Inhibition of DNA replication and repair by cadmium in mammalian cells. Protective interaction of zinc

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ABSTRACT

The effects of the treatment of cultured human and simian cells with cadmium (Cd), a toxic and carcinogenic metal, were first assayed on macromolecular synthesis. It was observed that DNA synthesis was inhibited by Cd concentrations considerably lower than those inhibiting protein and RNA synthesis. Because of the necessary occurrence of a DNA resynthesis step during the DNA excision repair process, the consequences of the exposure of cells to Cd were ulteriorly tested on different parameters measuring DNA repair after ultraviolet (UV) damage. UV-induced unscheduled DNA synthesis (UDS) was found 2-3 times lower in Cd (4 x 10^{-6} M) treated cells than in control cells for UV doses higher than 10 J/m^2. DNA breaks accumulated in UV-irradiated cells during post-exposure incubation in presence of Cd, whereas they were induced only transiently in control cells irradiated with the same dose. Cd inhibited in a concentration-dependent way the recovery of RNA transcription impaired by UV-irradiation. However, at concentrations used, Cd had no significant effects on DNA size and on rRNA synthesis in unirradiated cells. Finally, Cd was shown to inhibit the repair of potentially lethal damage during a 24 h liquid holding and to increase the toxicity of UV-irradiation.

The interactions between Cd and Zinc (Zn), an essential metal for many enzymatic proteins, were also analysed. Results showed that Zn, at 5 to 10 times higher concentrations, counteracts the inhibitory effects of Cd on DNA synthesis and restores, at least partially, the repair capability of cells and their survival. The possible molecular level and mechanism of action of these metals are discussed.

INTRODUCTION

Cd and Zn are elements of group II B in the periodic table. They have different ionic radius, but identical d^{10} s^2 electron configuration and, thus, similar chemical properties. Physiologically, Cd and Zn constitute a couple toxic metal/essential metal.

Cd is an extremely toxic element even if present as traces in cell DNA (1). In the animal it has sterilizing, teratogenic and carcinogenic effects (see for review 2). Very mutagenic for plants, data are however contradictory in mammals. Its direct carcinogenic activity for man is controversial, but it is admitted that Cd could increase the mutagenic action of other substances and thus constitute an important potential risk. In cell cultures Cd is cytotoxic (3, 4), mutagenic (5) and transformant (6). At the molecular level, Cd modifies DNA and RNA metabolism in a variety of organisms and cells in culture: RNA synthesis may be increased or decreased, thymidine
incorporation is always inhibited. In vitro, Cd affects the activity of DNA polymerase I of E. coli (7) and of human δ DNA polymerase (8) and decreases the fidelity of DNA synthesis by DNA polymerase of avian myeloblastosis virus (9). Repairable DNA single-strand breaks or unrepairable damage have been observed in E. coli (10) and human lymphocytes (11) exposed to Cd.

Zn is essential for the functioning and/or the structure of numerous enzymes, including RNA and DNA polymerases and superoxide dismutase (see for review 12-14). Zn is present in the DNA, particularly concentrated in reiterative regions, and in the RNA whose stability and probably metabolism it affects (1). The role of Zn in the biosynthesis of nucleic acids seems fundamental: it is indispensable to the activity of DNA (15, 16) and RNA polymerases of E. coli (17) and of reverse transcriptase of avian myeloblastosis virus in vitro (18); its deficiency inhibits the incorporation of DNA and RNA precursors in the animal (19) and in cultured cells (20), blocks cell division and provokes morphological and functional defects (21).

The existence of interactions between Zn and Cd in living systems (see for review 22) and their importance are demonstrated by the fact that a combined treatment with Zn can protect against the toxic (23) and carcinogenic effects (24) of Cd in rodents. Simultaneous administration of Zn reduces the toxicity of Cd on cultured cells (3, 4). Both Zn deficiency and Cd administration cause teratogenic effects in the animal and the effects of both are reversed by Zn (25).

