perhaps more importantly, the

Human Genome Organization (data base) also approved the symbols MT-1, MT-1A, MT-2 and the like. Further issues on nomenclature can be found on the website: http://www.expasy.org/cgi-bin/lists?metallo.txt.

During the mentioned first international meeting on metallothioneins held in Zürich consensus was reached not only about the nomenclature, but also about other issues, like methods for preparing the proteins. This first meeting was followed by another one in Zürich in 1985, which was more open but still of a workshop type. In-between a meeting had been arranged in Aberdeen in 1981. Other meetings have focused on various areas of interests, e.g., cadmium-binding proteins in non-mammalian species was brought to attention in an international meeting in 1984 [22] and pharmaceutical interests led to the third international meeting held in Japan in 1992 [23]. A variety of meetings with different themes and approaches followed (Table 1) [24–27].

Table 1. History of metallothionein and important workshops.

1941, 1957	Metallothionein, discovery	[3,4]
1960, 1961	Metallothionein, details about	[5,6]
1964	MT induction by cadmium	[9]
1971	Modification of Cd toxicity	[1]
1972	Amino acid composition	[7]
1976	Sequence	[24]
1978	1st International Metallothionein Meeting, Zürich, Switzerland; consensus on nomenclature	[21]
1979	Radioimmunoassay	[25]
1981	2nd International Metallothionein Meeting, Aberdeen, Scotland	
1983	1st International Meeting on Metallothionein and Cadmium Nephrotoxicity, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA	[26]
1984	High affinity metal-binding proteins in non-mammalian species	[22]
1985	International Workshop on metallothionein, Zürich, Switzerland	[27]
1992	3rd International Metallothionein Meeting with pharmaceutical implications, Tsukuba, Japan	[23]
1996	1st International Workshop on Metallothionein JRC/IRMM EU, Geel, Belgium	
1997	4th International Metallothionein Meeting, Kansas City, USA	
1999	2nd International Workshop on Metallothioneins (Euroconference) JRC/IRMM EU, Geel, Belgium	
2005	5th International Metallothionein Conference (MT-2005) (Metals and Metallothionein in Biology and Medicine), Beijing, China	

Research on metallothionein has now been going on for more than 50 years. Initially it followed two tracks, i.e., strict protein chemistry and toxicokinetics of the metals that constituted structural parts of the protein and those that turned on the synthesis. Among the metal ions especially cadmium and zinc were in the focus, but to some extent also copper. Of course, matters developed further and in 1986 a high intake of Cd-containing seafood and shellfish by human consumers in New Zealand was reported [28] and it was also shown that the chemical species containing Cd was different in two species of oysters. Cd-binding proteins identified in foodstuff have been reviewed by Petering and Fowler [29]. The chemical species containing Cd, particularly its binding to metallothionein-like proteins, is of importance for the uptake, distribution, and toxicity of Cd. These insights are of relevance with regard to the outcome of human exposure to metals in the form of MT.

The number of publications per year has increased over time and a recent search on Medline for the years 1950 to late 2007 gives nearly 7000 publications and the corresponding search in Pubmed provides almost 8200 hits. New techniques developed from molecular biology have confirmed earlier findings, opened new aspects, and made this rapid progress possible.

3. PROTEIN CHEMISTRY AND METAL BINDING

Metallothionein is a low molecular mass protein, characteristically with 6 to 7 KDa. Major hallmarks of MT are the amino acids that vary between 61–68. The typical MT consists of 20 cysteines (30%), methionine (N-terminal), alanine (C-terminal), no aromatics, no histidine and it has a unique amino acid sequence with a tertiary structure forming two domains of metal clusters, i.e., the α- and β- clusters. The metal content of Zn, Cd, Hg, and Cu can vary and may constitute 11% of its weight, the metal ions being bound by several sulfhydryl groups [30,31]. Specific absorption occurs at 225 (Zn), 250 (Cd), 300 (Hg), and 275 nm (Cu). Synthesis of MT-1 and -2 is induced by Cd^{2+} and Zn^{2+}. There are no disulfide bonds and MT is regarded as heat-stable. It is mainly localized in the cytoplasm. Metallothioneins exist in four major forms, MT-1 to MT-4. MT-3, present in brain and renal tissue (see Chapters 10 and 11), is not inducible by Cd as are MT-1 and 2. MT-1 also occurs in several isoforms and MT-4 is expressed in keratinocytes. In humans the gene is localized on chromosome 16 and in the mouse on chromosome 8 (see also Chapter 2). Metallothionein has been isolated from the liver and several other tissues of animals. Its synthesis is induced by Cd^{2+}, Zn^{2+}, and other metal ions or stress [20,32,33] (see Chapter 12).

The structure of MT-1 and -2 has two domains consisting of one cluster with 3 and one with 4 metal atoms and was first described by Winge and Miklossy [14]. The metallothionein gene is located on chromosomes varying

with species. For humans and other primates it is on chromosome 16 [34]. The protein consists of a number of isoforms coded by various alleles. The ratio of mRNA for MT-1 and MT-2 genes remains constant during induction by metals, e.g., Cd, Cu, and Zn. It was found that 1.4 times more MT-1 RNA than MT-2 RNA exists, indicating that the transcription rate is slightly higher for the MT-1 gene compared to the MT-2 gene [34]. MT-1A and MT-2A genes seem to be differentially regulated by metals. Lack of MT gene expression makes the organism sensitive to toxic effects. The MT gene becomes transcriptionally inactive as a consequence of DNA methylation.

