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Effect of Cadmium Chloride on Cell Division and Chromosomes in Chinese Hamster Ovary Cells

B. C. Lakkad, S. K. Nigam, A. B. Karnik, K. N. Thakore, and B. B. Chatterjee

National Institute of Occupational Health, Meghani Nagar, Ahmedabad-380016, India

Effect of cadmium chloride on cell division and chromosomes was studied in Chinese Hamster Ovary (CHO) cells in vitro. The cell cultures were exposed to various concentrations of cadmium chloride. Different treatment of cadmium chloride resulted in various cell division abnormalities like micronucleus formation, lagging chromosome, and chromatid bridges. At higher doses, an abundance of pyknotic nuclei in the monolayer was noteworthy. The 24 hours continuous treatment caused chromosomal aberrations like chromatid gaps, breaks, exchanges and chromatid separation.

Carcinogenic potential of the cadmium have been studied experimentally in animals (Gunn et al 1963, 1964; Haddow et al 1964; Heath and Daniel 1964; Heath and Webb 1967; Heath et al 1962; Kazantzis 1963; Kazantzis and Hanbury 1966; Kolonel 1976; Roe et al 1969). Cadmium has been shown to cause reproductive toxicity (Dwivedi et al 1977), testicular necrosis (Clegg and Carr 1967), and effects on meiosis (Fowler et al 1982). Recent reports suggest that chromosomal aberration analysis may prove to be a sensitive biological indicator of the mutagenic and carcinogenic potential of an environmental hazard (ICPEMC 1983; Evans 1977; Obe et al 1982).

There are very few reports which provide evidence that cadmium is able to induce genetic effects. Shiraishi and Yosida (1972) reported chromosome aberration in 'Itai-Itai' patients, whereas Bui et al (1975) could not demonstrate any such effects in cadmium exposed workers. Dekundt and Leonard (1975) found a significant increase in these anomalies. Gilliavod and Leonard (1975) have reported no chromosome abnormalities in mice exposed to cadmium in the dosage of 1.75mg/kg. Doyle et al (1974) exposed lambs to cadmium for 191 days and found a significant increase in chromosomal abnormalities. Available data thus do not show any consistent pattern and clearly indicate a need for further

study.

In the present experimental set up, an attempt has been made to study toxic effect of cadmium chloride on cell division and chromosomes using cell cultures.

MATERIALS AND METHODS

Chinese hamster ovary (CHO) cell line (stock from National Institute of Virology, Poona) was maintained in minimum essential medium with non-essential amino-acids supplemented with 15% goat serum and antibiotics (Penicillin 100 units/ml and Streptomycin 50 ug/ml).

In the experiments on cell division the cells were grown in Leighton tubes on coverslips (10x55mm). The cells were exposed to 0.1, 0.25, 0.5 and 1 ug of cadmium chloride at half confluency after changing medium. The cells were washed with phosphate buffer saline and then fixed *in situ* with 1:3 aceto methanol, at different time intervals.

Slide preparations of the cells were stained with 4% Geimsa stain in Sorenson's buffer pH 7.0, cleared and mounted in DPX. The slides were coded and scored at x 400 magnification. About 500 cells, for each dose and time, were counted to record different cytomorphological and cell division abnormalities.

The effects of $CdCl_2$ on chromosome were studied in to the cell cultures grown in bottles. The cells were exposed to different concentrations of cadmium chloride as mentioned earlier, continuously for 24 hours. Three hours before harvesting, the cultures were treated with colchicine (0.5 ug/ml) to arrest cell division at metaphase. The chromosome preparations were made according to the conventional air drying technique. The slides were stained in 2% Geimsa in Sorenson's buffer for 10-15 mins. About 100 well spread metaphase plates from each treatment dose were counted to note various chromosomal aberrations.

