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Protective effect of metallothionein on cadmium toxicity in isolated rat hepatocytes

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An isolated rat hepatocyte preparation was used to study the cellular toxicity of cadmium and the protective effects of metallothionein on cadmium-induced toxicity. Exposure of primary suspension cultures of isolated rat hepatocytes to Cd^{2+} (0–35.7 μM) for 15 min resulted in a dose-dependent reduction in the synthesis of cellular proteins during a subsequent 6 h incubation. Such inhibition could not be correlated with cellular lethality or gross membrane damage. Pre-induction of metallothionein in hepatocytes by zinc treatment *in vivo* of donor rats protected hepatocytes *in vitro* from cadmium-induced inhibition of protein synthesis. The protective effects in zinc-pre-induced hepatocytes are not due to alterations in the level of total cellular cadmium, but could be accounted for by the redistribution of intracellular cadmium in the presence of high levels of zinc-metallothionein. The data suggest that metallothionein exerts its protective effect by a kinetic detoxification mechanism, i.e. a decrease in reactive intracellular cadmium.

Cadmium toxicity and the induction of metallothionein by cadmium exposure have been extensively documented in humans and laboratory animals (Friberg *et al.*, 1974). Whatever the route of exposure, the liver represents an important organ for the initial accumulation of cadmium in the body (Frazier & Puglese, 1978) and is potentially susceptible to cadmium-induced cellular toxicities. Both morphological and biochemical lesions of hepatocytes have been reported in animals exposed to cadmium (Shinghal *et al.*, 1974; Hoffman *et al.*, 1975; Hidalgo *et al.*, 1976; Stoll *et al.*, 1976; Pence *et al.*, 1977; Roberts & Schnell, 1982; Dudley *et al.*, 1982).

The liver is one of the major sites where the metal-binding protein metallothionein is synthesized in response to cadmium exposure (Onosaka & Cherian, 1981). As a result, the liver controls the availability of cadmium to other target organs. Binding of cadmium to metallothionein in the liver has been linked to the development of cadmium tolerance in laboratory animals. Tolerance had been demonstrated to cadmium-induced lethality (Terhaar *et al.*, 1965; Leber & Miya, 1976; Probst *et al.*, 1977), testicular necrosis (Ito & Sawauchi, 1966; Nordberg, 1971), and alteration in hepatic drug metabolism (Roberts *et al.*, 1976; Roberts & Schnell, 1982). Protection of animals from cadmium toxicity has also been described in animals pretreated with zinc (Webb, 1972; Probst *et al.*, 1977). Induction of metallothionein was suggested as the mechanism of protection responsible for all of these observations. Furthermore, cadmium-resistant mutants have been isolated in many established cell lines (Rugstad & Norseth, 1975; Hilderbrand *et al.*, 1979; Corrigan & Huang, 1981). The mechanism of resistance has been attributed to the synthesis of metallothionein by the cadmium-exposed mutant (Griffith *et al.*, 1981).

The proposed mechanism of protection in these cases is kinetic detoxification, i.e. metallothionein serves as a scavenger of cellular cadmium and prevents cadmium from reaching target sites (Piscator, 1964; Yoshikawa, 1973). However, there is little direct evidence for such a mechanism. One reason is that the protective effect of metallothionein has been described only in systems where gross measurements of toxicity were observed, usually lethality. It is difficult to establish a dose–response relationship between such measurements and the distribution of cadmium. In addition, the influence of systemic effects is difficult to control *in vivo*, where protective effects of metallothionein are best documented.

The isolated hepatocyte system has provided an

invaluable tool in overcoming such difficulties. It provides a system in which the cellular toxicities of cadmium can be quantified. The role of pre-induced metallothionein in both the protection against cellular toxicity and the kinetic distribution of cadmium can be studied without systemic influences. The isolated hepatocyte has been previously employed to investigate the structural and metabolic changes associated with cadmium toxicity (Stacey *et al.*, 1980; Santone *et al.*, 1982). We have extended these studies by investigating the influence of pre-induced metallothionein on the relationship between cellular toxicity, as measured by protein synthesis, and cadmium kinetics in the isolated-rat-hepatocyte system.

