Biochemical Pharmacology, Vol. 36, No. 2, pp. 265–268, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

EFFECTS OF LEAD ACETATE ON DNA AND RNA SYNTHESIS BY INTACT HeLa CELLS, ISOLATED NUCLEI AND PURIFIED POLYMERASES

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(*Received* 4 *April* 1986; *accepted* 30 *June* 1986)

Abstract—The effects of lead acetate on DNA and RNA synthesis have been investigated with intact HeLa cells, isolated nuclei, and purified DNA and RNA polymerases. No inhibition of DNA or RNA synthesis in intact cells was found even after exposure to 0.5 mM lead acetate for 18 hr. In contrast, both DNA and RNA synthesis in isolated nuclei were inhibited by lead (with 50% inhibition at approximately 150 and 80 μM respectively). Similarly, both HeLa DNA polymerase α and RNA polymerase II were inhibited, with 50% inhibition obtained at approximately 150 and 20 μM lead acetate respectively. The inhibition of nucleic acid synthesis in isolated nuclei can thus be accounted for by inhibition of the polymerases. The sensitivity of *Escherichia coli* DNA polymerase I to lead acetate was found to be significantly greater than the HeLa DNA polymerase α (50% inhibition at only 10 μM), but the sensitivity of the *E. coli* RNA polymerase was the same as that of the HeLa enzyme.

Lead compounds have long been recognized as extremely serious health hazards [1, 2]. There have been epidemiological studies demonstrating toxicity in human beings [2], as well as direct toxicity studies in animals [3]. Relatively recently there has also been investigation into the potential genotoxicity of these compounds, that is, their mutagenicity, carcinogenicity, teratogenicity and clastogenicity [2, 4].

Inhibition of DNA and/or RNA synthesis, while not necessarily the primary cause of cell death, can be a significant toxic effect of a xenobiotic. Thus, we have been interested in the ability of lead to inhibit (or otherwise alter) cellular nucleic acid synthesis. Unfortunately, the existing literature does not offer a clear-cut picture as to the effect of lead at the cellular level. Thus, there have been several reports showing that nucleic acid synthesis is relatively insensitive to lead compounds, such that even high concentrations produce only a small degree of inhibition [5–7]. Other reports, however, have shown strong inhibition even at low concentrations [8, 9]. There have also been reports of stimulatory effects of lead on DNA synthesis under certain conditions [10–12]. These differing results may be ascribable to differing experimental conditions, or to differing cell types. Nevertheless, the question of whether lead can, in fact, inhibit nucleic acid synthesis remains unresolved. We therefore decided to re-investigate this question, by examining the effect of a lead compound not only on synthesis in intact cells, but also *in vitro* in isolated nuclei. The latter system permits examination of effects of the compound directly on the synthetic reactions, eliminating possible effects on transport and/or phosphorylation of the nucleoside precursors. It also eliminates possible differences between cells in permeability to the lead, thus allowing a relatively definitive determination as to whether the compound does, in fact, have inhibitory activity towards nucleic acid synthesis.

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METHODS

Calf thymus DNA and *Escherichia coli* DNA polymerase I were obtained from Cooper Biomedical. *E. coli* RNA polymerase and radioactive nucleosides and nucleoside triphosphates were purchased from New England Nuclear; unlabeled triphosphates were from PL Biochemical.

HeLa cells were grown in Dulbecco's Modified Eagle's Medium with 10% fetal calf serum (Gibco).

DNA and RNA syntheses in intact cells were measured by the incorporation of [^{3}H]thymidine or [^{3}H]uridine, respectively, as described previously [13]. Isolation of nuclei and the measurement of nucleic acid synthesis by the nuclei were carried out as described previously [13].

DNA polymerase α was purified from HeLa cells by DEAE-cellulose chromatography, as described previously [14]. RNA polymerase II was purified from HeLa cells, as described previously [13]. Enzyme assays were carried out as follows: for RNA polymerase, the reaction mixture (0.3 ml) contained: 50 mM Tris-acetate buffer (pH 7.9), 22 mM manganese acetate, 100 mM $(NH_4)_2SO_4$, 0.33 mM each of ATP, CTP, and GTP, 1 μM [^{3}H]UTP (final specific activity 32 Ci/mmole), and 50 μg/ml calf thymus DNA. For DNA polymerase the reaction mixture (0.3 ml) contained: 50 mM Tris-acetate buffer (pH 7.9), 10 mM magnesium acetate, 50 μM each of dATP, dCTP and dGTP, 0.5 μM [^{3}H]dTTP (final

specific activity 75 Ci/mmole) and 25 μg/ml calf thymus DNA. After incubation for 10 min at 37°, the reaction mixture was chilled, and 0.2 ml of salmon sperm DNA (0.25 mg/ml) and 0.5 ml of either 7% perchloric acid (for RNA polymerase) or 10% trichloroacetic acid (for DNA polymerase) were added. The precipitate was collected on a Whatman GF/C filter and washed with 6% trichloroacetic acid containing 0.1 M sodium pyrophosphate. The filter was dried and counted in a liquid scintillation counter.