RESULTS AND DISCUSSION

The continuous treatment resulted in various mitotic abnormalities like micronucleus formation (Fig.1B) lagging chromosomes (Fig.1C) and chromatid bridges between the separating sets of chromosomes (Fig.1D). The significant changes observed were multinucleated giant cells, heteropyknotic nuclei and degeneration of cells (Fig.1E). These effects were more prominent at the higher doses. The treated culture showed gradual increase in per cent abnormal cells. The abnormalities observed were time and dose dependent.

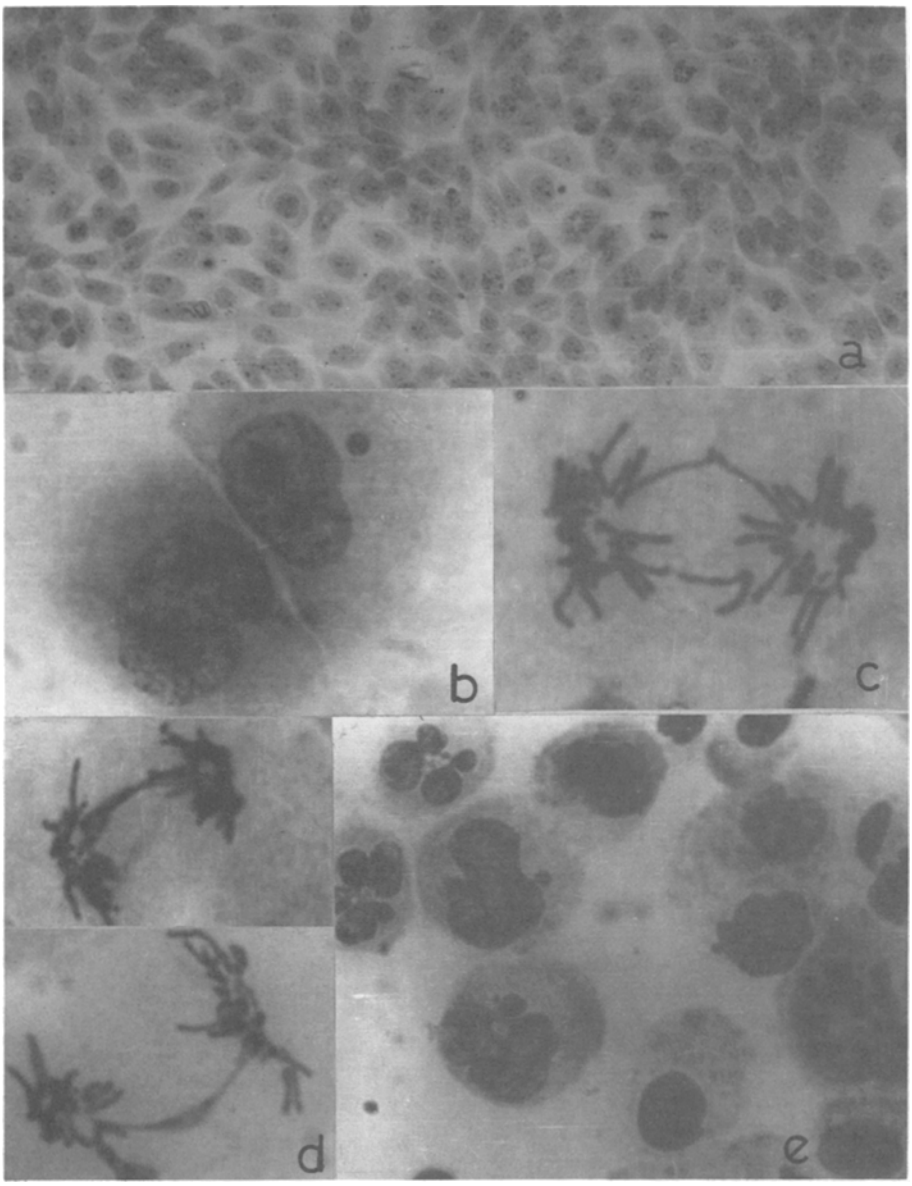

Figure 1. CHO cells showing various abnormalities (a) control (b) micronucleus formation (c) lagging chromosome (d) chromatin bridge and (e) cells with heteropyknotic, multiple nuclei.

