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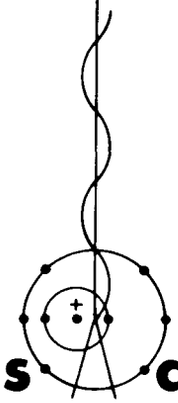
Larry L. Deaven
Evelyn W. Campbell



los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87545

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FACTORS AFFECTING THE INDUCTION OF CHROMOSOMAL ABERRATIONS BY CADMIUM IN CHINESE HAMSTER CELLS

by

Larry L. Deaven and Evelyn W. Campbell

ABSTRACT

Chinese hamster cells (line CHO) were examined for cadmium-induced growth rate perturbations and chromosome aberrations. Experimental protocols were designed to simulate conditions of cellular Cd^{++} exposure both *in vivo* and *in vitro*. CHO cells grown in the presence of nontoxic levels of Cd^{++} (2×10^{-7} M) for 12 wk became resistant to toxic levels (2×10^{-6} M) after this exposure. Metaphase cells in these resistant populations contained only background levels of chromosome aberrations. This suggests that individuals exposed to constant or intermittent low levels of Cd^{++} may be more resistant to the toxic effects of this metal and may not show chromosome aberrations as readily as nonexposed individuals. CHO cells grown in medium supplemented with several types of serum (fetal calf, newborn calf, and human) at concentrations commonly used for *in vitro* culture (5 to 20% v/v) or found in mammalian circulatory systems (50% v/v) differed markedly in Cd^{++} tolerance. Cells grown in medium containing 1×10^{-6} M Cd^{++} and supplemented with human or newborn calf serum were slightly protected against Cd^{++} damage at high serum concentrations (30 and 50% v/v) and accumulated approximately 90 μg $Cd^{++}/10^9$ cells in 48 h. Cells growing at low or high concentrations of fetal calf serum were completely protected from 1×10^{-6} M Cd^{++} and accumulated approximately 12 μg $Cd^{++}/10^9$ cells in 48 h. These results demonstrate the necessity for standardized protocols for cytogenetic investigations of Cd^{++} toxicity and should help to explain discrepancies between studies of chromosome damage in patients with high blood levels of cadmium.

I. INTRODUCTION

We summarized in our previous report[1] the reasons why certain heavy metals could become potential environmental hazards as a result of new energy initiatives by this country. We selected cadmium as an element of particular concern because of its known toxicity to humans, its usage in industry, and the possibility of increased amounts of it being released to the general environment. We demonstrated that, at relatively low concentrations, Cd^{++} can induce chromosome aberrations in mammalian cells grown *in vitro*; however, previous studies in other laboratories on the clastogenic properties of Cd^{++} are equivocal.

The positive[2-4] and negative[5-7] results in the available literature suggest that technical factors may be involved in the detection of cadmium-induced cytogenetic damage. Therefore, we initiated studies to determine the concentration of cadmium necessary to induce chromosome damage and if this threshold concentration can be altered by culture conditions. Specifically, we observed in our previous studies[1] that CHO cells grown in F-10 medium supplemented with 15% cadet calf serum began to show chromosome damage at 24 h after addition of 1×10^{-6} M $CdCl_2$. However, when CHO cells were grown in F-10 medium containing 20% fetal calf serum, the Cd^{++} concentration had to be increased to 2×10^{-6} M to induce chromosomal aberrations. It was not clear whether the observed difference was due to the concentration or to the type of serum in the medium, but the change in threshold concentration of Cd^{++} is potentially very important because of differences in serum supplementation in different laboratories and because serum concentrations are on the order of 50% v/v in mammalian circulatory systems.

Another report from this Laboratory[8] contains data on the partitioning of Cd^{++} between the nucleus

and cytoplasm of cells grown in vitro in the presence of cadmium chloride. The cadmium level and time of exposure necessary to induce chromosome damage are highly correlated with intracellular distribution. Continued elucidation of the cellular events initiated by Cd^{++} exposure should eventually provide a clear understanding of the relationship between Cd^{++} and chromosome damage and may determine whether or not chromosome analysis is an appropriate method for the detection of Cd^{++} toxicity.