Cells with extra copies of MT genes can be selected by exposure to a toxic concentration of cadmium. In metal-exposed mammals zinc is the dominating metal ion in MT and at least one zinc seems always to be present in MT (see Chapter 10). Various biological factors influence metal ion composition such as tissue of origin, age, and stage of development, This means that renal MT is higher in Cd and Cu in exposed animals than liver MT from the same organism. In an evaluation of several gels Sephadex G-75 and G-50 have been proven to be still the most efficient technique for purification of MT. Sometimes it is necessary to add mercaptoethanol to the samples in order to reduce MT back to non-polymerized MT [12,13].

Transgenic mice [35] have been introduced in order to gain new knowledge on MT and metal toxicity. Experimental laboratory animals (mainly mice) have shown that species differences with regard to sensitivity and resistance to metal toxicity exist [36]. By introduction of transgenic animals it is expected that the mechanism behind these differences will be elucidated.

4. METHODS FOR QUANTIFICATION OF METALLOTHIONEIN

A number of methods for quantification of MTs was reviewed in 2002 [37]. Several methods for measuring and quantifying metallothioneins are available [36,38] as displayed in Table 2 [39,40]. A review of various methods has also been published [41]. In addition to the information given in Table 2 it should be mentioned that metal-binding assays involving the binding of metals such as cadmium [39,42], mercury [17], and silver, together with pulse polarography [43] and immunoassays such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), based on the use of antibodies [40] are employed. Each of these methods has advantages and disadvantages. The radioimmunoassay needs specific antibodies that do not cross-react and this also applies to the immunoassay ELISA.

Most cadmium in urine of humans occurs bound to metallothionein. Urinary cadmium and metallothionein concentrations correlate well as shown in elderly women exposed to cadmium in the general environment

Table 2. Methods for the quantification of MT and MT mRNA.

After isolation:	
Freeze-drying	[7,30]
Calculation of amino acid analyses	[7,8]
Biuret method	[7]
In crude tissue fractions:	
In vitro binding of Hg	[17]
In vitro binding of Cd	[39]
Radioimmunoassay	[27]
ELISA	[40]

[44,45] and in occupationally exposed male cadmium workers [46,47], for whom values were reported of 2–155 ng MT/g urine and for plasma 2–11 ng MT/g. The level of MT by RIA in healthy humans is reported to be 1–16 μg/L in plasma or serum and 5–400 μg/g creatinine in urine [44,45]. In cadmium-exposed persons metallothionein in urine is a good indicator of urine concentration of cadmium and can also be assumed to be an index of the burden of cadmium.

Immunohistochemical staining of metallothionein in placental tissue indicated that metallothionein reflects the concentration of copper in this tissue. The method can be used for pre- and postnatal diagnosis of Menkes disease [48].

A gender difference is observed: Women have a higher metallothionein concentration in urine compared to men even at similar cadmium levels [49] as shown with RIA [49,50]. As presented in Table 3 [40,51–55,57] the range of normal concentrations of MT is defined by concentrations found among humans without proteinuria.

The normal concentrations of metallothionein in rat tissues as determined by ELISA are 18 μg/g in liver [53], 30 μg/g in kidney [53], and 35 μg/g in kidney cortex [56]. The detection limit for ELISA has been reported to be 100 pg MT [53].

The determination of metallothionein concentration in urine and blood has been found to be related with problems not observed in tissue analyses. This is likely due to the techniques used for sampling and storage of samples, procedures which are most crucial for the results of analyses. Usually urine samples are treated with bactericides in order to prevent bacterial contamination with a reduced pH as result. At low pH metallothionein loses the metals and free thionein is obtained; a change in configuration and instability of the protein follows. This is likely to influence the results. In experimental studies [57] excretion of MT, cadmium, calcium, and various

Table 3. Levels of metallothionein in humans.[a]

Method	Media	MT Concentration	Status	Reference
RIA	Sera	0.01–1 ng/g	Normal[b]	[46]
RIA	Sera (human)	> 2 ng/g	Abnormal[b]	[46]
RIA	Urine (human)	1–10 ng/g	Normal[b]	[46]
RIA	Urine (human)	> 10 ng/g	Abnormal[b]	[46]
RIA	Urine (human)	1880 μg/g CR	Itai-itai patients	[44,45]
		880 μg/g CR	Cd-polluted area	
		394 μg/g CR	Non-polluted areas	
ELISA	Urine (human)	120 and 210 μg/g CR	Normal[b]	[55]
		320 and 1050 μg/g CR	Abnormal[b]	

[a] Collected from data published in [51,52].
[b] Occupational exposure, abnormal denotes presence of low molecular weight proteinuria.

enzyme markers in urine were followed in male rats exposed to cadmium. MT was determined by ELISA. Interference with luminal and basolateral membranes and handling of calcium was demonstrated [58,59]. This is in accordance with the suggested model that Cd is released from CdMT after it is catabolized in lysosomes and appears in the cytoplasm of renal tubule cells, where it may change the electrochemical gradient across the luminal membrane giving rise to decreased calcium absorption.

Thus, it is necessary to evaluate and standardize the methods for urine and blood. As the body fluids are used for biological monitoring of many metals, it would be most valuable to measure metallothionein as well and to relate the concentration of metals to the concentration of metallothionein.