In all experiments involving isolated nuclei and polymerases, care was taken to avoid the introduction of compounds such as sulfhydryls and chelating agents. In particular, prior to use enzymes were dialyzed and/or diluted into buffers free of such compounds.

RESULTS

Intact cells. Lead acetate was added to HeLa cell cultures simultaneously with either [^{3}H]thymidine or [^{3}H]uridine, and the acid precipitable radioactivity was measured after 1 hr. The results (Fig. 1A) show that the lead acetate had no inhibitory effect on DNA or RNA synthesis, even at a concentration as high as 0.5 mM. In this experiment the incubation of the cells with lead acetate was carried out in medium containing serum, which might contain compounds which could interact with the lead acetate, thus leading to an apparent, and artifactual, lack of effect of the lead compound. To examine this possibility we carried out a similar experiment in which the incubation of the cells with lead acetate and the radioactive nucleoside was carried out in serum-free medium. The results in Fig. 1B show that, even in the absence of serum, there was no inhibition by the lead acetate. To examine whether a longer exposure of the cells to lead would result in inhibition of nucleic acid synthesis, we pre-exposed the cells to the compound for 18 hr, then added the radioactive nucleoside and measured incorporation after one additional hour of incubation. The results (Fig. 1C) show that even after such a relatively long exposure there was no significant inhibition of DNA or RNA synthesis.

Isolated nuclei. A lack of an inhibitory effect on synthesis in intact cells, while of obvious significance in terms of the cellular effects of lead, does not necessarily imply that the compound has no activity against the cellular nucleic acid synthetic apparatus. Rather it could simply reflect the inability of the compound to gain access to the site of synthesis, i.e. it could reflect a lack of permeability of the cell plasma membrane to the lead. We therefore examined the effect of lead acetate on nucleic acid synthesis in isolated nuclei. This system has the added advantage of utilizing as substrates the nucleoside triphosphates, the immediate precursors of nucleic acids, and thus of measuring the synthetic reactions directly. The effect of lead acetate on DNA synthesis in isolated nuclei is shown in Fig. 2. In spite of the complete resistance of DNA synthesis in intact cells to lead (Fig. 1), in isolated nuclei DNA synthesis was inhibited significantly by the compound, with 50% inhibition achieved at approximately 150 μM. The effect of lead on RNA synthesis in isolated nuclei is shown in Fig. 3. The results are presented in terms of total RNA synthesis as well as broken down into two categories: synthesis which was inhibited by α-amanitin [15] (which is catalyzed by RNA polymerase II) and synthesis which was resistant to α-amanitin [15] (which is catalyzed by RNA polymerases I and III). Lead inhibited both types of

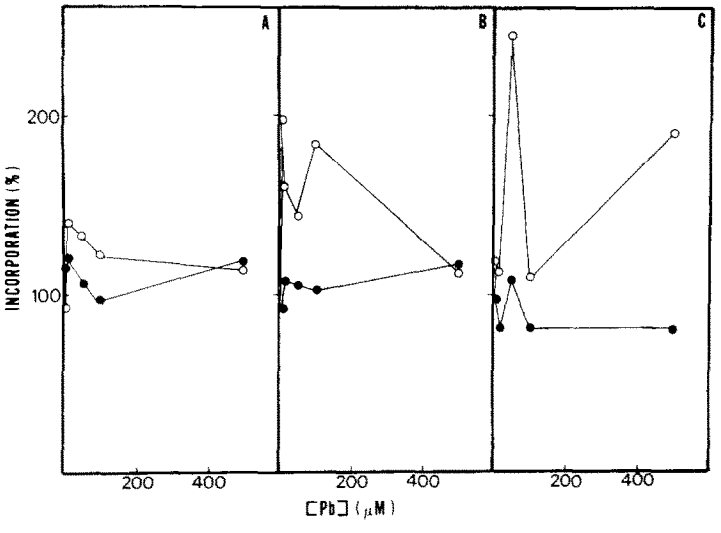

Fig. 1. Effect of lead acetate on DNA (○) and RNA (●) syntheses in intact HeLa cells. The incorporation of [^{3}H]thymidine or [^{3}H]uridine into acid precipitable material in 1 hr was measured in the presence of the indicated concentration of lead acetate. (A) Lead acetate and radiolabeled precursors were added to the cultures simultaneously, in complete medium. (B) Lead acetate and radiolabeled precursors were added to the cultures simultaneously in medium without serum. (C) Lead acetate was added to the cultures, in complete medium, and after 18 hr the radiolabeled precursors were added. Results are expressed as percentage of the total incorporation in the absence of lead acetate. (One hundred percent = 3.9 to 5.4 × 10^4 cpm for DNA synthesis, and 18.2 to 19.8 × 10^4 cpm for RNA synthesis.)

