LA-6451-PR
Progress Report

UC-48
Issued: August 1976

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January 1—June 30, 1976

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UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
CONTRACT W-7405-ENG. 36

This is the first report in this series.

This work was supported by the US Energy Research and Development Administration and the US Environmental Protection Agency, LASL Project R251, EPA Agreement EPA-IAG-D5-0681.

Printed in the United States of America. Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161
Price: Printed Copy $3.50 Microfiche $2.25

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I. INTRODUCTION

Chromosome aberration analysis may prove to be an effective biological dosimeter for the mutagenic potential of environmental hazards. It is the goal of this project to develop rapid and inexpensive methods to assess the extent of chromosome damage induced by toxic agents associated with energy production, conversion, or utilization. Our first presentation[1] of data concerned current efforts directed toward automated chromosome analysis using flow systems. This report will summarize data on the chromosomal effects of cadmium, a toxic agent associated with coal processing and combustion. When these studies are completed, we will attempt to use the flow-system technology to evaluate the chromosomal damage in cell populations induced by cadmium. Such a system could then be applied to other environmental toxic agents associated with energy production. We selected cadmium for a number of reasons.

(1) Additional quantities of cadmium will be presented to the environment as a result of increased coal utilization and oil shale extraction.

(2) Cadmium is known to be toxic to man (acute toxicity results from ingestion of 3 g or inhalation of 40 mg, while chronic exposures may result in pulmonary disorders, renal damage, or osteoporosis).

(3) The existing data on cytogenetic effects of cadmium are equivocal, with some investigators demonstrating chromosome damage while others do not.

(4) Another project at this Laboratory[2] is concerned with the cellular localization of cadmium and its effects on informational macromolecules.

II. METHODS

Chinese hamster ovary cells were grown as monolayers in Ham's F-10 medium supplemented with 20% fetal calf serum. Cadmium chloride stock solutions were prepared in 0.1 M HCl and diluted with sterile H_2O in polyethylene containers. Cadmium was added to exponentially growing cell cultures at concentrations ranging from 1×10^{-4} M to 1×10^{-7} M. At the highest concentrations (10^{-4} and 10^{-5} M), cell growth stops immediately after adding Cd^{++}; at the lowest concentrations (10^{-6} and 10^{-7} M), cells continue to grow for long periods of time (> 3 wk). Therefore, different protocols were used to examine the effects of high and low Cd^{++} concentrations. Cells exposed to high concentrations of Cd^{++} were reversed after a short exposure (2 h) and analyzed for chromosome aberrations during a recovery period of 24 h, whereas cells exposed to lower concentrations of Cd^{++} were analyzed for chromosome aberrations at 12-h intervals after addition of the cadmium chloride.

III. RESULTS AND DISCUSSION

The type of chromosome damage induced by Cd^{++} can be demonstrated by treating the cells at a concentration of 2×10^{-6} M. Under these conditions, cells show no damage up to 24 h after addition of the metal (Fig. 1A). At 48 h after treatment, about 40% of the cells have chromatid damage in one or more chromosomes (Fig. 1B); a few of the cells at this time have multiple chromatid aberrations affecting many chromosomes (Fig. 1C). At 72 h, 80% of the metaphase cells have shattered chromosomes (Fig. 1D). At higher concentrations (10^{-4} M, 10^{-5} M), the same type of aberrations are found in the cell populations for up to 24 h after reversal of the insult. In contrast to these results are cells treated at a concentration of 1×10^{-6} M $CdCl_2$. These cells continue to grow for at least 3 wk and show only occasional (> 1%) chromatid deletions, but after treatment for 1 wk, an increased number of tetraploid cells appear in the population (6%).

All concentrations of Cd^{++} studied to date have a mitotic inhibitory effect, indicating that this element in some way disrupts the mitotic spindle apparatus. Electron microscopic studies are now in progress to determine the mechanism of this disruption. Other studies in progress include comparative analyses between flow microfluorometry of whole cells and chromosome aberration induction to correlate cell-cycle effects with chromosome effects and analysis of sister chromatid exchange rates in cells treated with low levels of Cd^{++} but which do not show