The results of cell division study have clearly showed mitodepressive and mitotoxic effects of cadmium. Cadmium have high affinity towards the sulfhydryl and disulfide groups. The effect of cadmium on cell division

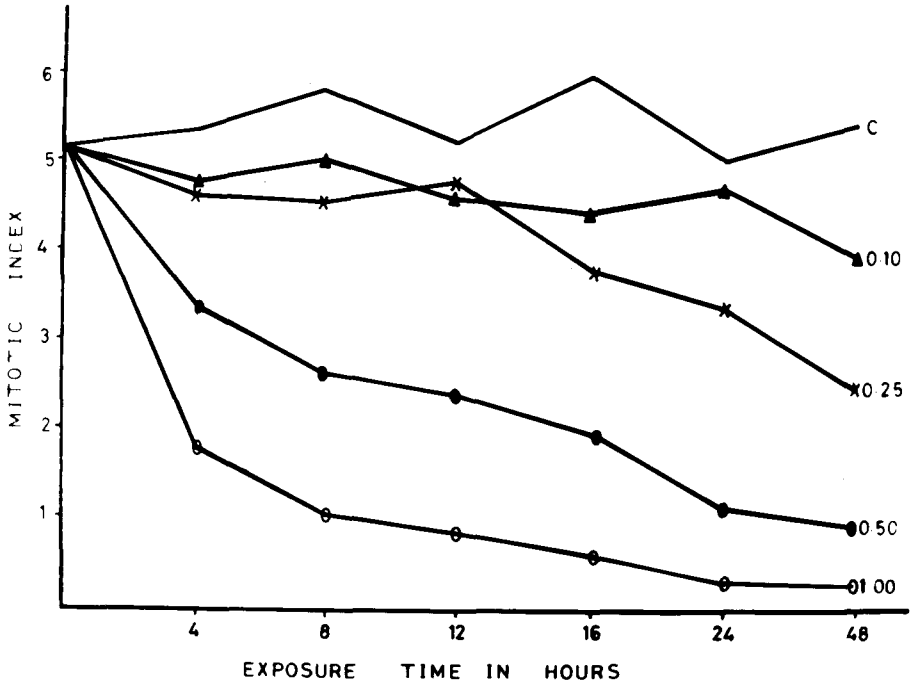

Figure 2. Effect of $CdCl_2$ treatment on Mitotic index in CHO Cells.

seen in the present study may presumably be due to interaction with the sulfhydryl groups of the spindle forming proteins. It is clear from this study that cadmium chloride show dose dependent toxicity in CHO cells. At higher concentration (1.0 ug) mitodepressive effect is distinct by 4 hours (Fig.2). The cytotoxic morphological manifestations in treated cells were micronuclei formation, multinucleated cells, increase in cell volumes and the presence of heteropyknotic nuclei of treated cells. Similar changes were observed by Rohr and Bauchinger (1976).

Cell exposed to cadmium chloride continuously for 24 hrs developed a variety of chromosomal abnormalities. These include chromatid gaps (Fig.3B), breaks, fragments (Fig.3C), exchanges (Fig.3D) and partial and/or total pulverisation (Fig.3E). There is a progressive increase in the number of total chromosomal aberrations and relative number of metaphases with affected chromosomes, with increases in the intensity of exposure to cadmium (Table 1).

The results of chromosome analysis in the present investigation is also similar to the earlier reports of Shirashi and Yosida (1972) and Rohr and Bauchinger