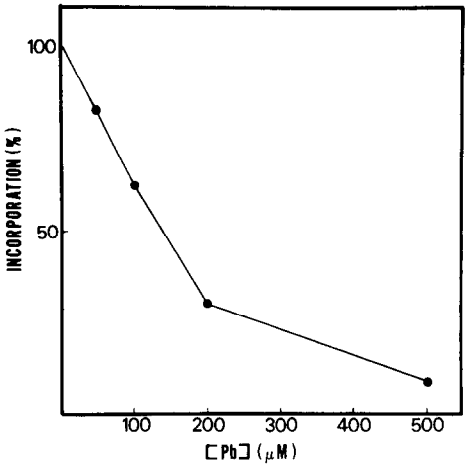

Fig. 2. Effect of lead acetate on DNA synthesis in isolated nuclei. Incorporation of [^{3}H]thymidine triphosphate into acid precipitable material by isolated HeLa nuclei was measured in the presence of the indicated concentration of lead acetate. Results are expressed as percentage of the total incorporation in the absence of lead acetate. (One hundred percent = 4.5×10^4 cpm.)

synthesis equally well, with 50% inhibition achieved at approximately 80 μM.

Purified polymerases. The inhibition by lead of nucleic acid synthesis in isolated nuclei could result from a direct inhibitory effect on the polymerases, the enzymes which catalyze the synthetic reactions. Alternatively, inhibition could result from an effect of lead on another factor (or factors) which are required for the synthesis. To examine this question, the effect of lead on the activity of the purified polymerases was examined.

The DNA synthesis which occurs in isolated nuclei has been shown to be primarily replicative (as opposed to repair) and to be catalyzed by DNA polymerase α[16]. As shown in Fig. 4, purified DNA polymerase α was inhibited by lead acetate, with 50% inhibition obtained at approximately 150 μM. Thus, the inhibitory effect of the lead on DNA synthesis in isolated nuclei can be accounted for by an effect on the enzyme. For purposes of comparison, we also examined the effect of lead on a procaryotic enzyme, DNA polymerase I of *E. coli*. This enzyme was significantly more sensitive to lead than the eucaryotic polymerase (Fig. 4).

In a preliminary experiment with the *E. coli* polymerase we found that lead appeared to increase the apparent K_m of the enzyme for the deoxyribonucleoside triphosphates but did not alter the V_{max} of the enzyme. This kinetic result is compatible with a competitive type of mechanism, but it is also compatible with a mechanism of substrate depletion, whereby the lead decreases the effective concentration of the triphosphates in the reaction [17]. To test this possibility we examined the effect of lead in the presence of excess enzyme. Under these conditions, inhibition by substrate depletion would still be expected to occur, whereas a decrease by the inhibitor of the amount of active enzyme would not be expected to result in a decrease in the rate of the reaction. We found that, in the presence of excess enzyme, there was no decrease in the reaction rate in the presence of 10 μM lead, a concentration which inhibited the reaction rate significantly under conditions of limiting enzyme (see Fig. 4). This result supports the idea that the enzyme is the target of the inhibitory effect of the lead.

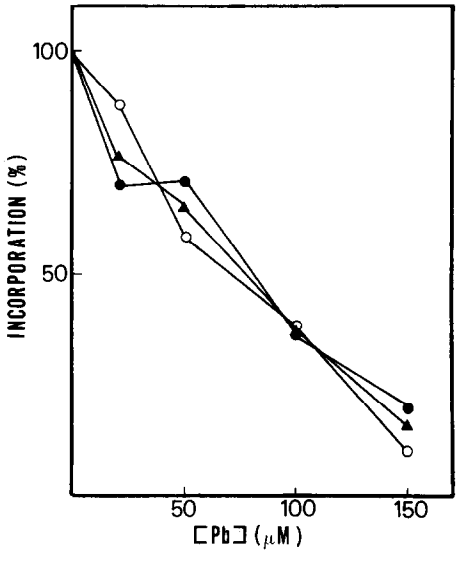

Fig. 3. Effect of lead acetate on RNA synthesis in isolated nuclei. Incorporation of [^{3}H]uridine triphosphate into acid precipitable material by isolated HeLa nuclei was measured in the presence of the indicated concentration of lead acetate. Results are expressed as percentage of the total incorporation in the absence of lead acetate. Key: (▲) total synthesis (100% = 4.6×10^4 cpm); (○) synthesis resistant to α-amanitin (0.5 μg/ml) (100% = 2.1×10^4 cpm); and (●) synthesis sensitive to α-amanitin (100% = 2.5×10^4 cpm).