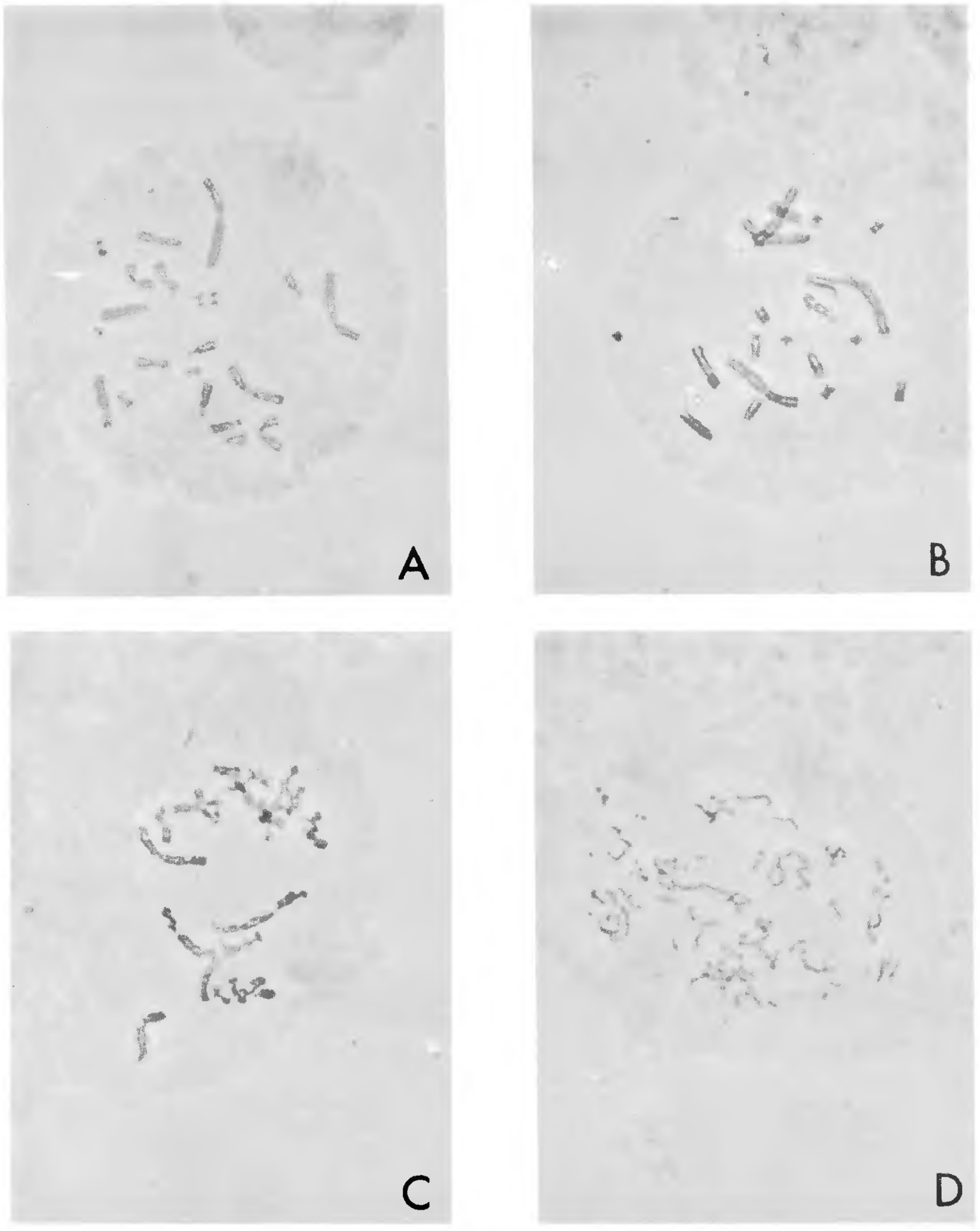

Fig. 1.
Chromosomes of Chinese hamster ovary cells after exposure to 2×10^{-6} M Cd^{++} for 24 h (A), for 48 h (B) and (C), and for 72 h (D).

visible chromosomal damage. Our results clearly indicate that relatively low levels of cadmium can induce chromosome aberrations in mammalian cells *in vitro*. It is also known that certain tissues (e.g., kidneys, liver) can produce excess amounts of thionein which binds cadmium as a nontoxic complex. We plan to use Chinese hamster kidney cultures to see what effect this binding has on chromosome aberrations and on cell toxicity.

Cadmium appears to be a toxic agent which may be detected in low levels by chromosome analysis. Because increased amounts may be released into the environment by new energy technologies, it warrants further study. At the same time, these studies may help to further our understanding of chromosome breaks *per se* and of the effects of these breaks on cells and organisms.

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