Materials and methods

Animals

Male Wistar Rats (Charles River, Wilmington, MA, U.S.A.), weighing 150–200g were used in all studies. Commercial rodent chow (Formula RMH-1000; Charles River) and tap water were provided *ad libitum*.

Chemicals

All chemicals used were of reagent grade and purchased from Fisher Chemical Co. (Fairlawn, NJ, U.S.A.), unless otherwise specified. Radioisotopes were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). Trace-metal contamination of all buffers was removed by ion-exchange on Chelex-100 (Bio-Rad Laboratories, Richmond, CA, U.S.A.). All apparatus and glassware involved in metal analysis were soaked in either 10% (v/v) HNO_3 or 2% (w/v) EDTA solution overnight and rinsed thoroughly with distilled, deionized water before use.

Isolation of hepatocytes

Hepatocytes were prepared by the two-step perfusion technique. The procedure is briefly summarized here (see Seglen, 1976, for details). Rats were anaesthesized with sodium pentobarbital (65mg/kg, intraperitoneally). The liver was flushed *in situ* with 350ml of Ca^{2+}-free perfusion medium [110mM-NaCl/4.4mM-KCl/1.1mM-KH_2PO_4/1.1mM-$MgSO_4$,$7H_2O$/25mM-NaH-CO_3/25mM-sodium glutamate (Sigma Chemical Co., St. Louis, MO, U.S.A.), 11mM-glucose (Sigma) and 5mM-sodium pyruvate (Sigma)]. Liver connective tissue was digested with 0.025% collagenase (Type II; Worthington Biochemical Corp., Freehold, NJ, U.S.A.) in 200ml of recirculating perfusion medium, supplemented with 5mM-$CaCl_2$ and continuously bubbled with O_2/CO_2 (19:1). At the end of the digestion period (15–20min), the liver was dispersed in 80ml of fresh Ca^{2+}-containing perfusion medium, supplemented with 1% dialysed albumin (fraction V, Sigma) and then incubated (37°C, 200 oscillations/min) for a further 10min. Finally, hepatocytes were purified by filtration through four layers of gauze and then a nylon mesh (Nitex 153; Tetko, Elmsford, NY, U.S.A.), followed by three washes with incubation media. Hepatocyte viability was assessed by the Trypan Blue exclusion test. Hepatocytes prepared from untreated donor rats are referred to as 'naïve' hepatocytes.

Incubation of isolated hepatocytes

Experiments with successful preparations (viability more than 90%) were commenced within 15min of obtaining the final cell suspension and carried out in 25ml plastic Erlenmeyer flasks. Each flask contained 3×10^7 viable cells in 10ml of incubation medium [RPMI media 1640 (Gibco, Grand Island, NY, U.S.A.)], supplemented with 1% dialysed albumin and 90munits of bovine pancreatic insulin (Sigma)/ml, and was incubated at 37°C in a shaking water-bath (150 oscillations/min), and equilibrated with O_2/CO_2 (19:1).

Isolation of zinc-pre-induced hepatocytes

Rats were injected subcutaneously with 20mg of Zn^{2+}/kg body weight as zinc acetate. After 24h the rats were used as liver donors for the isolation of hepatocytes. The presence of metallothionein in pre-induced hepatocytes was evaluated by fractionation of hepatocyte cytosol (100000*g*/60min supernatant fraction) on a Sephadex G-75 column after saturation of metallothionein with stable cadmium. Each fraction was analysed for cadmium by flame atomic-absorption spectrophotometry (Varian Techtron AA-5) and the cellular metallothionein content computed as nmol of metallothionein/3×10^7 cells (assuming that seven cadmium ions are bound per molecule of metallothionein).