To get further insight on the level and the mechanism of action of Cd, its effects on DNA, RNA and protein synthesis were, first, studied in cultured cells. The influence of simultaneous or delayed additions of Zn to the culture medium was also examined. In view of results indicating that Cd acts by preferentially inhibiting DNA synthesis, other experiments were designed to test a possible effect of Cd on DNA repair after UV damage, since this process requires the occurrence of some DNA synthesis, even if non semi-conservative in nature (26). Therefore, the repair capability of UV-irradiated cells submitted to low concentrations of metal ions was determined by three different approaches: i) by autoradiographic measurement of UDS addressing to the step of repair replication following excision of lesions (26); ii) by alkaline elution analysis (27) of induction of single-strand breaks and of their subsequent disappearance as gap filling and ligation occur (28); and iii) by transcriptional analysis of the template capability of cell DNA impaired by UV-damage and eventually restored by a repair process in a fully functional way (29). Finally the effects of metal ions on DNA repair of UV-irradiated cells were determined by their consequences on cell survival.

Results reported here show that Cd has an important inhibitory effect on the DNA repair process(es) and that Zn, at 5-10 times higher concentrations, is able to restore, at least partially, the repair capability of irradiated cells.
MATERIALS AND METHODS

Cells

A diploid fibroblast strain Jac derived from a normal child, obtained from D. Pham Dim (Paris), and a continuous line of african green monkey kidney cells CV-1 have been employed. Both human and monkey cells were cultured in Eagle's minimal essential medium (MEM) supplemented with 15% fetal calf serum or with 5% new-born calf serum, respectively, and with 20 μg/l gentamycin at 37°C in an atmosphere of 5% CO₂ : air.

Metal ions

Chloride salts (Merck) of the metals were used. Molar stock solutions were in distilled water. Metal ions were diluted just before use to the indicated concentrations in complete medium and added to cultures for the appropriate time interval according to the experiment.

Macromolecular synthesis

Exponentially growing CV-1 cells were incubated at 37°C with metal ions at different concentrations in complete medium for the indicated time intervals. Thereafter [³H]-thymidine (4 μCi/ml, 74 Ci/m mole), [³H]-uridine (4 μCi/ml, 26 Ci/m mole) or [³H]-tyrosine (5 μCi/ml, 52 Ci/m mole) were added for 1 h to label DNA, RNA or protein. After the radiolabelling pulse, cultures were washed with cold phosphate-buffered saline (PBS) and lyzed in 0.5 M NaOH, when labelled in their DNA, or trypsinized, when labelled in their RNA or proteins. Acid-insoluble material was precipitated in 10% trichloro-acetic acid (TCA), filtered on GF/C Whatman glass fibre filters, thoroughly washed with 5% TCA and 95% ethanol and dried. The precipitates on filters were dissolved overnight with 0.2 ml solulyte (3. T. Baker Chemicals). The radioactivity (dpm) was measured after addition of scintillation fluid (Econofluor NEN) in a LKB 1216 Rackbeta II spectrometer.

Cell survival

The clonogenicity of UV-irradiated CV-1 cells was tested immediately after exposure or after 24 h of liquid holding recovery in complete medium in presence or in absence of metal ions. After treatment, cells were rinsed, trypsinized and seeded in appropriate number in Petri dishes (6 cm diameter) containing 3 x 10⁴ feeder CV-1 cells irradiated at 45 Gy X-rays, in MEM medium supplemented with 5% fetal calf serum. Colonies were counted after 2 weeks.

Unscheduled DNA synthesis

Experiments were carried out in confluent Jac cells. Cultures were UV-irradiated and then labelled for 3 h with [³H]-thymidine (10 μCi/ml, 74 Ci/m mole) in medium containing or not metal ions. Thereafter cultures were washed with PBS, fixed
with 3% glutaraldehyde and rinsed with distilled water. Autoradiographs were prepared with K12 Ilford emulsion.