II. METHODS

Chinese hamster ovary (line CHO) cells were maintained free of Mycoplasma as monolayers or in suspension culture in Ham's F-10 medium supplemented with fetal calf, newborn calf, cadet calf, or human serum, penicillin, and streptomycin. Commercial sources of sera are Reheis, Division of Armour Pharmaceutical Company, fetal calf; Biocell Laboratories, cadet calf and newborn calf; and Grand Island Biological Company, human. To simulate cellular environment in a circulatory system, as well as conditions commonly used for in vitro cell culture, serum concentrations in the F-10 medium were varied from 5 to 50% v/v. Cadmium chloride stock solutions were prepared in 0.1 M HCl and diluted with sterile H_2O in polyethylene containers.

Cadmium is known to induce the synthesis of a specific protein, metallothionein, which sequesters cadmium and by this mechanism stores it in the tissue of certain organs (e.g., liver, kidney).[2] Cadmium exposure, then, may include constant or intermittent low levels of the metal in the circulatory system, with higher levels accumulating in the kidney and/or liver. To simulate these conditions in vitro, cells were grown in the presence of low levels of cadmium which do not induce chromosome damage[1] for relatively long periods of time (12 wk) to see if this treatment would have any effect on cellular resistance to higher levels. Final concentrations of cadmium for these experiments varied from 2×10^{-7} M to 2×10^{-6} M. In all experiments, cell concentration was determined by electronic particle counting.

III. RESULTS

Cells grown in F-10 medium supplemented with 15% cadet calf serum and challenged with 2×10^{-6} M Cd^{++} began to show growth rate effects at 4 h after addition of the cadmium and, by 20 h, growth nearly ceased (Fig. 1). A few of these cells continued to progress to metaphase until 72 h after Cd^{++} addition. Chromosome aberrations, primarily of the chromatid type, began to appear at 24 h after treatment and, by 48 h, approximately 40% of the cells had chromatid damage in one or more chromosomes. By 72 h, 80% of the cells in metaphase had shattered chromosomes. While a picture of increasing effect over time emerges from these studies, it should be pointed out that the effects were not uniformly distributed among all cells (i.e., some normal metaphase cells can be found at each of the collection points).

When the Cd^{++} concentration was lowered to 2×10^{-7} M, cell growth rate remained unaffected and metaphase cells in these populations showed no chromosome abnormalities even after 12 wk of exposure. When these cells were challenged with a lethal dose of Cd^{++} (2×10^{-6} M), they continued to grow at normal rates (Fig. 2) and did not sustain any chromosome damage. This induced resistance has been shown by Enger et al.[9] to be correlated with an increase in production of metallothionein.

The effects of increasing concentrations of human serum in F-10 medium are illustrated in Figs. 3 and 4. Figure 3 shows the effect of adding

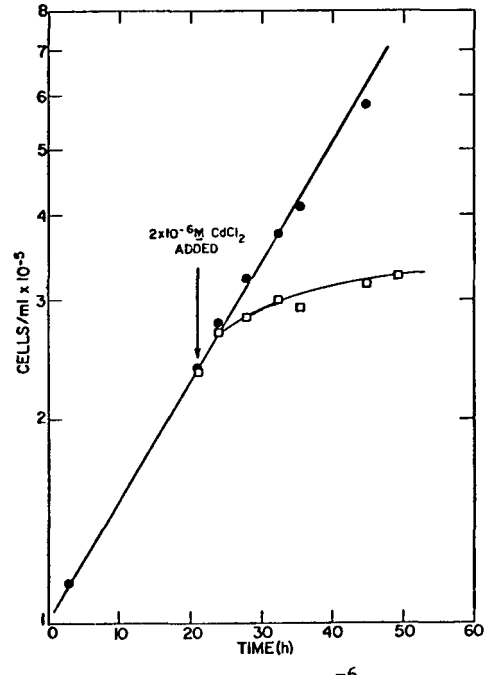

Fig. 1. Growth effects of 2×10^{-6} M $CdCl_2$ on Chinese hamster (line CHO) cells: (-●-) control and (-□-) 2×10^{-6} M $CdCl_2$.