Uptake of cadmium

Freshly isolated hepatocytes were suspended in the complete incubation media. Cadmium as $CdCl_2$ (Mallinckrodt, Paris, KY, U.S.A.), was added to each hepatocyte suspension to obtain final Cd^{2+} concentrations of 2.7, 4.4, 7.1, 8.9 or 17.9μM. After 15min of exposure, the hepatocytes were harvested and washed twice with fresh incubation media. A 4ml portion of 50mM-Tris buffer (pH7.4 at 25°C; Sigma) containing 0.9% NaCl was added to each 1g of wet cells and subsequently homogenized in a Potter–Elvehjem glass homogenizer with a motor-driven pestle. Portions of the homogenate were frozen at −20°C for later DNA determination (Burton, 1956). The remaining homogenate was wet-digested in Ultrex

HNO_3 plus 30% (v/v) H_2O_2 and then analysed for cadmium by atomic-absorption spectrometry using the 228.8nm spectral line. Bovine liver (Standard Reference Material 1644; National Bureau of Standards, Rockville, MD, U.S.A.) was used for quality control.

Toxicities of cadmium

At the end of the 15min exposure to 0, 4.4, 8.9, 17.9 or 35.7 μM-Cd^{2+}, hepatocytes were harvested and washed twice with fresh media. Each final cell pellet was resuspended in cadmium-free medium and incubated as described above. The possibility of delayed cadmium toxicity was monitored in all suspensions during the next 6h of incubation by testing the ability of the cells to exclude Trypan Blue (Seglen, 1976) and the extent of leakage of cytosolic lactate dehydrogenase (Sigma Chemical Co., 1980).

Synthesis of cellular proteins

After 15min exposure to cadmium, 0.1 μCi of [^{3}H]leucine (sp. radioactivity 52.3Ci/mmol) was added to each flask of washed and resuspended hepatocytes and incubated for 6h. At the end of the incubation period, cells from each flask were harvested, washed twice, and homogenized in 4vol. of saline (0.9% NaCl). Portions of the homogenate were frozen at −20°C for DNA determination. The remaining homogenate was precipitated with 10% (w/v) trichloroacetic acid. The pellet (10000*g*, 10min) was solubilized with Protosol (New England Nuclear Corp.) and radioactivity was determined in a Tri-Carb liquid-scintillation spectrometer (Packard).

Uptake of α-amino[1-^{14}C]isobutyric acid

After 15min exposure to cadmium, hepatocytes were washed and resuspended in fresh cadmium-free medium. α-Amino[1-^{14}C]isobutyric acid (0.5 μCi/flask; sp. radioactivity 52.6mCi/mmol) was added and the incubation performed as described above. Cells were harvested after either a 15min or a 2h incubation. The cell pellets were washed twice with fresh media and subsequently homogenized as described above. The homogenate was precipitated with 10% (w/v) trichloroacetic acid and the supernatant was analysed for ^{14}C radioactivity. The 10%-trichloroacetic acid precipitate was analysed for ^{14}C radioactivity after solubilization. In a second set of experiments investigating the possibility of delayed effects of cadmium exposure on α-aminoisobutyric acid uptake, α-aminoisobutyric acid was added to hepatocyte suspensions 2h after 15min exposure to cadmium.

Subcellular and cytosolic distribution of cadmium

Immediately after 15min exposure to 4.4, 8.9 or 17.9 μM-Cd^{2+} containing 5 μCi of ^{109}Cd (carrier-free)/mg of Cd^{2+}, isolated hepatocytes were harvested and homogenized as described above. Subcellular fractionations were obtained by differential centrifugation (Krack *et al.*, 1980). The P1 fraction (cellular debris and nuclei) was obtained by centrifuging the homogenate at 600*g* for 10min and washing twice with buffer. The P2 fraction (mitochondria and lysosomes) was obtained by centrifugation at 10000*g* for 20min. Finally the P3 fraction (microsomal) was obtained as the pellet after centrifugation at 100000*g* for 60min, with the supernatant corresponding to the soluble cytosol. Each fraction was measured for ^{109}Cd radioactivity by liquid-scintillation spectrometry (Packard). The soluble cytosol was further fractionated on a column (1.6cm × 100cm) packed with 200ml of Sephadex G-75 (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) and equilibrated with 50mM-Tris buffer (pH7.4 at 25°C, Sigma) containing 0.9% NaCl. Fractions (2.5ml each) were collected and assayed for ^{109}Cd radioactivity.