**Alkaline elution**

Jac cells (3 x 10^5) were seeded into Petri dishes (6 cm diameter) and labelled with I^Cl-thymidine (0.4 μCi/ml, 52 Ci/mole). After 48-72 h and 25 h before the starting of alkaline elution, considered as time zero, radioactive label was removed and replaced with fresh medium containing or not metal ions. At different time intervals (24 h, 3 h or 0 h) before time zero, cells were UV-irradiated with a single dose of 254 nm UV light. After irradiation, the medium previously removed was added back to the cultures which were incubated in the dark at 37°C until alkaline elution of their DNA was carried out according to the technique of Kohn et al. (27). Cells were scraped off the dishes, mixed with an appropriate number of I^3Hl-thymidine-labelled V-79 cells irradiated on ice with 2 Gy of X-rays to serve as internal standard of elution, and washed onto polycarbonate filters (2 μm pore size) with ice-cold PBS. Lysis solution (5 ml, 2% sodium dodecyl sulfate (SDS), 0.025 M ethylenediamine tetracetic acid (EDTA), pH 9.7) was added 15 min before being allowed to run through the filter under gravity. The lysate was washed with 3 ml of 0.02 M EDTA, pH 10. The DNA was then eluted from the filters in the dark by pumping the elution solution (2% tetrapropylammonium hydroxyde, 0.02 M EDTA, 0.1% SDS, pH 12.1) at a constant speed of 2.4 ml/h. Fractions were collected every 90 min. Filters were counted after depurination in 1 N HCl at 60°C for 1 h followed by neutralisation with 0.4 N NaOH. The I^4C and I^3H dpm for each fraction, automatically corrected for spillover, were plotted as the log percentage I^4C-DNA versus the log percentage I^3Hl-DNA retained on the filter.

**RNA transcription analysis**

For transcription analysis, the medium of confluent monolayers of cells (CV-1 or Jac) was replaced with fresh medium containing or not metal ions at 25 h before labelling. The labelling time with I^3Hl-uridine was considered as time zero. At time - 24 h or - 15 min, different lots of cultures were irradiated with a single dose of 254 nm-UV light. After irradiation, the medium previously removed was added back to the cultures which were incubated in the dark at 37°C until time zero. Cultures were labelled with 25 μCi/ml of I^3Hl-uridine (25 Ci/mmole) for 1 h at 37°C. They were then washed with PBS and chased for 6 h in medium containing a 1000-fold excess of unlabelled uridine. Labelling and chase medium also contained metal ions.

RNA extraction was carried out at room temperature. After the chase, cells were rinsed with PBS and lysed with 2 ml of lysis buffer (10 mM tris-hydroxymethyl aminomethane-chloride (Tris) (pH 7.5), 50 mM NaCl, 1% Na tri-isopropylhydrasulphonic acid, 6% Na 4-amino-salicylate, 6% 2-butanol). The lysate was transferred to a
centrifuge tube containing 2 ml of phenol buffer (phenol, water, 8-hydroxyquinoline, m-cresol : 50 v ; 10 v ; 0.05 v ; 7 v) stirred, and centrifuged 10 min at 2000 g. The organic phase was removed. The phenol layer and the interphase were adjusted to a final concentration of 0.5 M NaCl and reextracted, first, with phenol buffer and subsequently with chloroform-isooamyl alcohol (24:1). The aqueous phase was collected and nucleic acids were precipitated with 2 volumes of ethanol and centrifuged for 20 min at 15000 g. The pellet was dissolved in 0.2 ml of 0.1 M Na acetate and raised to 3 M Na acetate. The solution was maintained 24 h at 4°C. The precipitated RNA was pelleted by centrifugation and analysed by electrophoresis. Electrophoresis was carried out in 1.7% polyacrylamide - 0.5% agarose, cylindrical gels (20 cm long, 0.6 cm diameter) previously soaked overnight in electrophoresis buffer (89 mM Tris, 2.5 mM EDTA disodium salt, 89 mM boric acid, 2% SDS). Gels were loaded with 75 μl of sample, containing about 1 mg RNA/ml electrophoresis buffer plus 15% glycerol, and run for 5 h.