One problem with the estimation of metallothionein in tissues and body fluids is how to manage to express the true concentration and relate it to biological events. For a long time this has been done in the form of μg MT/g wet weight tissue. Since the concentration of MT varies with many factors this has to be further expressed in relation to something that is stable in the cell. The cellular concentration of MT is age-dependent and also dependent on exposure to numerous agents [29]. Several methods and pitfalls with various methods for MT determination have been summarized by contributions from many scientists in MT research [41]. It is urgent to develop a method for MT quantification with high precision, accuracy, and specificity. To test the specificity, a known amount of MT may be added to the samples. In spiking the samples, several questions are raised: How should the various forms of MT be quantitated? They might reflect various biological functions that are related to both age and exposure as suggested previously [12,13]. Isoelectric focusing [60] is a rapid, quick, and good method for the preparation

Table 4. Media and methods for the detection of MT and MT mRNA upon exposure to Cd.

Metallothionein and MT mRNA as Biomarker in Environmental and Occupational Cadmium Exposure	
Peripheral lymphocytes	MT mRNA RT-PCR
Plasma or Serum	MT by ELISA or RIA
Urine	MT by ELISA or RIA

of the various isoforms of metallothionein. Commercially available metallothionein has to be checked with regard to purity even if a well recognized method has been used for preparation. It has been noticed that the metal concentration in some shippings from commercial purchases has been extremely low, indicating a low purity of the protein.

Commercial antibodies are available for MT-1, -2 and -3. Monoclonal antibodies are believed not to be specific enough to be taken for ELISA assays.

Methods from molecular biology offer possibilities to quantitate MT in eukaryotes and prokaryotes. MT gene transcription and RT-PCR to measure MT mRNA can be used. It may be mentioned that it is also possible to use a DNA probe for RNA synthesis and translated DNA [37]. MT-2 expression in lymphocytes can be performed by PCR techniques [37].

A question to be solved is how the determination and estimation of MT in body fluids and tissues should be performed and how to relate the concentration of MT to effects? A RT-PCR method for MT mRNA primers and oligo probes are commercially available. Basal and *in vitro* induced MT mRNA is significantly higher in Cd-exposed groups than in controls. Quantification of MT by ELISA demands a good antibody. Detection of expression of MT by Western blot demands a specific antibody. Detection of mRNA expression by RT-PCR demands specific primers (see Table 4).

5. ROLE OF METALLOTHIONEIN IN METAL METABOLISM AND TOXICOLOGY

Metallothionein in the physiological system has not only one but several roles, especially in the metabolism and kinetics of metals (see also Chapter 10). These are

– transport of metal ions
– detoxification of metal ions
– protection from metal toxicity

– free radical scavenging
– storage of metal ions
– metabolism of essential metal ions
– immune response
– genotoxicity and carcinogenicity

The mechanisms by which MT protects cells from toxicity include binding of metals to proteins and localization in the cell where MT is mainly present in the cytoplasm, but also in the nucleus and in the lysosomes of the kidney. MT stands for detoxification! The protection against adverse effects caused by cadmium exposure follows from the ratio of non-MT-bound to MT-bound cadmium [1]. Such binding may also occur with other metals, e.g., Hg [16], and is thus a mechanism for detoxification.

Another function of metallothionein, in addition to its involvement to transport metals, is as a free radical scavenger and it also stores metals like Zn, Cd, Cu, and Hg. In the immune response MT acts as a Zn donator. Most likely MT is also involved in genotoxicity and carcinogenicity processes (see Chapter 13).

After absorption from the lungs or the gut, cadmium is transported via blood to other parts of the body. In blood cadmium is mainly found in the blood cells [36], where a high molecular weight and a low molecular weight fraction occurs [2]. Further studies [12,13,61] have shown that the latter fraction is similar to metallothionein, which also binds cadmium in plasma [2] and which has an important role in the transport of cadmium in the body of animals and humans [62,63]. The low molecular weight of metallothionein enables this protein to be filtered through the kidney glomerular membrane. Like other proteins in the primary urine, metallothionein is reabsorbed into proximal tubular cells. The transport of cadmium bound to metallothionein from blood to renal tubular cells is rapid and almost complete [19,64]. Cadmium not bound to metallothionein does not enter the kidneys to the same extent. A similar difference was seen in animals fed cadmium-metallothionein and cadmium chloride [65]. The former gave rise to a much higher accumulation of cadmium in the renal cortex than the latter, most probably because Cd^{2+} from chloride binds to albumin in blood plasma [12,13].

Cadmium exposure induces the synthesis of metallothionein in a number of tissues [31]. During the first 12 hours after a high acute exposure to cadmium (not bound to metallothionein), there will be an increase over time of cadmium bound to metallothionein due to the increased production of the protein [1,66]. As the transport of cadmium to the kidney is dependent on its metallothionein binding in plasma, the distribution of cadmium within the body found after an acute exposure will be different from that found after repeated exposures. Figure 1 summarizes the transport of Cd in blood and its uptake in kidney tubules.