Statistical analysis

Data were subjected to analysis of variance and to a *post hoc* test that evaluated the critical differences based on Student's *t* test.

Results

The dose-dependent uptake of cadmium in isolated rat hepatocytes is illustrated in Fig. 1. Cadmium accumulation in hepatocytes increases with increasing cadmium concentration in the media; however, the percentage of the dose that is taken up during the 15min exposure decreases with increasing dose, suggesting saturable kinetics. When the 15min uptake of cadmium is compared between naïve hepatocytes and zinc-pre-induced hepatocytes, there is no significant difference between the two groups for all concentrations studied (results not shown).

Exposures to cadmium (4.4–35.7 μM) of 15min do not affect the viability of hepatocytes, as measured by Trypan Blue exclusion and lactate dehydrogenase leakage, for up to 6h after exposure (results not shown). However, exposure to cadmium (4.4–35.7 μM) for 15min significantly decreases the ability of the isolated hepatocytes to incorporate [^{3}H]leucine into trichloroacetic acid-precipitable total cellular proteins measured 6h after exposure (Fig. 2). The inhibition is significant at cadmium concentrations as low as 10 μM. In the highest-cadmium-treatment (35.7 μM) group, the total amount of [^{3}H]leucine incorporated was only

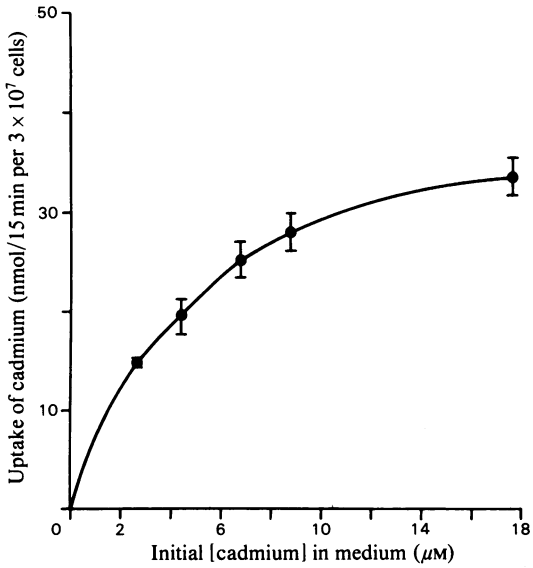

Fig. 1. *Dose-dependence of cadmium uptake in isolated rat hepatocytes*

After a 15 min exposure to the indicated cadmium concentrations, homogenates of the cell pellets were wet-digested and the cadmium content analysed by atomic-absorption spectrometry. Data are expressed as nmol of cadmium accumulated/15 min per 3×10^7 cells. Each data point is the mean ± S.D. for four hepatocyte preparations.

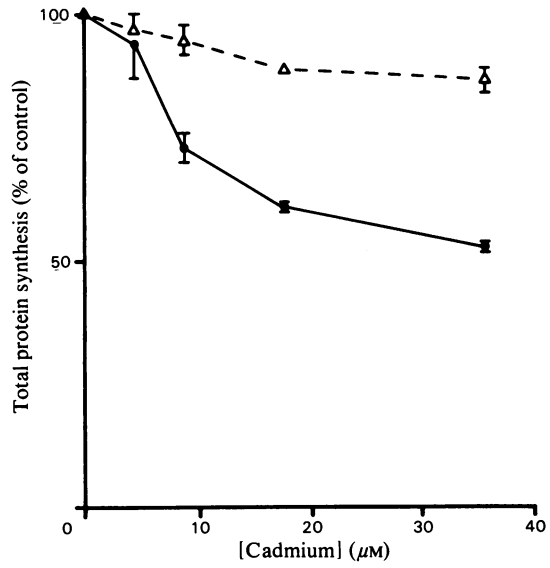

Fig. 2. *Inhibition of protein synthesis in isolated rat hepatocytes by cadmium*

After a 15 min pulse exposure to various cadmium concentrations, isolated hepatocytes were incubated in the presence of [^{3}H]leucine for 6 h. Total [^{3}H]leucine incorporated into cellular proteins was precipitated from the cell homogenate by 10% trichloroacetic acid and the radioactivity determined by liquid-scintillation spectrometry. Data are corrected for the number of cells by DNA quantification and expressed as a percentage of the control value, which corresponded to 1.5×10^5 d.p.m./3×10^7 cells. Naïve hepatocytes (●) were isolated from untreated rats and pre-induced hepatocytes (△) from Zn^{2+}-injected rats as described in the text.