Fig. 1 : DNA, RNA and protein synthesis in CV-1 cells after 2 h treatment with different concentrations of metal ions. Each data point represents the average of two separate determinations.
at 8 V/cm. Gels were then scanned in order to record their absorbance at 260 nm and cut into 2 mm segments. Slices were treated with 0.15 ml solulyte overnight at room temperature and assayed for radioactivity. Planimetric measurements of absorbance profiles were used to check if nearly identical amounts of RNA had been loaded on the different gels. When necessary, the radioactivity of RNA from irradiated cells was normalized to the control level.

**RESULTS**

**Effects of Cd and Zn on macromolecular synthesis**

The rates of DNA, RNA and protein synthesis of exponentially dividing CV-1 cells incubated for 2 h in presence of different concentrations of metal ions are indicated as percentage of control in Fig. 1. It was observed that DNA synthesis is inhibited by Cd concentrations considerably lower than those inhibiting RNA synthesis. Protein synthesis has an intermediate sensitivity. Inhibitions of DNA, RNA and protein synthesis by Zn occur at higher ionic concentrations with about equal effectiveness (Fig. 1). The inhibitory effect of Cd on DNA synthesis can be reversed by a concomitant treatment with Zn at 5 to 10 times higher, but still non toxic, concentrations (Fig. 2). The reduction of the Cd effects is equally observed when Zn administration is delayed 1 hr with regard to that of Cd (data not shown).

These results suggest a reversible action of Cd on some enzymatic activity.

![Graph](image_url)

*Fig. 2:* DNA synthesis in CV-1 cells treated (2.5 h) with Cd and concomitantly with Zn at indicated concentrations. Each datum shows the mean value of duplicate determinations.
involved on DNA metabolism, such as thymidine kinase or DNA polymerases, rather than an induction of lesions at the DNA level (see discussion) and raise the question of a possible effect of Cd on excision repair of lesions, because of the requirement of a DNA synthesis step in this process.

**Effect of Cd and Zn on DNA repair**

The repair capability of CV-1 cells and of human fibroblasts submitted to low concentrations of metal ions was determined after UV 254 nm damage.

**Unscheduled DNA synthesis.** The synthesis of DNA in non-S phase cells (Fig. 3), representing the insertion of new bases into DNA to replace excised photoproducts, was reduced by a factor of 2 to 3 in Cd (4 x 10^{-6} M) treated cells compared to control cells for UV damaging doses higher than 10 J/m^2. Treatment of cells with Zn at 8 x 10^{-5} M did not considerably affect UDS (data not shown). The concomitant administration of Zn at 10 times higher concentration than Cd restores, at least partially, the repair replication in irradiated cells.

**Induction and repair of single-strand DNA breaks.** In UV-irradiated cells, single-strand DNA breaks are produced as a consequence of the incision step of repair process. Such

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**Fig. 3:** Unscheduled DNA synthesis in Jac fibroblasts as a function of UV-dose and of ionic environment. Autoradiographs were exposed 3 weeks. Each point represents the mean grain count for 100 nuclei. Vertical bars indicate standard error of the mean.
breaks disappear after repair replication and ligation have occurred. By the analysis of DNA alkaline elution rates (Fig. 4), it was observed that in control cells the DNA size decreases in the first few hours following UV-irradiation and then progressively recovers. This indicates that, in agreement with the above scheme (28), DNA breaks are induced only transiently.

On the contrary, in the presence of Cd the DNA size of irradiated cells continues to decrease during post-exposure incubation indicating that DNA breaks accumulate in these cells. Cd, at concentrations employed here, has no effect on the elution rate of DNA of unirradiated cells. When irradiated cells were concomitantly incubated in the presence of Cd and Zn at 10 times higher concentrations, the patterns of alkaline elution approach those observed for irradiated cultures incubated in normal medium, showing a partial restoration of cell repair capability by Zn. The presence of Zn alone at the same concentration had no appreciable effect on repair.