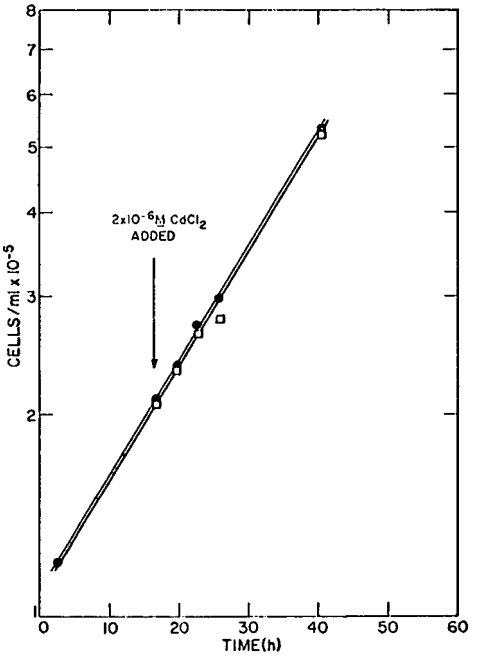

Fig. 2. Growth effects of 2 x 10^{-6} M $CdCl_2$ on Chinese hamster (line CHO) cells previously grown in the presence of 2 x 10^{-7} M $CdCl_2$; (-●-) control and (-○-) 2 x 10^{-6} M $CdCl_2$.

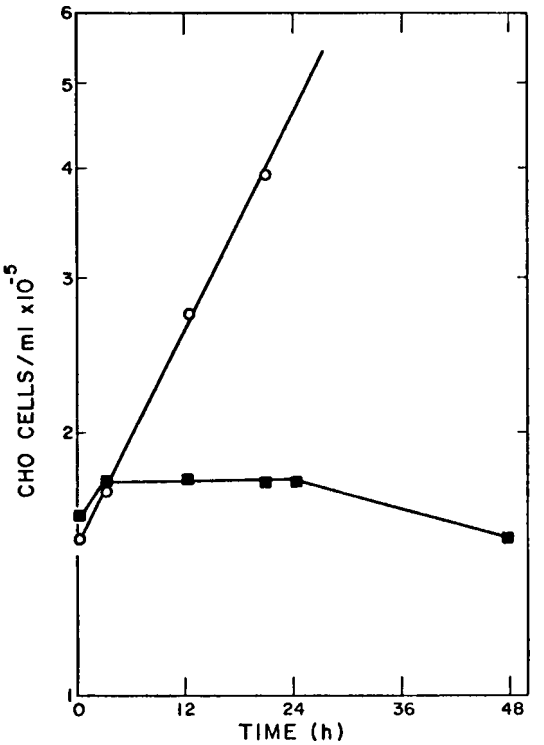

Fig. 3. Growth effects of 1 x 10^{-6} M $CdCl_2$ on Chinese hamster (line CHO) cells growing in F-10 medium supplemented with 15% human serum: (-○-) control and (-■-) 1 x 10^{-6} M $CdCl_2$.