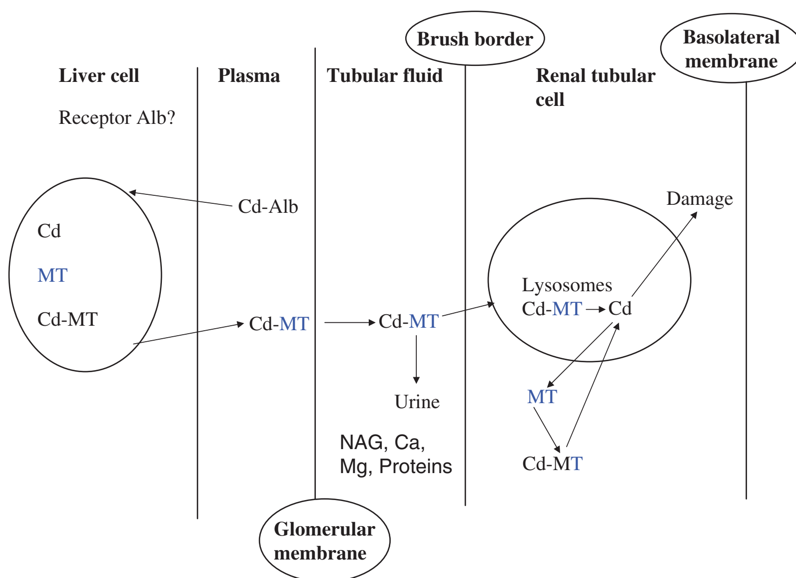

Figure 1. Transport of Cd in blood and uptake in kidney tubules.

Metallothionein-bound cadmium in plasma is filtered through the renal glomeruli and reabsorbed in the tubuli, where cadmium is released. If CdMT is injected in animals the released cadmium causes damage to the kidney tubule because there is insufficient tissue MT available to protect the kidney [67]. Upon a long-term exposure, the unbound cadmium stimulates new metallothionein production which binds cadmium and protects the renal tubular cells. When this process is insufficient, toxic effects occur, possibly because of cadmium interference with zinc-dependent enzymes and membrane functions.

The scheme in Figure 1 was suggested in early studies by Nordberg et al. in 1971 [1,2] and continued to be used for cadmium [68,69] as well as later on for copper as summarized by Bremner in 1987 [70].

A long time after a single exposure, or in long-term exposure, a considerable proportion of plasma Cd is bound to metallothionein [12,13]. Uptake of CdMT may become more efficient in cells pre-exposed to Cd compared to non pre-Cd-exposed cells [71]. In long-term exposure there is a slow release of CdMT from the liver to the blood. This transport phenomenon has gained support from studies where Cd-containing livers were transplanted to non-Cd-exposed animals, which showed a gradual uptake of Cd in the kidney [53] and from studies demonstrating a lower Cd accumulation in kidneys of MT-null mice [72]. Inorganic cadmium compounds are

known to cause toxicity to the kidney after long-term exposure. In animal experiments it has been shown that Cd administered as MT may cause similar renal damage at a tissue concentration of Cd at 10 μg/g wet weight [19] compared to 70–200 μg/g wet weight when administered as Cd ions.

In addition to the early findings regarding the modifications of cadmium toxicity by metallothionein induction [1,9], data have also been provided concerning the binding of Cd to metallothionein in blood [12,13,19,27]. The identification of bound forms of Cd in blood plasma and studies by autoradiography showing that Cd is distributed selectively to the kidney after administration of CdMT, while it is predominantly taken up by the liver after injection as Cd^{2+} or as Cd-albumin, provided a background for the mechanistic model of cadmium kinetics [19,68,69]. Immediately after uptake in blood, cadmium is bound to albumin in blood plasma, distributed and taken up in the liver (Figure 1). It is speculated that an albumin receptor is present on the surface of liver cells. Once in the liver, cadmium binds to already present metallothionein by exchanging zinc. Then, cadmium induces the synthesis of metallothionein and the newly synthesized MT is sequestering cadmium from other binding sites, thus protecting liver cells from toxicity. Cadmium-metallothionein is released to the blood stream and transported to the kidney where it is filtered through the glomerulus and taken up by adsorptive endocytosis [73]. Metallothionein is catabolized in the lysosomes of the tubules [74] and the free cadmium ions induce then the new synthesis of metallothionein in the cell and, of course, cadmium may also react with other sensitive sites.

Cadmium has a biological half-time in humans of 10–15 years which is regarded as very long. This observation may be explained by the property of Cd^{2+} to induce synthesis of metallothionein. The described model has been further developed [75,76]: CdMT-induced kidney damage was shown to decrease uptake and binding of calcium in membrane vesicles isolated from animals injected with CdMT [75]. Rats given a combined exposure by injection of CdMT (0.25 mg/kg), Zn (12.5 mg/kg), and Cu (6.25 mg/kg) had considerably increased levels of MT in the renal cortex, i.e., up to 746 μg/g wet weight. These high concentrations of MT were considered to be of major importance explaining the protection against renal damage from Cd in these animals [56].

Repeated injections of CdMT given with a short time interval gave rise to a considerably prolonged and possibly irreversible calciuria in rats [76]. Increased excretion of magnesium has been found in rats with CdMT-induced nephrotoxicity [77]. A possible contribution of endogenous intestinal metallothionein to renal accumulation of cadmium was studied in rats fed with cadmium [78]. To distinguish between exogenous and endogenous metallothionein isoforms from rat and pig, differences in chromatographic behavior were used [79]. Cadmium may also possibly be bound in small

