STIMULATION OF INFORMOSOMAL RNA SYNTHESIS IN CULTURED CHINESE HAMSTER CELLS EXPOSED TO LOW LEVELS OF CADMIUM

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1. Introduction

Cadmium is a trace element that is quite toxic [1–6]. Exposure to 40 mg by inhalation or to between 0.35 and 3.5 g by ingestion can be lethal [3]. Incidents of such acute effects are infrequent [1,3,4]. However, chronic exposure represents a unique phenomenon in toxicology because of the ubiquity of human exposure and its continuous, cumulative nature [1,3,5]. Cadmium is widely dissipated in the environment due to (a) pollution from various industrial processes such as ore smelting and burning of coal, oil, and plastics [1,3] and (b) the fact that it is not recycled in most of its applications [3,4]. This environmental presence is translated to human exposure mainly through foodstuffs [1,3]. The average diet in the United States contains 30–180 μg daily [3], 3–8% of which is retained [1,3–5]. Since biological turnover is very low [1,3,6], cadmium accumulates in the body. An adult may have a body burden of 30 mg [1,4,5]. Most cadmium is found in the liver and kidneys [1,5], which appear to be exceptionally competent in their ability to produce a specific cadmium-binding protein called thionein [1,2,4,6]. Such bound cadmium is relatively nontoxic [1,4,6]. Kidney failure does not occur until the concentration of thionein-bound cadmium in the kidney cortical cells reaches 200 ppm (~ 2 mM) [1,4]. Since the normal adult content is one-fourth to one-sixth this level [1,4], the margin of safety is low and has been exceeded in populations exposed to relatively high environmental levels [1,3,4]. Toxic effects due to the normal burden of cadmium or to exposure of cells more sensitive than those in the liver or kidneys to transient high levels of pollution are not defined. Assessment of such effects suffers from a relative lack of knowledge of cadmium response at the cellular and molecular level [3]. The present studies define the effects of low levels of cadmium on the growth and RNA metabolism of cultured Chinese hamster cells (CHO). This model system benefits from the absence of secondary effects encountered in vivo due to hormonal effects that may attend cadmium induced reduction in pancreatic functions [5], adrenal hypertrophy [5], and altered cyclic AMP levels [5].

Abbreviations: CHO, an established line of cultured Chinese hamster ovary cells; mRNA, messenger RNA; mlRNA, messenger-like RNA; RNP, ribonucleoprotein; tRNA, transfer RNA; rRNA, ribosomal RNA.

2. Materials and methods

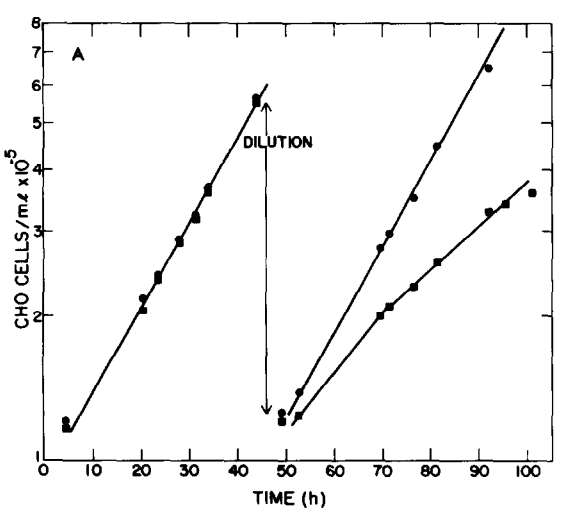
Cultured Chinese hamster ovary cells (line CHO) were grown in spinner culture in calcium-free Ham's F-10 medium supplemented with calf and fetal calf serum. Growth was monitored using a Coulter counter. Cadmium was added to the cultures as the chloride salt. Either analytical grade (Mallinckrodt) or ultra pure (Alfa) salt was used. Stock solutions were made up in 0.1 M HCl, and dilutions with sterile water were effected in polyallomer or polyethylene containers to reduce adsorptive loss. The cadmium content of exposed cells was determined by atomic absorption analysis or by following the incorporation of ^{109}Cd (New England Nuclear) of known specific activity. Labeled cadmium was counted using liquid scintillation spectrometry and PCS scintillation fluid.

The wet weight of CHO cells was determined as described for pig kidney cells by Webb and Daniel [7]. This determination gave values for the wet weight of cultured CHO cells that ranged from 3–4 g/10^9 cells. The actual mass per cell, as grown in spinner culture, was derived from the known volume and density of CHO cells which are such that 10^9 cells ≅ 1 g [8,9].

The polysome contents of CHO cultures and the incorporation of radiolabeled precursors into messenger-related RNAs were determined as reported previously [10,11].