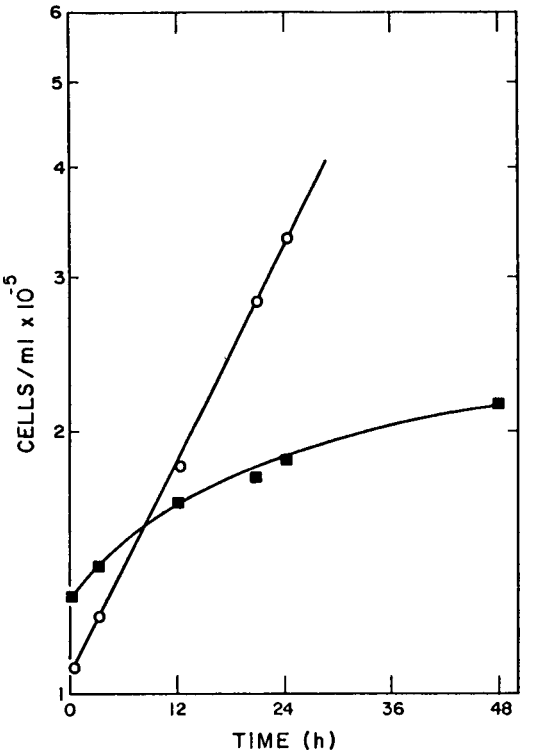

Fig. 4. Growth effects of 1 x 10^{-6} M $CdCl_2$ on Chinese hamster (line CHO) cells growing in F-10 medium supplemented with 50% human serum: (-○-) control and (-■-) 1 x 10^{-6} M $CdCl_2$.

1 x 10^{-6} M $CdCl_2$ to exponentially growing CHO cells in medium supplemented with 15% human serum. Cell growth stopped shortly (3 h) after the addition of Cd^{++} and, by 24 h, cell concentration began to decrease. A similar result occurred in cell populations grown in medium containing 20% human serum. At higher serum concentrations of 30 and 50% (see Fig. 4), cells were partially protected, at least in terms of growth rate. Growth studies with increasing concentrations of newborn calf serum gave similar results to those with human serum.

When cells were grown in increasing concentrations of fetal calf serum, however, there was a sharp increase in protection against the damaging effects of Cd^{++}, even at low concentrations of 15% serum (Fig. 5). At each serum concentration examined (15, 20, 30, and 50%), the addition of 1 x 10^{-6} M Cd^{++} had no effect on cell proliferation.

Greater insight into the effects of serum type and concentration on Cd^{++} toxicity was obtained by measuring the $^{109}Cd^{++}$ uptake as a function of time, serum concentration, and serum type. The data are

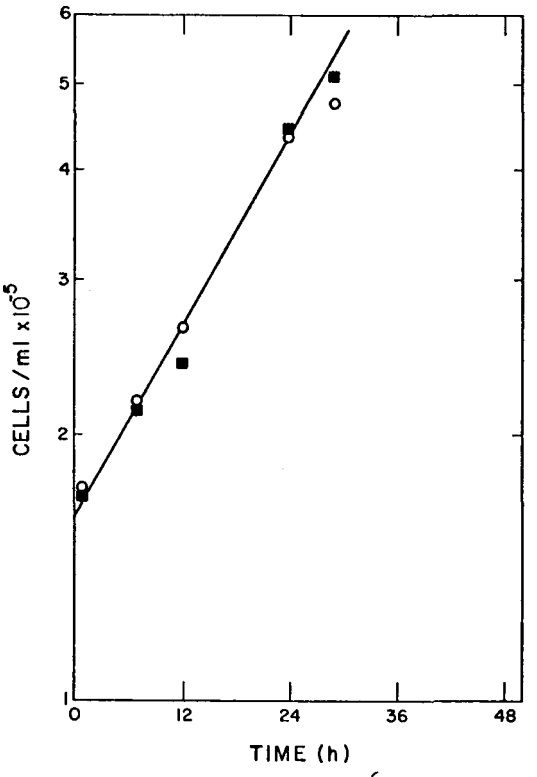

Fig. 5. Growth effects of 1×10^{-6} M $CdCl_2$ on Chinese hamster (line CHO) cells growing in F-10 medium supplemented with 15% fetal calf serum: (-O-) control and (-■-) 1×10^{-6} M $CdCl_2$.

presented in Table I. These results are in good agreement with the growth rate data. If these values are averaged, it is clear that more cadmium enters CHO cells grown in human serum (95 $\mu g/10^9$ cells) or newborn calf serum (86 $\mu g/10^9$ cells) than cells grown in fetal calf serum (12 $\mu g/10^9$ cells).