50% of the value in the control group. Hepatocytes containing zinc-pre-induced levels of metallothionein (4.2 ± 2.2 nmol/3×10^7 cells) are less sensitive to the inhibitory effects of cadmium on protein synthesis, as shown in Fig. 2. At the highest cadmium concentration (35.7 μM), the amount of [^{3}H]leucine incorporation in zinc-pre-induced hepatocytes is maintained at 85% of the control value. In order to decrease the amount of [^{3}H]-leucine incorporation to 50% of the control value in zinc-pre-induced hepatocytes the cadmium concentration in the media had to be increased to 140 μM, a value four times higher than that required for naïve hepatocytes.

The effects of cadmium treatment on the uptake of the amino acid analogue α-aminoisobutyric acid were investigated after treatment with 4.4 and 17.9 μM-cadmium. Cadmium treatment does not affect the uptake rate (15 min measurement) or the steady-state level (2 h measurement) of α-aminoisobutyric acid in isolated rat hepatocytes (results not shown). A possible late effect was investigated by measuring both the 15 min uptake rate and the subsequent 2 h steady-state level in 2 h-old suspensions. Again, no difference is found in the uptake rate or steady-state level of α-aminoisobutyric acid in either treatment groups compared with the control. No significant radioactivity was found in the 10%-trichloroacetic acid precipitate, confirming that α-aminoisobutyric acid is not incorporated into cellular proteins.

The subcellular distribution of cadmium was investigated immediately after exposure to cadmium for 15 min in both naïve and zinc-pre-induced hepatocytes. The results for naïve hepatocytes are illustrated in Fig. 3(*a*). The data suggest that the subcellular distribution of cadmium is dose-dependent. At the lowest dose (4.4 μM), most of the cellular cadmium is found in the cytosol (64%). At the highest dose (17.9 μM), the P1 fraction becomes the dominant pool of cellular cadmium (52%). With increasing cadmium exposure, cadmium accumulates preferentially in the P1, P2 and P3 fractions in naïve hepatocytes, which contain only basal levels of metallothionein. The subcellular distribution of cadmium in hepatocytes from zinc-pre-induced rats is quite different from that in