3. Results and discussion

When CHO cells were grown at a medium concentration of 4×10^{-7} M Cd^{2+}, growth rate effects were apparent after 70 h, compared to 17 h for the control. Since cadmium could adsorb to serum proteins [2] or form insoluble complexes [3] such as carbonates with many constituents of the growht medium, it was important to determine the actual intracellular content of cells exposed to a given extracellular concentration. The 70 h culture was found to have a cellular cadmium content (based on actual cells mass of 1 g/10^9 cells) of 36 ppm. The content on a wet weight basis would be 9–12 ppm. The abrupt growth rate change seen after 3 days in culture at 4×10^{-7} M was reproducible. This dose-response contrasts markedly with that seen in K7 adult pig kidney cells in vitro, which have been reported to grow normally for 8 days in 4.4×10^{-6} M Cd; at the end of which time, the intracellular content (wet weight) was found to be 470 ppm [7]. The relative sensitivity of CHO cells to growth inhibition by low levels of cadmium suggests that their ability to produce protective amounts of thionein is not comparable to that of kidney cells. This possibility is being delimited by analysis of the partitioning of ^{109}Cd among thionein- and nonthionein-bound forms in CHO cells as a function of dose and time of exposure.



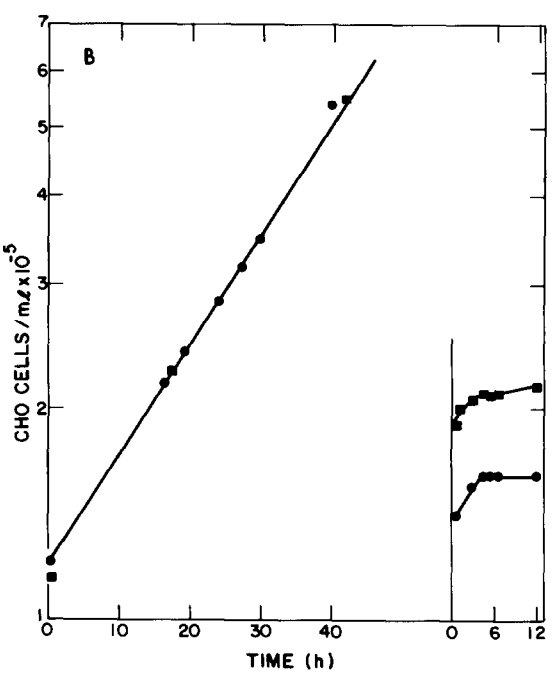


Fig.1. (A) Growth of CHO cells in 4×10^{-7} M Cd: (-•-) control and (-▪-) cells cultured at 4×10^{-7} M $CdCl_2$ and diluted with medium containing 4×10^{-7} M $CdCl_2$. (B) Growth of cultured CHO cells at permissive and inhibitory levels of Cd: (left) growth of control (-•-) and culture exposed to 2×10^{-7} M Cd (-▪-) for 2 weeks. Cultures were established in regular F-10 or F-10 containing 2×10^{-7} M $CdCl_2$. Each was diluted every 48 h, from 400 000–500 000/ml back to 50 000/ml, using regular F-10 medium for the control and F-10 medium containing 2×10^{-7} M $CdCl_2$ for the treated culture. Cultures were maintained for 2 weeks; growth during 2 days following the last dilution is shown (left). (right) Control, untreated CHO cells (-•-) and CHO cells cultured in the presence of 2×10^{-7} M $CdCl_2$ for 8 days (-▪-) were exposed to 3×10^{-6} M $CdCl_2$. Growth during 12 h following treatment is shown.

At a medium concentration of 2×10^{-7} M Cd^{2+} (fig.1B), CHO cells grew for at least 2 weeks with the same doubling time or, in some instances, faster than controls. These cultures were maintained by adding fresh F-10 medium containing 2×10^{-7} M Cd^{2+} at each dilution. The actual intracellular content of cadmium in such cultures was found to range from 10–20 ppm. On a wet weight basis, the cadmium concentration in cells grown in 2×10^{-7} M Cd^{2+} would be on the order of 2.5–5.0 ppm.

Increasing the cadmium content to 2 or 3×10^{-6} M caused cessation of growth within one generation time (fig.1B, right side). This occurred both with untreated cultures and with cultures pregrown 8 days at 2×10^{-7} M. These data suggest that exposure of CHO cells to a low dose of cadmium does not induce the production of protective amounts of thionein. The verity of this suggestion is being tested also by analyzing the intracellular partitioning of ^{109}Cd, in this instance using a large dose with or without pregrowth in 2×10^{-7} M Cd.