**Functional integrity of DNA.** The template activity of DNA was used as a probe for monitoring DNA damage and repair. In fact, UV-induced lesions constitute termination sites for RNA polymerase impairing transcription (30). On the other hand, a recovery of RNA synthesis, accompanied by the reestablishment of a normal ratio between two
Fig. 5: Typical inhibition and recovery of 28s and 18s RNA synthesis in UV-irradiated Jac fibroblasts in absence (panels A) or in presence of different concentrations of metal ions: 2 x 10^{-6} M Cd (panels B); 3 x 10^{-6} M Cd (panels C); 3 x 10^{-6} M Cd/3 x 10^{-5} M Zn (panels D). Separate stationary cultures were irradiated with a dose of 20 or 30 J/m^2 at 24 h or 15 min before labelling (= time 0). A1, B1, C1, D1: unirradiated controls; A2, B2, C2, D2: 20 J/m^2 at t = - 15 min; A3, B3, C3, D3: 30 J/m^2 at t = - 15 min; A4, B4, C4, D4: 20 J/m^2 at t = - 24 h; A5, B5, C5, D5: 30 J/m^2 at t = - 24 h.

Transcriptionally linked genes, occurs during post-exposure incubation and positively correlates in cell systems used with excision repair of damage and survival recovery (29, 31). Here, the rate of RNA synthesis was measured for the ribosomal RNA species.
Fig. 6: Dose-response curves of the colony forming ability of CV-1 cells irradiated with UV-light as confluent monolayers, and plated immediately after irradiation (●) or 24 h later after holding in normal medium (○) or in presence of different concentrations of metal ions (●, ▲, △, ★, ★). The cloning efficiency of unirradiated cells ranged from 74% for 8 x 10^{-6} M Cd-treated cells to about 100% for Zn-treated cells. For each group, survival of irradiated cells was calculated as percentage of the cloning efficiency of the unirradiated control. The mean values of survival from 6 dishes per point are reported.

28s and 18s at different times after UV-irradiation comparatively in cells incubated in presence or absence of metal ions.

Typical results obtained with Jac human fibroblasts are shown in Fig. 5. In panels A, cultures were incubated in normal medium. Panel A1 presents the electrophoretic patterns of 28s and 18s RNA synthesized in unirradiated cultures. Panels A2 and A3 show the dose-dependent, differential inhibition of 28s and 18s RNA
synthesis as a consequence of transcription terminating lesions induced in the polycistronic rDNA by UV light. Panels A4 and A5 depict the recovery of rRNA synthesis during 24 h of post exposure incubation. The extent of this recovery is inversely related to the initial damage. The increase of 28s RNA production is relatively greater than that of 18s RNA indicating that the restoration of rDNA template activity by the repair process(es) is accompanied by a reestablishment of the normal expression of these transcriptionally linked genes (see 29).

Panels B and C represent results obtained in Cd treated cultures at concentration of $2 \times 10^{-6}$ M and $3 \times 10^{-6}$ M, respectively. Panels B1 and C1 show that the incubation of cells in presence of Cd ions at indicated concentrations for 25 h does not affect their RNA synthesis. In these cells, immediate effects of 20 or 30 J/m$^2$ of UV radiation (panels B2 and B3, C2 and C3) are similar to those observed in corresponding irradiated untreated cultures. Nevertheless, the capability of cells to recover RNA synthesis during 24 h of post-exposure incubation in presence of Cd is severely reduced (panels B4 and B5) or even abolished (panels C4 and C5), indicating the great sensitivity of the repair process(es) to Cd.