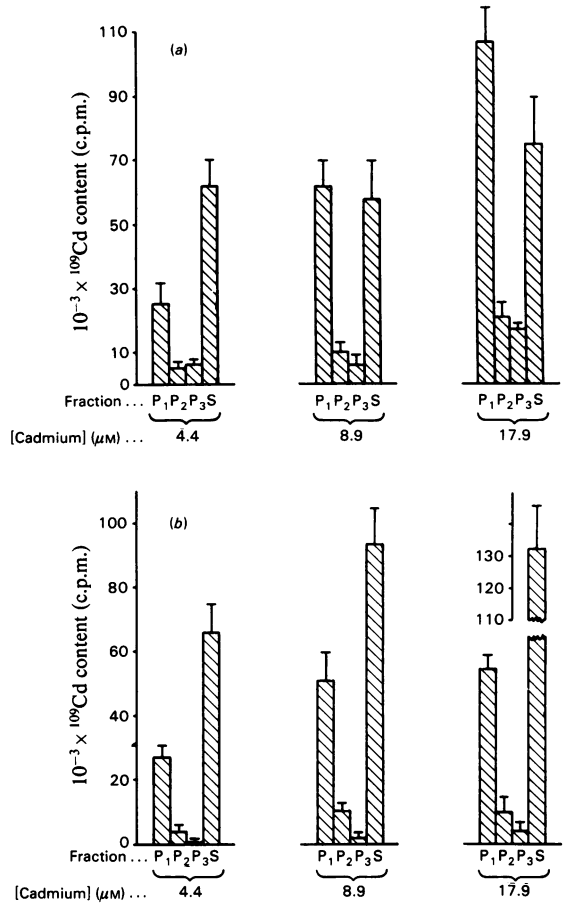

Fig. 3. *Subcellular distribution of cadmium in isolated rat hepatocytes*

After a 15min exposure to the indicated cadmium concentrations (containing 5μCi of ^{109}Cd/mg of Cd^{2+}), the cells were separated into P1 (nuclei and cellular debris), P2 (mitochondria and lysosomes), P3 (microsomal) and S (soluble cytoplasm) fractions as described in the text. The ^{109}Cd radioactivity in each fraction was determined by liquid-scintillation spectrometry. The dose-dependent distribution of ^{109}Cd radioactivity in each of the fractions is shown for naïve hepatocytes (*a*) and for zinc-pre-induced hepatocytes (*b*). Each value represents the mean ± S.D. for four hepatocyte preparations. Recovery during the fractionation procedure was more than 90% in all preparations.

naïve hepatocytes, as Fig. 3(*b*) shows. At the lowest dose (4.4μM), the distribution of cadmium is similar in the two groups of isolated hepatocytes. With increasing cadmium concentrations, most of the additional cadmium is associated with the cytosolic fraction. Little increase is observed in the P1, P2 and P3 fractions. These data suggest that, in hepatocytes from zinc-pre-induced rats, the binding capacity in the cytosol is greater and preferentially accumulates cadmium.

The distribution of cadmium in the cytosol immediately after cadmium exposure was further analysed by fractionation on Sephadex G-75. Two major peaks of cadmium are identified in both profiles, a high-M_r peak eluted at the void volume (V_o) and a metallothionein peak eluted at a V_e/V_o ratio of 1.9. The percentage distribution of cytosolic cadmium between these two peaks was computed by integrating the areas under the two peaks and the results are shown in Table 1. At a cadmium concentration of 4.4μM, only 10% of the cytosolic cadmium is bound to metallothionein and 90% to the high-M_r peak in naïve hepatocytes, whereas 95.9% of the cytosolic cadmium is bound to metallothionein and only 4.1% to the high-M_r peak in hepatocytes from Zn-pre-induced rats. With increasing cadmium doses, increasing percentages of cytosolic cadmium are bound to the high-M_r macromolecules in both groups of isolated hepatocytes.

Data from the subcellular and cytosolic distributions enabled us to compute the percentage of total cellular cadmium that is bound to metallothionein and, by difference, that which is non-metallothionein bound. The computed results are presented in Table 2. In naïve hepatocytes, 93.6% of the total cellular cadmium is not bound to metallothionein at the lowest dose, but increases to almost 99% at the highest dose. In the zinc-pre-induced hepatocytes, 37% of the total cellular cadmium is not bound to metallothionein, and this pool of labile cadmium increases to only 47% at the highest dose.

In order to explore the interaction between cadmium toxicity and its cellular distribution, the extent of cadmium-induced inhibition of protein synthesis, measured at 6h after exposure (Fig. 2) was plotted against the non-metallothionein-bound cadmium in the hepatocytes immediately after exposure to cadmium. Total non-metallothionein-bound intracellular cadmium was computed by multiplying the percentage of total cellular cadmium that was non-metallothionein-bound (Table 2) by the total cellular cadmium accumulation (Fig. 1). The results (Fig. 4) suggest that there is a unique relationship between cadmium-induced toxicity and the amount of non-metallothionein-bound cadmium in the hepatocytes independent of their origin. A threshold effect is observed. Once the critical concentration of non-metallothionein-bound cadmium (approx. 15nmol/3×10^7 cells) is exceeded in either group of isolated hepatocytes, the extent of inhibition in protein synthesis becomes a function of the amount of non-metallothionein-bound cadmium

Table 1. *Percentage distribution of cytosolic cadmium between high-M_r macromolecules and metallothionein*
The area under the high-M_r and metallothionein (MT) peaks in the Sephadex G-75 cytosolic cadmium profiles was determined. The percentage distribution of cytosolic cadmium profiles was determined. The percentage distribution of cytosolic cadmium was obtained as follows: 100 × (amount of cadmium in each peak/total cytosolic cadmium). Each value is the mean ± S.D. for four hepatocyte preparations.