When the polysome contents of cadmium-treated and control cultures were determined, a significantly greater polysome mass was found in the cadmium-treated cultures relative to the untreated cultures. The polysome mass of cells grown in medium containing 2×10^{-7} M Cd for 3 days (fig.2) was 1.26–1.28 times as great as the control in two instances. Thus, there appears to be a stimulation of macromolecular (RNA and protein) synthesis in treated cultures.

In an attempt to determine whether there is any specificity in cadmium stimulation of RNA or protein synthesis, the appearance of messenger RNA into cytoplasmic ribosome-bound (polysomal mRNA) or free (informosomal [12] mlRNA) nucleoprotein forms was followed. The RNAs of these RNPs were selectively labeled with radioactive nucleoside precursors in the presence of a low concentration of actinomycin (0.05 μg/ml). As the informosomal mlRNP and polysomal mRNPs were incompletely

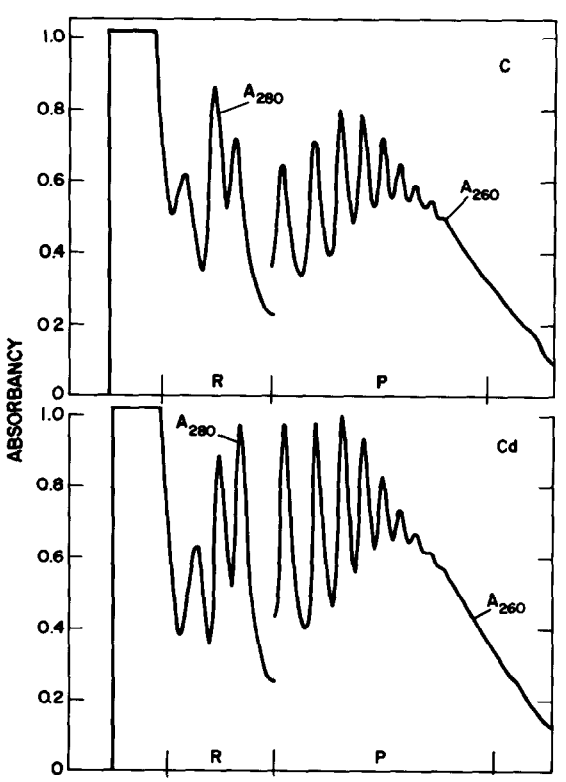


Fig.2. Zone sedimentation of cytoplasm derived from control (C) and cadmium-treated (Cd) CHO cells. At 72 h after addition of cadmium to the treated culture, 300 ml of each culture was made to 0.05 μg/ml in actinomycin D; 30 min later, 1 mCi [2,8-^{3}H] adenosine (New England Nuclear, 5 mCi/0.033 mg in H_2O) was added to each. After 90 min exposure to the labeled precursor, incorporation was terminated by pouring the cultures over frozen 0.25 M sucrose. The cells were collected by centrifugation, washed once with cold 0.25 M sucrose, and frozen in $K_{10}T_{10}M_{1.5}$ (10 mM KCl; 10 mM Tris, pH 7.4, at 25°C; 1.5 mM $MgCl_2$) buffer. Upon thawing, the cells were lysed with Nonidet P-40 nonionic detergent [1], the cytoplasm was stripped from the nuclei by the further action of sodium deoxycholate (0.5%), the nuclei were removed by sedimentation, the cytoplasm was adjusted to 100 mM in KCl by the addition of $K_{800}T_{10}M_{1.5}$, and then ribosomes and polysomes were resolved by zone sedimentation through a 10–50% sucrose gradient made up in $K_{100}T_{10}M_{1.5}$ buffer. Fractions R and P were collected as indicated. Aliquots of each were (1) ethanol-precipitated with carrier-RNA prior to extraction of RNA and analysis of RNA species labeled by zone sedimentation; (2) precipitated with trichloroacetic acid for determination of total incorporation; and (3) fixed with one-fourth volume of neutralized 30% formaldehyde for 24 h prior to dialysis and resolution of informosomal and polysomal species of labeled RNP by isopycnic (CsCl) centrifugation. A similar experiment was performed using a 1.5 h pulse [5-^{3}H]uridine (300 μCi/300 ml culture) in control and 44 h cadmium-treated (2×10^{-7} M) CHO cultures.

resolved by zone sedimentation, the relative contribution of each nucleoprotein species (mRNP or mlRNP) to radioactivity incorporated into RNA of the polysome region was determined by isopycnic banding of formaldehyde-fixed material. Figure 3 illustrates the distribution of incorporated [^{3}H]uridine of [^{3}H]-adenosine between polysomes and informosomes in ribosomal (R) and polysomal (P) fractions of control cells and cells exposed to 2×10^{-7} M Cd for 44 h. The results of these determinations for control (C) and cadmium-treated (Cd) cultures are itemized in table 1. These data indicate that, in the cadmium-treated cultures, messenger and messenger-like RNA incorporation is stimulated, with a greater stimulation of incorporation into informosomal species (176% and 214% of control) relative to polysome-bound species (146% and 107% of control).