Results reported in panels D on cells incubated in presence of $3 \times 10^{-6}$ M Cd and of $3 \times 10^{-5}$ M Zn show that the deleterious effects of Cd on DNA repair are considerably attenuated by an excess of Zn simultaneously administrated. Zn alone at $3 \times 10^{-5}$ M has no effect on inhibition and recovery of RNA synthesis (data not shown) in the same experimental conditions. Experiments performed with CV-1 cells gave identical results to those with human fibroblasts.

**Effects on survival**

To test the biological consequences of the effects on DNA repair of Cd and Zn, plateau-phase CV-1 cells were UV-irradiated and their clonogenicity was measured by plating them either immediately after exposure, or after a 24 h holding in normal or metal supplemented medium. Results are shown in Fig. 6. The survival of irradiated control cells is considerably increased by the delayed plating, reflecting the repair of potentially lethal damage which occurred during post-exposure liquid holding. The treatment of cultures for the same period with Zn at $8 \times 10^{-5}$ M did not affect the improvement of survival, except slightly for the highest UV-dose. In contrast, holding in presence of Cd decreases the survival of irradiated cells to a lower level than that of untreated cultures plated immediately after irradiation. This decrease is Cd concentration dependent. On the other hand, the clonogenicity of irradiated cells incubated after exposure in the simultaneous presence of Cd and Zn at 10 times greater concentrations is largely restored. Holding the cells for 48 h in the different conditions gave similar results (data not shown).
It is here reported that the administration of Cd to cultured cells inhibits especially their DNA synthesis and that higher, non toxic concentrations of Zn counteract this effect of Cd (Fig. 1 and 2). This finding could thus account at the molecular level for the toxic effect of Cd and the protective action of Zn observed in the animal (23) and in cell cultures (3, 4). The underlying mechanism of this antagonistic interaction between Cd and Zn is likely to reside in a competition for common binding sites at the level of some enzymatic proteins involved in DNA synthesis. The observation that the inhibitory effect of Cd, at least at concentrations employed here, can be reversed by delayed administrations of Zn is in fact in favour of a direct, reversible inhibition by Cd of enzymes, such as DNA polymerases or thymidine kinase, rather than of the induction of lesions at the DNA level responsible for decreased DNA synthesis. Furthermore, measurements of molecular size by alkaline elution and of template capability of DNA from Cd-treated cells (Fig. 4 and 5) failed to show any significant level of DNA damage induced by Cd alone in experimental conditions employed here.

It has been reported (8) that Cd inhibits human DNA polymerase B in vitro at low concentrations through direct reaction with the enzymatic protein. This inhibition is reversible by EDTA and monothiols but not by Zn. It thus seems that the interaction of Cd with polymerase B is not at the site which presumably binds a Zn atom. However, Zn is probably present in all DNA polymerases (15, 16) and it could be possible that in polymerase α, playing a central role in DNA replication and repair (31), and, eventually, in polymerase γ, Cd-inhibition takes place by displacing the essential Zn atoms.

The specific activity of thymidine kinase, which phosphorylates thymidine prior to its incorporation in the DNA, was reported to be reduced by Zn dietary deficiency in rats (32). Unless this reduction is a consequence of a feedback regulation of thymidine kinase synthesis or activity by pyrimidine phosphates which accumulate in non dividing cells, this result indicates that thymidine kinase is also a Zn-requiring enzyme and, thus, susceptible to be inhibited by Cd. In this case, an interaction of Cd with thymidine kinase, limiting the pool of thymidine phosphates, could also explain the reduction of DNA replication in Cd-treated cells.