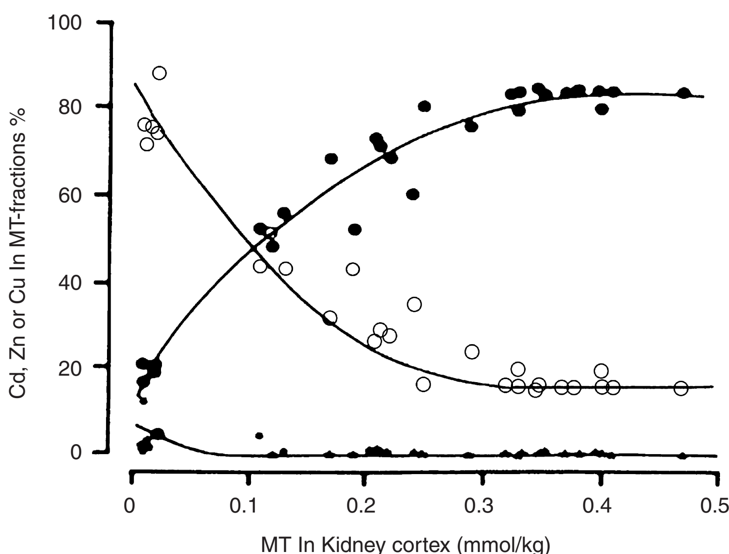

Figure 2. Relative concentrations (%) of cadmium, zinc, and copper in MT fractions in relation to the total MT concentration. MT isolated from kidney cortex of rabbits with varying exposure to Cd. (●) Cd; (○) Zn; (∗) Cu. Reproduced from [81] by permission of Elsevier; copyright (1987).

amounts to low molecular weight SH-rich compounds such as glutathione and cysteine [80] (see also Chapter 14) although evidence for such binding in plasma of mammals exposed to cadmium salts is limited and the main transporting protein for cadmium to the kidney most probably is metallothionein [63]. As mentioned above, a similar pathway was shown for copper [70].

In normal human beings, the increase in cadmium in the renal cortex with age is accompanied by an equimolar increase in zinc. This is thought to be due to the metallothionein stored in the kidney, which contains equimolar amounts of the two metals. In Figure 2 it is seen that the intersection of Cd and Zn in MT in kidney cortex gives a MT concentration in kidney which is equal to the critical concentration of Cd [81].

Another important function for metallothionein is the cellular defense mechanism against free radicals where methionine might serve as free radical scavenger as discussed in many publications [68,70]. Furthermore, metallothionein may protect DNA by sequestering copper and preventing its participation in redox reactions and thus inhibit the formation of free radicals as pointed out by Cai, Koropatnick, and Cherian [82].

Metallothionein regulates the toxicity of various metals and trace elements, and as we have seen, copper and zinc are examples of this. With regard to high-tech metals, e.g., gallium, germanium, indium, antimony, tellurium, yttrium, niobium, thallium, and bismuth and some more recently introduced high-tech compounds used in superconductors such as yttrium-barium-copper-oxide (YBCO) and $Bi_2Sr_2Ca_2Cu_3O_{10}$ (BSCCO), it may well be, if taken up, that their content of Bi and Cu may induce MT and that BiMT and CuMT are formed. It cannot be excluded that among exposed people MT levels might increase and an evaluation in relation to MT is warranted in the electronics industry when handling some of the semiconductor and superconductor materials [83].

Another aspect of toxicity is exposure via food. MT and MT-like proteins have been described to occur in various foodstuff. Questions related to such problems have received some attention [50,84].

6. METALLOTHIONEIN AND DNA, GENETIC POLYMORPHISM, GENDER PERSPECTIVES

Does genetic polymorphism, i.e., several genes for MT on the same chromosome, code for specific MT functions? Literature data on metallothionein, genetic polymorphism, and gender perspectives are scarce. Metallothionein is a ligand for zinc, cadmium, and various other metals. In humans fourteen different genes are located in a gene cluster of about 82 kb [85] on chromosome 16. Six of these have been identified to be functional and two are not [86]. The genes have been identified on the basis of nucleotide sequencing. As several genes coding for metallothionein are present on the same chromosome, this might indicate that the various codes are for a specific purpose, i.e., a specific biological function as was suggested already during the first international meeting on metallothionein [21] (see also Chapter 2).

An age-dependent change of metal composition in metallothionein also indicates specific functions. In the fetus no cadmium is found, but the concentration of metallothionein is high. During gestation and in the newborn a high concentration of intracellular metallothionein rich in copper and zinc is mainly present in the liver [50,87]. This copper probably has an important function in providing Cu during the first period of life when the tissue copper concentration declines to the concentration characteristic of adult life if no metabolic disorders are present. A similar situation occurs for zinc-metallothionein. Immunohistochemical localization of MT shows MT in the nucleus of hepatocytes in neonates for several days and later on MT is present as a cytoplasmic protein during postnatal development as studied in rats [88].

That DNA synthesis and cell growth is stimulated by very low concentrations of cadmium [89] was shown in cultured mammalian cells. Human brain tissue has been found to be rich in MT-3 [90]. A growth inhibitory factor (GIF) from this tissue was identified to be a metallothionein. MT-3 shows tissue specificity and a structure that differs by having six glutamic acids inserted near the C-terminal and one additional insert in the N-terminal. Expression of MT-3 is not regulated by metals. MT-3 is downregulated in Alzheimer's disease. So far this is the only metallothionein that has a function in relation to growth. The GIF/MT-3 gene like other MT genes is located on chromosome 16 in humans.

The occurrence of metallothionein in growing tissue in tumors was reported by Cherian [91] (see also Chapter 13). Metallothionein concentrations measured by the Cd saturation method displays a clear age dependence. A decline of metallothionein in kidneys of humans after age 60 is in accordance with findings for cadmium kinetics. It has been postulated [33] that the capacity of renal tissue to produce metallothionein is age-dependent and that protein synthesis is possibly less efficient at older biological age. Epidemiological and experimental studies in laboratory animals show that females are more vulnerable to cadmium toxicity than men [49,53] (see also Chapter 10).