		Distribution (%)					
		Naïve hepatocytes			Zinc-pre-induced hepatocytes		
Peak	Cadmium dose (μM) ...	4.4	8.9	17.9	4.4	8.9	17.9
High-M_r		90.8 ± 3.0	93.0 ± 2.0	95.5 ± 2.5	5.0 ± 1.5	10.1 ± 2.1	17.3 ± 1.2
MT		9.2 ± 2.5	7.0 ± 1.5	4.5 ± 0.8	95.0 ± 1.6	89.9 ± 3.2	82.8 ± 3.4

Table 2. *Relationship between cadmium dose and cellular non-metallothionein(MT)-bound cadmium*
The subcellular and cytosolic distribution of cadmium was determined immediately after a 15 min exposure to the indicated cadmium concentrations as described in the text. Values (D) are percentages of cellular cadmium present as non-metallothionein-bound cadmium in the hepatocytes and are computed as follows: $D = 100 \times (1 - XY)$, where X is the fraction of cellular cadmium in the cytosol and Y is the fraction of cytosolic cadmium bound to metallothionein. Each value represents the mean ± S.D. for four different hepatocyte preparations.

		Non-MT-bound cadmium (%)		
Hepatocytes	Cadmium dose (μM) ...	4.4	8.9	17.9
Naïve		93.6 ± 2.5	97.5 ± 3.7	98.6 ± 4.2
Zinc-pre-induced		37.0 ± 1.9	44.9 ± 2.4	47.2 ± 2.1

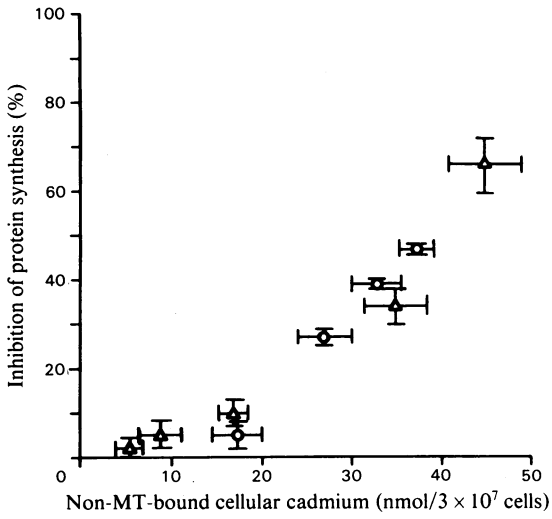

Fig. 4. *Relationship between the extent of cadmium-induced inhibition of protein synthesis and non-metallothionein(MT)-bound intracellular cadmium in isolated hepatocytes*
The extent of cadmium-induced inhibition of protein synthesis was measured at 6 h after exposure (Fig. 2). Non-metallothionein-bound cellular cadmium was determined immediately after exposure to cadmium and computed as described in the text. The horizontal and vertical bars represent S.D. of the respective measurements. △, zinc-preinduced hepatocytes; ○, naïve hepatocytes.

and is not related to total cellular cadmium concentration.

Discussion

Inhibition of protein synthesis by cadmium had been reported both in systems *in vivo* (Hidalgo *et al.*, 1976; Stoll *et al.*, 1976) and in the isolated perfused liver system (Zak & Dubin, 1978). The present data demonstrate that inhibition of protein synthesis is a sensitive index of cadmium cytotoxicity in cultured rat hepatocytes. It has also been shown that cadmium exposure has no effect on the amino-acid-transport system responsible for α-aminoisobutyric acid uptake. Since more than one transport system has been characterized for the uptake of amino acids in isolated rat hepatocytes (Kilberg, 1982), these data cannot be extrapolated to all amino-acid-transport mechanisms. However, the data suggest that the effect of cadmium on protein synthesis is unlikely to be a consequence of its effect on the transport of amino acids. Possible mechanisms for decreased protein synthesis include dysfunction at the level of transcription or translation. Cadmium is known to bind to specific sites in chromatin, and such binding might possibly interfere with the transcription of mRNA. It has also been reported that cadmium can disrupt the structure of polyribosomes (Gamulin *et al.*, 1977) and therefore could potentially affect the

translation of mRNA. Furthermore, Stacey *et al.* (1980) have reported that cadmium causes lipid peroxidation. Such damage to the endoplasmic reticulum might also affect the synthesis of secretory proteins. In order to understand the mechanism of the inhibitory action of cadmium on protein synthesis and its specificity, additional research is required.