Thus, a stimulation of incorporation of nucleoside precursor into messenger-related RNAs of CHO cytoplasm occurs in cadmium-treated cultures, with incorporation into nonpolysomal mlRNA being increased to a significantly greater extent than that into polysomal mRNA. This effect could be due to altered transcriptional mechanisms (increased synthesis of RNA destined to appear in informosomes) or to changes in post-transcriptional mechanisms such as increased selection of pre-mlRNA transcripts for processing, and it could represent an increment in existing informational sequences or appearance of new sequences. RNA–cDNA hybridization analyses should be performed to distinguish among these possibilities.

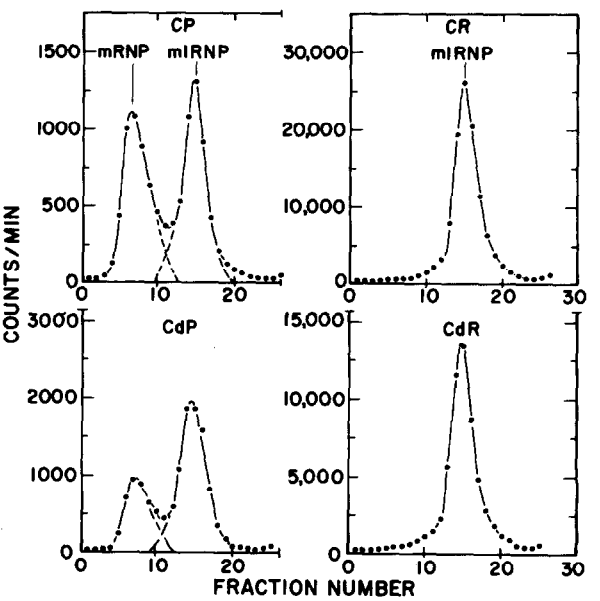


Fig.3. Isopycnic/CsCl banding of ribosome and polysome fractions of control (C) and cadmium-treated (Cd) cultures exposed to [5-^{3}H]uridine for 1.5 h after 30 min pretreatment with 0.05 μg/ml actinomycin D.

Table 1
Incorporation of RNA precursors into polysomal messenger or informosomal messenger-like RNAs of CHO cells during a 1.5 h pulse in the presence of actinomycin D

	Total informosomal mlRNA labeled (as % control)	Total polysomal mRNA labeled (as % control)
[2,8-^{3}H] Adenosine pulse 72 h in 2×10^{-7} M Cd	176	146
[5-^{3}H] Uridine pulse 44 h in 2×10^{-7} M Cd	214	107

The amount of labeled RNA of the polysome region that is in polysomes or informosomes is the product of the percentage banding at the respective density (1.4 g/cc for mlRNP, 1.52 g/cc for mRNP) times the total radiolabel in RNA extracted from the region that sediments in a sucrose gradient as mRNA does. Total mlRNA is the sum of its contribution to the polysome (P) region plus all messenger-like RNA of the ribosome region (R). Incorporation of label into appropriate species (mRNA or mlRNA as opposed to tRNA or rRNA) was verified by zone sedimentation analysis of RNAs extracted from the R and P fractions.

Singhal et al. [5] noted an effect of cadmium on cAMP metabolism in animals and postulated that such effects might 'regulate gene expression through cAMP-dependent phosphorylation of nucleoproteins by protein kinase.' Future experiments could address the phenomenon of specific stimulation of RNA metabolism in this context (i.e., whether the stimulation may be activated or mediated by effectors of cyclic AMP productionor protein phosphorylation).

Exposure to low levels of cadmium is the third condition found to alter the ratio of nucleoside precursor incorporation into RNAs of polysomes and informosomes. In the first instance, it was noted that polysomal messenger RNA incorporation increased relative to informosomal RNA incorporation as cells progressed through interphase in synchronized CHO cultures [11]. Second, in the period of resumed cell division following X-irradiation, incorporation of nucleoside precursors into cytoplasmic polysomal mRNA was selectively reduced [13]. It is not yet known whether these alterations reflect changes in RNA synthesis or processing, or both. However, these independent variations suggest that informosomes are not simple, obligate precursors to polysome-bound mRNP and may provide experimental systems for further definition of the relationship between these ribosome-bound and free mRNPs.

Regardless of mechanism, it is clear that a relatively low level of cadmium, one which does not affect growth, can alter RNA metabolism in CHO cells to a significant extent. Such an alteration could modify the nature of cell development and could possibly provide a basis for the known teratogenic effects of sublethal doses of cadmium in hamsters [14].

Acknowledgments

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