Women have a higher cadmium concentration in blood [92] compared to men even if differences for cadmium levels in blood is overwhelmed by smoking habits [92] and they also have a higher concentration in the liver. The iron status is of importance for cadmium and metallothionein concentration: A low iron status increases the absorption of cadmium [36]. Iron deficiency also increases the concentration of MT-1 in bone marrow in rats exposed to cadmium revealing an effect on the bone marrow, as was also suggested by Piscator [9] for rabbits with hemolytic anemia. The concentration of MT in liver is unchanged but the renal concentration is reduced in animals with iron deficiency [93]. However, that the cadmium concentration seems to be higher in aged women compared to men is contradictory to the observation that men have higher MT levels than women. A gender perspective is present in MT.

The involvement in signal transduction has not yet been described in the literature. MT-3 or -4 might, however, be involved in this. MT-4 is expressed in stratified squamous epithelia differentiating cells. The metallothionein part from the transport of metals such as cadmium and copper in the cell also functions as a free radical scavenger.

Regulation of MT gene expression in mammalian species involves the metal-responsive transcription factor (MTF1), a nuclear receptor [94]. MT gene expression was studied in humans either exposed to cadmium in the working environment or in the general environment. In these studies MT mRNA levels were measured using RT-PCR [95–98]. As will be described in more detail in

Section 7, the results indicate that metallothionein gene expression in peripheral blood lymphocytes (PBLs) may be used as a biomarker for cadmium exposure and the related susceptibility to renal dysfunction.

7. METALLOTHIONEINS AND DISEASE

7.1. General Aspects and Disease Etiology

The role of MT in the etiology of kidney disease has been described in Section 5. Binding of Cd to MT implies efficient transport to the kidney where, however, de novo synthesized MT counteracts the toxic effect of Cd and a high tissue level of Cd can be tolerated, particularly in persons that are able to synthesize MT efficiently. Those who have a poor ability to synthesize MT will suffer kidney damage at a lower tissue concentration of Cd.

There are diseases that are linked to metabolic disorders of handling metals genetically transferred from generation to generation. Examples of such diseases are Menkes' and Wilson's disease (Table 5). Menkes disease, an inherited X-linked recessive disturbed copper metabolism with mutation in ATP7A results in an accumulated copper concentration in many tissues; however, in spite of the accumulation of CuMT, the lack of ATP7A makes copper unavailable for many copper-dependent enzymes. Another inherited disorder with regard to copper accumulation is Wilson's disease with a

Table 5. Metallothionein and disease.[a,b]

Agent/ Metal	Organ	Illness
Cadmium	Kidney	Proteinuria, calciuria
		Itai-itai disease
	Kidney, pancreatic islets	Diabetes type-2/increased kidney disease
Copper	Liver	Wilson's disease
		Indian liver cirrhosis
	Intestine	MT-induction in treatment of Wilson's disease
	Placenta	Menkes disease
GIF[c]	CNS	Neurodegenerative diseases
	Brain	Alzheimer's disease

[a] Modified and compiled from [100].
[b] Cancer of several organs: MT as marker of malignancy and tumor sensitivity to chemotherapy (see also Chapter 13).
[c] Growth inhibitor factor, MT-3.

mutation in ATP7B. Patients suffering from Wilson's disease have a failure in excretion of excess copper in bile from liver leading to excessive copper accumulation in the liver, CNS, and other organs and toxicities result, e.g., in neurological disorders (see Chapter 11). The excessive Cu is stored in tissues partly bound to MT [99,100].

Metabolic disorders related to copper have been studied in animal models with similar genetic deficiencies as in human Wilson's disease. It is proposed that non-MT bound copper is transferred to ceruloplasmin. The toxicity from tissue accumulation of copper is explained by participation of MT and active oxygen species that are produced upon reactions involving copper [101].

Livers of patients with diabetes mellitus have a high concentration of metallothionein. In humans approximately 600 μg MT/g and 60 μg Zn/g have been reported in the liver. The susceptibility of spontaneously diabetic mice to CdMT nephrotoxicity was studied [102]: In these mice CdMT injections gave rise to renal damage at considerable lower doses than in normal mice [102]. Iron status in subjects exposed to various metals like Cd is linked to metal toxicity with an increased uptake of the metal and thus an increased concentration of CdMT will be seen. Iron deficiency increases MT-1 in bone marrow, with unchanged MT in liver, and decreased MT in kidney which indicates a mechanism that needs further explanation [93].

Ushida et al. [90] found that the concentration of growth inhibiting factors (GIF), shown to be identical to MT-3, is decreased in brain tissue from Alzheimer patients. Neurotropic activity of neonatal rat brain tissue was inhibited by GIF/MT-3 [103].

Presently much attention is paid to an interesting research direction on metallothionein and its potential role in neurodegenerative disorders. Molecular pathways of neuroprotection and regeneration are metallothionein-mediated. MTs expressed in astrocytes after CNS injury are reported to serve both neuroprotective and neuroregenerative roles critical for the outcome of recovery [104]. MTs lacking signal peptides, scavenge free radicals and bind toxic metals and because of this they have a neuroprotective function intracellularly. Neuroprotective functions of MTs may also involve an extracellular component. A possible significant therapeutic potential of MT in the context of current understanding of the role of MT in astrocyte-neuron interactions in the injured brain has been brought forward [105]. MT-3 is predominantly expressed in Zn-containing neurons of the hippocampus. Disturbed MT homeostasis can lead to changes in brain concentrations of Zn (see Chapters 10 and 11). Since the intracellular concentration of Zn is mediated by complexing with apo-thionein to form MT, there has been great interest in ascertaining whether disordered MT regulation plays a role in the etiology of neurodegenerative disorders. Abnormalities in MT and/or Zn homeostasis have been reported in multiple