Development of cadmium tolerance has been reported in intact animals that had been injected with sub-lethal doses of cadmium or pretreated with zinc, and in mutants isolated from established cell lines. The common denominator for all these observations seems to reside in the induction of the metal-binding protein metallothionein. The present paper is the first report in which the protective role of metallothionein against cadmium toxicity has been demonstrated with respect to a specific cellular function, protein synthesis, in an isolated cellular system, as opposed to general indicators of lethality or gross tissue damage. The fact that pre-induction of hepatocytes has no effect on the cellular uptake of cadmium rules out the possibility that metallothionein may protect isolated hepatocytes from cadmium toxicity by affecting the cellular level of cadmium. However, the presence of metallothionein significantly affects the subcellular and cytosolic distribution of cellular cadmium. In naïve hepatocytes the cytosol has a surprisingly limited capacity for binding cadmium when compared with that of the P1 fraction. The total level of non-metallothionein-bound cadmium in the cells increases rapidly with increasing cadmium doses. These data imply that there must be cadmium-binding ligands within these organelles that have higher affinity for cadmium than do the cytosolic proteins, in the absence of metallothionein. Binding of cadmium to these ligands may be important for both the induction of metallothionein and/or the mechanisms of cadmium toxicity. In the zinc-pre-induced hepatocytes, the cytosolic capacity for cadmium is greatly increased. The presence of pre-induced metallothionein in the cytosol prevents the accumulation of cadmium in other cellular fractions and reduces the total level of non-metallothionein-bound cadmium in the cells. This observation is consistent with the data reported by Goering & Klaassen (1983) for intact rats. In their study they concluded that pre-induction did not affect the absorption, nor alter tissue distribution, of a subsequent cadmium dose. Instead, it altered the hepatic subcellular distribution of cadmium.

A unique relationship was observed when the extent of cadmium-induced inhibition of protein synthesis was plotted against the total amount of non-metallothionein-bound cadmium in the isolated rat hepatocytes for each dose of cadmium in the medium. Such a relationship suggests the presence of a threshold level for cadmium-induced toxicity and may have implications on the mechanism of toxicity. Above a critical concentration of cellular non-metallothionein-bound cadmium there is a direct correlation between the amount of non-metallothionein-bound cadmium and the extent of inhibition in protein synthesis in both naïve and zinc-pre-induced hepatocytes. The data provide direct evidence for the hypothesis that the protective mechanism of metallothionein involves kinetic detoxification. i.e. metallothionein protects isolated rat hepatocytes from cadmium toxicity by reducing the amount of labile or potentially reactive cadmium in the cells. Once the binding capacity of metallothionein is taken into account, cadmium exerts the same degree of toxicity in pre-induced hepatocytes as in the naïve hepatocytes. No correlation is observed when the extent of inhibition of protein synthesis is compared with the cytosolic non-metallothionein-bound cadmium alone. This further reinforces the view that binding of cadmium to ligands in cellular compartments other than the cytosol is probably important in cadmium-induced toxicity.

In conclusion, the present study demonstrates that inhibition of protein synthesis is a sensitive index of cadmium toxicity in isolated rat hepatocytes and that incorporation of [^{3}H]leucine into trichloroacetic acid-precipitable cellular proteins is a potentially useful experimental technique for monitoring such toxicity. In addition, results of detailed studies of intracellular cadmium kinetics are consistent with the hypothesis that intracellular metallothionein exerts its protective effects by kinetic detoxification and that this mechanism alone is adequate to account for the observed effects of preinduction of intracellular zinc-metallothionein on the inhibition of protein synthesis.

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