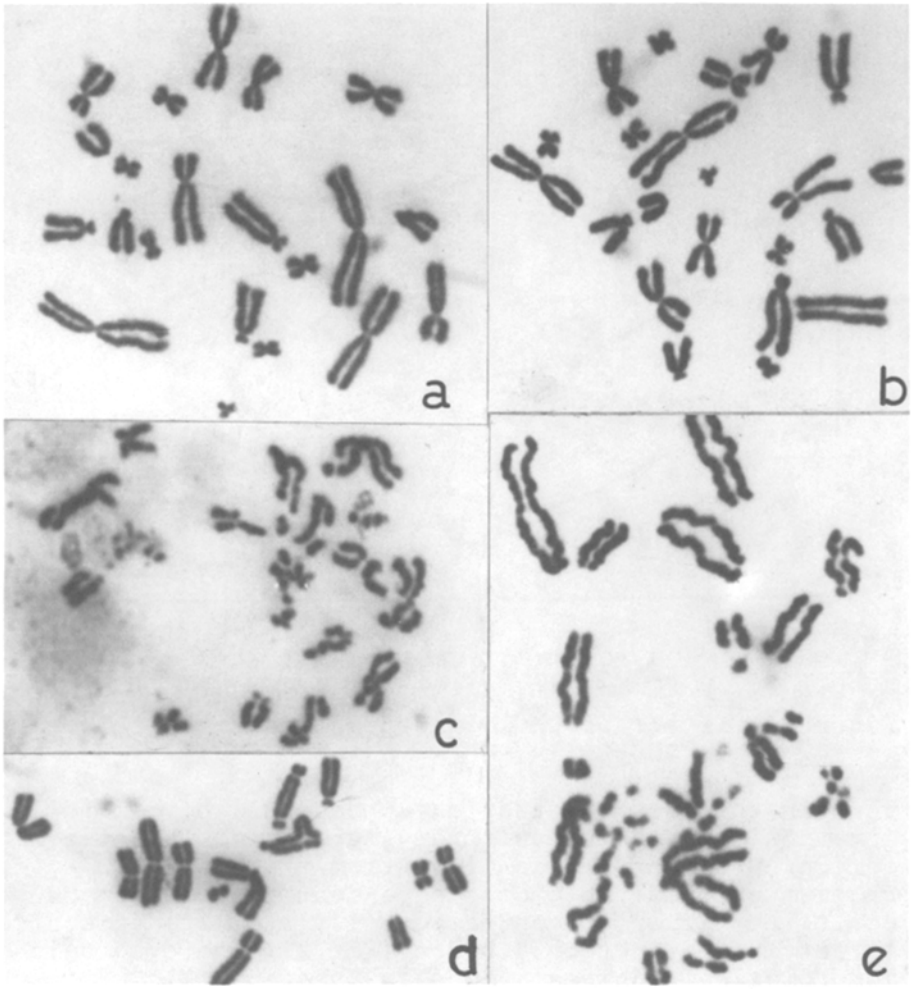

Figure 3. Chromosomal abnormalities caused by $CdCl_2$ treatment (a) control (b) gap and acentric fragment (c) breaks (d) exchange figure and dicentric chromosome and (e) pulverization.

(1976) who reported structural chromosomal abnormalities as also chromosomal stickiness and pyknosis. At the cellular level cadmium has been shown to cause chromatin condensation followed by accumulation of perichromatin granules. Thus the chromosomal aberrations observed in the present study and also reported by the above authors and also by Shirashi et al (1972) clearly indicate the toxipotential of cadmium. The mechanism of chromosomal aberration as observed in the study may be due either to direct action of cadmium on chromatin causing irreversible condensation (Morsolt et al 1983) or due to an indirect effect (Derenzini

Table 1. Effect of cadmium chloride on CHO chromosomes

Treatment* (ug/ml)	Total cells counted	Chromatid gaps	Chromatid breaks	Chromatin fragments	Dicentric & exchanges	Acute damage
Control	100	2	1	-	-	-
0.10	98	6	8	2	12	-
0.25	99	5	10	5	14	12
0.50	82	7	11	6	15	19
1.00	52	1	2	8	4	30

* = 24 hours continuous treatment.

et al 1981).

These anomalies are of much significance in evaluating the cytotoxic effects of cadmium and its salts. On the basis of the present study it may be concluded that cadmium chloride is mitodepressive, mitotoxic and cytotoxic and,therefore, precautionary measures are strongly advocated in controlling exposure to the metal at work places and also potential exposures from the ambient environment.

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