The evidence of an action of Cd on DNA synthesis has raised the question of its consequences on the repair of damaged DNA and, indeed, this process induced here by UV-irradiation, was found to be quite sensitive to the presence of Cd in cells environment. The incision of DNA in the neighborhood of UV photoproducts, the first essential step of excision repair, does not seem impaired by Cd. This is shown by the similar reduction in the size of DNA of irradiated cells incubated either in presence or in absence of Cd ions during the first hours following exposure. On the contrary, repair
replication appears considerably affected, as measured by autoradiography of UDS occurring in irradiated and Cd-treated cultures in comparison with irradiated control cells (Fig. 3). The only partial gap filling, in conjunction with a normal rate of damage excision, could finally account for the abnormal accumulation of breaks observed with time of post-exposure incubation in the DNA of these cells (Fig. 4). This is exactly what is expected in the case of an inhibition of DNA polymerase(s) by Cd. In this connection, it may be remarked that various DNA polymerase inhibitors such as aphidicolin or cytosine arabinoside have effects analogous to those of Cd on excision repair-related DNA breaks (33, 34). On the other hand, the possibility to ascribe the reduced repair replication to the inhibition by Cd of thymidine kinase seems unlikely: actually, the number of precursors required for this process is much lower than for semi-conservative replication and the demands of repair could be met by the residual pool. It was observed, in fact, that the recovery of ribosomal RNA synthesis after UV damage is not affected by fluorodeoxyuridine (29), an inhibitor of DNA precursor synthesis, whereas it is completely abolished by aphidicolin (31), a very specific inhibitor of α polymerase. However, under some circumstances, drugs inhibiting DNA precursor synthesis (notably hydroxyurea, but also fluorodeoxyuridine) do inhibit or slow down DNA repair, leading to significant accumulation of DNA breaks (see for review 35). So a similar role for Cd cannot be completely excluded. A reduced ligation efficiency could also contribute to the accumulation of breaks in the DNA of UV-irradiated cells incubated in presence of Cd, but this step was not assayed here.

Zasukina et al. (11) reported that Cd does not inhibit repair of DNA damage induced in human lymphocytes by γ-irradiation. A possible explanation of this result could be that these lesions, contrarily to UV photoproducts, do not involve long patch excision and thus their repair is less dependent on DNA polymerasing activities. On the other hand, this observation seems to indicate that DNA ligase is not affected by Cd. These authors also observed a degradation of DNA in cells treated for 18 h with a relatively high concentration of Cd (2.5 x 10^{-5} M) and concluded that Cd is likely to induce non-repairable DNA damage, since DNA lesions were not rejoined by a further 18 h culture in the absence of Cd. However, as shown by Mitra and Bernstein (10), although the exposure of bacteria DNA to Cd (3 x 10^{-6} M) in vivo results in single strand breaks, which are repairable in their experimental conditions, exposure in vitro produces no breaks. The degradation of bacteria DNA and especially of human lymphocyte DNA was thus probably a consequence of some severe impairment by Cd of DNA syntheses in these actively dividing cells. No significant level of breakage was, on the contrary, observed with techniques employed here in the DNA of confluent unirradiated cells maintained for 24 h in presence of Cd at relatively low concentrations.
The physiological consequences of the impairment of DNA repair were expressed as an alteration of essential cellular functions such as RNA synthesis. In fact, the immediate effects of irradiation on this function were even aggravated with time of post-exposure incubation in presence of Cd. Cd concentrations eliciting this effect do not produce any inhibition of RNA synthesis in unirradiated cultures and, thus, are not responsible for direct DNA lesions or RNA polymerase inhibition.

The inhibition of DNA repair by Cd administered for 24 h following UV-irradiation has an important final consequence, namely a strong decrease in the survival of damaged cells (Fig. 6). Cd-treated cells not only do not present any repair of potential lethal damage during 24 h of liquid holding but they have a much lower clonogenicity than irradiated control cells not allowed to recover before plating. This result highlights that even a transient exposure of a biological system to Cd leads to an increase in the toxicity of other damaging agents. Moreover, it is worth noting that the inhibition of DNA repair could constitute another mechanism contributing, in addition to base misincorporation induced by Cd (9), to its mutagenicity and eventually carcinogenicity.

Finally, the observed reversion by Zn of the deleterious effects of Cd on DNA repair and survival of UV damaged cells is in agreement with the idea of an antagonistic interaction of the two metals for common binding sites particularly on DNA polymerising enzyme(s) involved in DNA replication and/or repair.

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