neurological disorders, e.g., Alzheimer's disease [106]. A possible role of MT and metals in other neurodegenerative diseases, e.g., in amyotrophic lateral sclerosis is suggested by the finding of increased levels of metals in cerebrospinal fluid (CSF) among such cases [107]. MT-binding was studied by chromatographic separation and metal analyses [108]. Silencing of genes changes the expression of the protein that is encoded by the gene. If the protein is not present in the tissue, a different physiological/toxicological pattern is seen even if the gene is present. Thus it can be questioned what happens when MT genes are silenced. MT-3 is seen in the brain but mRNA for MT-3 synthesis has been identified in the kidney though no MT-3 has been found. This raises the question if MT-3 can be involved in neurodegenerative disorders by disturbed expression or silencing of the gene.

7.2. Metallothionein-Related Biomonitoring in Diseases

Metallothionein in urine correlates well with Cd in urine. Both measurements can be used as a biomarker of cumulative cadmium doses in long-term exposures [52]. Basal and induced MT gene expression levels in PBLs are closely related with Cd exposure. MT gene expression in PBLs thus may be used as biomarker of Cd exposure, but more direct methods for this purpose are determinations of Cd in blood or urine [52].

7.2.1. Biomarker of Susceptibility

Induced MT mRNA levels in PBLs seem to reflect the renal ability for MT induction, thus providing a possible index of susceptibility to the adverse effect of Cd on the kidney [96–98]. Because it is not possible in routine biomonitoring to measure the renal MT gene expression *in vivo*, *in vitro* induced MT gene expression levels in PBLs would serve as a potential suitable index for this. A dose-effect relationship between the internal dose of cadmium and the MT mRNA level confirmed the validity of MT gene expression in PBLs as a biomarker of cadmium exposure. Both studies on Cd workers and environmentally Cd-exposed persons have measured the *in vitro* induced MT mRNA level in PBLs sampled from exposed persons as an indicator reflecting the ability of the body to synthesize MT upon cadmium exposure. A negative correlation between urinary N-acetyl-β-D-glucosaminidase (UNAG), a sensitive indicator for renal effects of cadmium exposure and the *in vitro* induced MT mRNA level in the subjects with high UCd level (over 10 μg/g creatinine) was shown. The lower proportion of individuals expected to have exceeded their individual critical concentration in the renal cortex at UCd levels below 10 μg/g creatinine explains that no statistically

significant correlation was observed between the *in vitro* induced MT mRNA level and UNAG in 2–10 μg/g creatinine UCd groups.

A reverse relationship between *in vitro* induced MT mRNA level in PBLs and UNAG indicates that MT gene expression in PBLs can be used as a biomarker inversely related to the susceptibility to renal toxicity of cadmium. It is suggested to apply MT gene expression in PBLs for the risk assessment of cadmium exposure [96–98]. Studies of MT mRNA in human lymphocytes provide evidence of a lower prevalence of tubular proteinuria among Cd-exposed persons with high ability to express tissue metallothionein compared to persons with such a low ability. These studies provide support in humans for the important role of MT in Cd toxicology [96–98]. Metallothionein in urine can be used as a sensitive biomarker for metal induced nephrotoxicity [109].

Metallothionein gene expression in peripheral blood lymphocytes as a biomarker of susceptibility to renal Cd toxicity in humans is an example of how advances in molecular epidemiology may increase delineation of human health risks from exposure to this element.

The presence of MT-ab in blood plasma is a significant indicator for the occurrence of tubular dysfunction among diabetic subjects. Among Cd-exposed workers and among persons suffering type-2 diabetes, elevated levels of MT-ab was associated with a higher prevalence of tubular dysfunction compared to those with lower MT-ab levels [109–111].

It was shown that the cellular localization of MT is different in some cancer cells compared to normal cells (see Chapter 13). This observation forms the basis for the use of MT as biomarker of cancer malignancy [91]. There is an extensive recent literature on the use of MT immunostaining in tumor diagnosis, e.g., in adenoid cystic carcinomas MT immunolocalization may be important [112].

7.3. Metallothionein in the Treatment of Diseases

Different MT synthesis in tumor tissue compared to normal tissue has been suggested as a basis for treatment. The possible use of cadmium as a cancer treatment agent in some forms of liver tumors was discussed [113] based on observations in mice. Animals that were treated with combinations of Cd and NDEA (N-nitrosodiethylamine) did not develop liver tumors in contrast to animals treated only with the tumorigenic agent NDEA. MT levels were markedly reduced in the livers of tumor bearing animals compared to normal animals. The effect of cadmium treatment on these liver tumors, thus can be explained by their higher sensitivity to Cd due to the lack of MT expression.

It has been shown in animal experiments that bismuth treatment will induce MT in the kidney and reduce toxicity of *Cisplatin* to the kidney

[114,115]. This observation may serve as a basis for administering higher doses of this therapeutic agent with retained patient safety when the combination with bismuth is used in cancer therapy.

Oral treatment with zinc has been suggested [116] and is now recommended as a standard treatment in Wilson's disease. The conditions of patients improved by a mechanism that zinc induces synthesis of intestinal metallothionein that sequesters copper for the structure and thus blocks the intestinal uptake of Cu.