IV. DISCUSSION

It is clear from the studies on induction of resistance to Cd^{++} that the history of Cd^{++} exposure plays an important role in cellular response with regard to growth and appearance of chromosome aberrations. It has been suggested that the ability for cells to synthesize high levels of metallothionein is limited to cells from the liver or kidney.[10] However, human fetal skin and muscle fibroblasts have been reported to develop resistance via this mechanism,[11] and these data plus the results reported herein with CHO cells suggest that this property may be common to mammalian cells regardless of cell type. It is conceivable that certain cell lines *in vitro* may be more resistant than others on the basis of their origin and previous Cd^{++} exposure. This variability could result in the establishment of variable threshold values for induction of chromosome damage. Likewise, *in vivo* studies could be misleading if similar mechanisms are involved. Individuals who are constantly exposed to low levels of Cd^{++} might show more resistance to chromosome and/or cellular damage by Cd^{++} than those not previously exposed.

The studies on serum type and concentration suggest that cell growth perturbation and induction of chromosome aberrations by Cd^{++} are related to culture conditions. This observation is especially important because established protocols for the cultivation of human lymphocytes may utilize human, fetal calf, or newborn calf sera. Cell culture media are usually supplemented with fetal or newborn

TABLE I

EFFECTS OF SERUM TYPE AND CONCENTRATION ON CADMIUM UPTAKE[a] BY CHO CELLS

Serum Concentration (%)	Hours after Cd^{++} Addition	Human Serum	Newborn Calf Serum	Fetal Calf Serum
15	24	78	90	17
15	48	74	83	16
20	24	89	94	10
20	48	80	100	23
30	24	102	82	4
30	48	105	90	12
50	24	114	74	4
50	48	118	78	8

[a]Expressed as $\mu g\ Cd^{++}/10^9$ cells.

calf sera or combinations of the two in variable concentrations. It is clear from the results presented in this report that standardized protocols must be established for experimental results to be comparable between laboratories. Although it is not clear at the present time why fetal calf serum is more protective than adult serum, this characteristic may be related to the high levels of protein-glutathione mixed disulfides found in fetal calf serum.[12] These high levels vary with gestation time and decrease to below detectable levels in near-term calves.

If the data in Table I are compared to growth rate data, they suggest that toxicity is related to cellular uptake of Cd^{++}. However, if the data for human serum are compared with Figs. 3 and 4, this relationship does not hold. Rather, the partial protection of high concentrations of human serum (Fig. 4) is correlated with an increase in intracellular Cd^{++}. This may suggest that there are at least two mechanisms for serum protection against Cd^{++} toxicity. One type of protection involves metal binding by serum proteins which are not accumulated by cells (fetal calf serum), and the other involves metal binding proteins which enter the cell but which maintain Cd^{++} in a nontoxic state (human serum).

An alternative explanation for the gross differences in protection against Cd^{++} between adult and fetal serum may involve the heat inactivation necessary for adult but not for fetal serum. This possibility is being examined, but it should be pointed out that our present results are relevant to *in vitro* culture conditions regardless of the outcome of this study. Normal treatment of adult serum involves heat inactivation, while fetal serum is utilized in tissue culture without heat treatment.

Further studies will involve examination of cells derived from several different tissues from one animal and of the cytogenetic response to Cd^{++} by several commonly used mammalian cell lines. We have demonstrated in this report that culture conditions can alter cellular response to Cd^{++}, but the question of genetic predisposition of certain cells has not been addressed. All these variables demonstrate the need for detailed analysis of the effects of toxic metals at the cellular level before meaningful interpretations of *in vivo* data can be made.

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