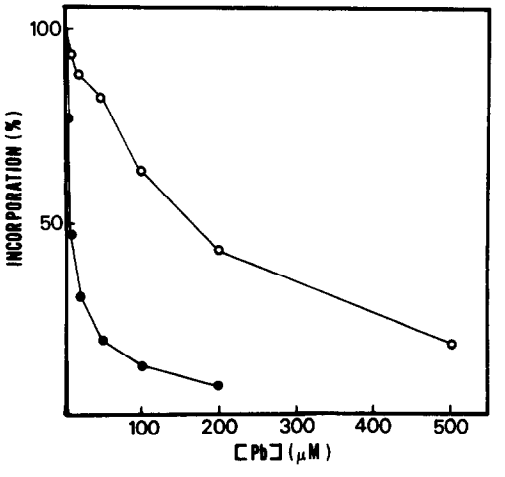

Fig. 4. Effect of lead acetate on DNA polymerase. The activity of HeLa DNA polymerase α (○) or *E. coli* DNA polymerase I (●) was measured in the presence of the indicated concentration of lead acetate. Results are expressed as percentage of the total incorporation in the absence of lead acetate. (One hundred percent = 1.3×10^4 cpm and 42×10^4 cpm for the HeLa and *E. coli* polymerases respectively.)

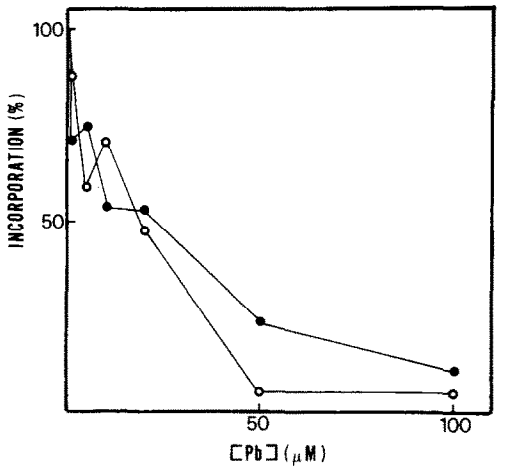

Fig. 5. Effect of lead acetate on RNA polymerase. The activity of HeLa RNA polymerase II (○) or *E. coli* RNA polymerase (●) was measured in the presence of the indicated concentration of lead acetate. Results are expressed as percentage of the total incorporation in the absence of lead acetate. (One hundred percent = 1.5×10^3 cpm and 4.5×10^4 cpm for the HeLa and *E. coli* polymerases respectively.)

We have also examined the effect of lead on the activity of RNA polymerase II, purified from HeLa cells, was well as the *E. coli* RNA polymerase. As shown in Fig. 5, both of these enzymes were inhibited by lead, with about equal sensitivity. Again in the case of RNA synthesis, the effect of lead on the polymerase can account for its inhibitory effect in isolated nuclei.

DISCUSSION

Our results have demonstrated that lead acetate is, in fact, able to inhibit DNA and RNA syntheses, in an *in vitro* system of isolated nuclei. Furthermore, this inhibition can be accounted for by a direct inhibitory effect of the lead on the DNA and RNA polymerases which catalyze this synthesis. The lack of an inhibitory effect of the lead on synthesis in intact cells, therefore, was not due to its inactivity vis-à-vis nucleic acid synthesis but, most likely, rather due to its inability to penetrate the cell to the site of synthesis. This suggests that agents which increase the permeability of the cell to lead compounds may be expected to affect profoundly their potencies in the inhibition of nucleic acid synthesis and, perhaps, in cytotoxicity as well. This possibility is currently under investigation.

With intact HeLa cells no inhibition was detectable, even at relatively high concentrations of lead acetate. This is consistent with those earlier studies which reported relatively low sensitivity of nucleic acid synthesis to inhibition by lead [5–7]. [We have also apparently detected (Fig. 1) the stimulatory effect which has been reported to occur under certain conditions [10–12]. The mechanism of this effect is not known [10–22].] Our result is not consistent with earlier reports of high sensitivity of nucleic acid synthesis to inhibition by lead [8, 9]. The reason for the apparent discrepancies is not clear, although we tend to ascribe them to differences in cell type. Since our results suggest that the insensitivity of intact HeLa cells may result from a lack of permeability to the lead compound (see above), differences between cell types could reflect differing permeabilities to lead compounds. We are currently examining other cell types for their sensitivity to inhibition by lead compounds, in order to investigate this possibility.

Acknowledgements—This work was supported in part by Grant ES-03413 from the National Institutes of Health. This is publication number 19 from the Department of Zoology and Physiology of Rutgers-Newark.

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