8. FUTURE ASPECTS ON METALLOTHIONEINS

Already at the first meeting on MT in Zürich in 1978 it was discussed to set up a bank or producer of MT in order to have pure and standardized MT probes available. Discussions dealt with the question if interlaboratory exchange and a quality control program would solve some difficulties that might occur due to reports on the varying purity of MT. In order to be able to compare results in MT research and reported quantifications from different laboratories it is necessary to include some quality control. This should include standard material or reference material of MT. Due to the lack of the latter mostly commercially available MT is employed as a substitute of a standard in MT quantification. However, the quality of MT employed as such can be questioned. Several reports about the quality have ended with results that are difficult to interpret. The large number of isoforms and subisoforms within the MT family makes application of immunological techniques to biological samples difficult. In order to increase the selectivity and sensitivity of these methods, the specificity of the antibodies is important. However, the complete family of MT lacks common antibodies for the different isoforms and subisoforms, and this could lead to underestimations when total MT is measured by, e.g., ELISA [37]. Purification of MT by the individual research groups seems to yield high quality MT.

In order to gain more information on MT it is necessary to harmonize methods for its quantification. Several methods for estimating and measuring the concentration of metallothionein in tissues and body fluids have been developed and are at present more or less successfully in use. It is necessary to have in mind factors that influence the concentration of metallothionein in tissues and fluids. Metallothionein is increasingly used, but reported levels in tissues and fluids vary among research groups. Like other biomarkers, MTs may have to be related to some other factors. Exposure to many agents induce MT synthesis and metals are inducers with high potency; cadmium being the strongest one.

Little is known about the biological aging in synthesizing metallothionein. Studies on cadmium concentration in renal tissue have shown a decrease

after the age of 50–60 years in human beings. A method to measure the concentration of metallothionein should be protein-specific and manage to measure changes such as increase or decrease of the normal or, more precisely, basic concentration. Cadmium concentration increases upon exposure and particularly with age as the new born has a low concentration of cadmium since this metal does not pass the placental barrier. The concentration of metallothionein in the cell is influenced by so many parameters and factors that a method to quantitate it has to be standardized with respect to many factors. Just to measure the concentration in relation to exposure to some inducing agent, many of the previously mentioned methods are quite acceptable. However, in order to use MT values in routine biomonitoring, ''normal'' values in matrices such as blood, urine, and CSF need to be established. Measurement of MT mRNA in peripheral blood lymphocytes has been suggested as a biomarker of susceptibility in metal exposures. In order for this method to be used more widely, it has to be standardized and adapted to field conditions. Metallothionein autoantibodies in blood plasma appear to be a strong indicator of susceptibility to kidney effects of cadmium and may serve a similar role in other metal-induced diseases. Future studies concerning such uses would be of great interest.

Metallothionein is still a protein that demands further research and attracts scientists from many fields. A number of aspects have been brought to attention. The state of present knowledge and seen from the historical point of view indicates that results of future research on metallothionein will contribute to explain many rather different biological effects.

9. CONCLUSIONS

Summarizing half a century of research on MT shows that metallothionein remains a fascinating protein with several physiological functions and that MT can be described as a Camelot protein. MTs are a family of ubiquitous low molecular weight proteins with a high thiolate sulfur and metal content (Zn(II), Cu(I)), on the order of 10% (w/w). In conclusion, the experimental difficulties with the quantification of MT from the perspectives of three different fields, that is electrochemistry, chromatography, and immunochemistry, shows that even if advances in the development of the equipment occurred during the last five years, problems still exist in the determination of MT and particularly in the quantification in various complex media such as tissues and biological fluids. A number of newly developed techniques and equipments with increasing sensitivity and specificity has recently been applied to MT determinations in various media and greatly improved the possibilities for accurate measurements. Although MT

is stable under specific circumstances it can be degraded or complexed with other proteins under other commonly occurring circumstances.

Knowledge and training in handling the protein is most crucial in order to avoid confusing results that are tricky to interpret. MT can be used as an indicator in both environmental and biological monitoring reflecting exposure to metals, and as a good biomarker of renal dysfunction. When values can be set for the normal concentration of MT, the protein could also serve in relation to medical aspects and assist in the calculation of an allowable intake or exposure limit for Cd. MT is an established biomarker in biomontoring of human Cd exposure and may also be useful in the risk assessment of other metal exposures. MT mRNA in lymphocytes in humans has been suggested as an indicator of susceptible groups in relation to metal exposure; the development of practical procedures to measure MT in biological samples, e.g., blood, urine, biopsies of tumors, etc., is highly desirable. MT-ab appears to be an important biomarker for Cd-related tubular dysfunction.

ABBREVIATIONS

Alb	albumin
BAL	British anti-lewisite, 2,3-dimercaptopropanol (dimercaprol)
CdMT	metallothionein containing Cd^{2+}
CMB	p-chloromercuribenzoate
CNS	central nervous system
CR	creatinine
CSF	cerebrospinal fluid
DMSA	meso-2,3-dimercaptosuccinic acid (succimer)
EDTA	ethylenediamine-N,N,N′,N′-tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
GIF	growth inhibitor factor
MCBI	Medical Center for Biotechnology Information
MT	metallothionein
MT-ab	metallothionein autoantibody
MTF	metal-responsive transcription factor
NDEA	N-nitrosodiethylamine
PBLs	peripheral blood lymphocytes
PCR	polymerase chain reaction
RIA	radio-immunoassay
RT-PCR	real-time polymerase chain reaction
Tris	tris-(hydroxymethyl)-aminomethane
UCd	urinary cadmium
UNAG	urinary N-acetyl-β-D-glucosaminidase
YBCO	yttrium-barium-copper-oxide

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