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A COMPARATIVE STUDY OF THE EFFECTS OF MERCURY COMPOUNDS ON CELL VIABILITY AND NUCLEIC ACID SYNTHESIS IN HeLa CELLS

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Abstract—The effects of various mercury compounds on HeLa cell viability and DNA and RNA syntheses in intact cells and in isolated nuclei have been studied. The compounds examined were: methylmercuric chloride, ethylmercuric chloride, dimethylmercury, phenylmercuric acetate, *p*-hydroxymercuribenzoate, *p*-hydroxymercuribenzenesulfonate, $HgCl_2$, $HgSO_4$, $Hg(ClO_4)_2$ and $Hg_2(ClO_4)_2$. All of the compounds except dimethylmercury inhibited colony formation as well as DNA synthesis in intact cells and in isolated nuclei. RNA synthesis in intact cells was inhibited by all the compounds except dimethylmercury, *p*-hydroxymercuribenzoate and $Hg(ClO_4)_2$. In isolated nuclei, α-amanitin-resistant RNA synthesis was inhibited by all the compounds except dimethylmercury. α-Amanitin-sensitive RNA synthesis was stimulated by some compounds, inhibited by some, and unaffected by others. The effects of two non-mercurial sulfhydryl reagents, *N*-ethylmaleimide and iodoacetic acid, were also examined. These compounds showed a pattern of effects on nucleic acid synthesis which differed considerably from that of the mercury compounds. Neither compound significantly inhibited α-amanitin-resistant RNA synthesis in isolated nuclei, although both inhibited RNA synthesis in intact cells. Iodoacetic acid had no inhibitory effect on DNA synthesis in isolated nuclei but strongly inhibited DNA synthesis in intact cells.

Environmental contamination with mercury compounds has been a source of concern for a number of years, and the chemical, ecological, toxicological and biochemical properties of these compounds have been investigated extensively [1]. In general, however, the genetic effects of mercury compounds have not received a great degree of attention, perhaps because they have not been demonstrated to be strongly mutagenic or carcinogenic [2]. Nevertheless, there is good evidence that these compounds can damage chromosomes [3–6], interact with DNA and chromatin [7–12], and interfere with genetic processes such as replication and transcription [13–15]. They have also been reported to be weakly mutagenic in mammalian cell cultures [16].

Of the wide variety of relatively common mercury-containing compounds, both inorganic and organic, the most widely studied have been $HgCl_2$ and methyl mercury, perhaps because of their environmental relevance [1]. Because of our interest in the effects of mercury compounds on DNA replication and transcription, we have compared the effects of ten mercury-containing compounds (inorganic, alkyl organic and aryl organic) on these genetic processes. These studies were carried out with HeLa cells, a permanent line of human cells derived from a cervical carcinoma. In addition to studying the effects of the mercury compounds on DNA and RNA syntheses in intact cells in culture, we have also examined their effects on synthesis in isolated nuclei [17, 18]. Since nucleoside triphosphates are utilized as substrates in this system, any potential effects of an exogenous agent on transport or phosphorylation of precursors, such as may occur in intact cells, are obviated.

MATERIALS AND METHODS

$Hg_2(ClO_4)_2$ was obtained from the Alfa Chemical Co. (Danvers, MA), and IA† from Matheson, Coleman & Bell (Gibbstown, NJ). Sources for all other chemicals and reagents used in this study have been described previously [19].

DNA polymerase α was purified from HeLa cells (grown in suspension) by chromatography on DEAE-cellulose [20] and assayed as described previously [20].

HeLa cells were grown either in monolayer cultures in Dulbecco's Modified Eagle's Medium (GIBCO) containing 10% heat-inactivated fetal calf serum or in suspension cultures in Joklik's minimal essential medium (GIBCO) containing 5% horse serum.

Nucleic acid synthesis in intact cells was measured in monolayer cultures by the incorporation of [^{3}H] thymidine or [^{3}H]uridine, using the general procedure described previously [21]. In the experiments described here, the mercury compound and the radiolabeled precursor were added to the cells simultaneously, and acid precipitable radioactivity was measured after incubation for 30 min at 37°. All

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† Abbreviations: IA, iodoacetic acid; MeHgCl, methylmercuric chloride; EtHgCl, ethylmercuric chloride; Me_2Hg, dimethylmercury; ØHgAc, phenylmercuric acetate; pHMB, *p*-hydroxymercuribenzoate; pHMBS, *p*-hydroxymercuribenzenesulfonate; and NEM, *N*-ethylmaleimide.

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Table 1. Cytotoxicity of mercury compounds for HeLa cells

Compound	IC_{50}* (μM)
MeHgCl	0.03
EtHgCl	0.02
Me_2Hg	NI (200)†
ØHgAc	0.004
pHMB	1.5
pHMBS	3.1
$HgCl_2$	0.6
$HgSO_4$	0.5
$Hg(ClO_4)_2$	0.7
$Hg_2(ClO_4)_2$	0.4

* Concentration which results in 50% inhibition of colony formation (see Materials and Methods).

† NI = no inhibition at indicated concentration.

determinations were done in duplicate; individual determinations varied no more than 15% from the mean.

Nuclei were isolated from monolayer cultures as described previously [21]. Synthetic reactions measuring incorporation of [^{3}H]dTTP or [^{3}H]UTP into DNA or RNA, respectively, were carried out at 20° for 8 min as described previously [21].

Cytotoxicity was determined by inhibition of colony formation. HeLa cells were seeded into 50 mm dishes at a concentration of 360 cells per dish, with 3.6 ml of Dulbecco's Modified Eagle's Medium with 10% heat-inactivated fetal calf serum. After incubation of the cultures at 37° for 24 hr, 0.4 ml of a series of 2-fold dilutions of a freshly made 2 mM solution of the mercury compound was added to the cultures. After 9 days of further incubation the cultures were washed with 4 ml of phosphate-buffered saline (pH 7.6), fixed with methanol, and stained with Giemsa, and the number of colonies per dish was determined. Six replicates were done for each concentration.

RESULTS

Cytotoxicity. The effects of ten mercury compounds on the ability of HeLa cells to form colonies were examined. All the compounds, with the exception of Me_2Hg, had an inhibitory effect. The relative potencies of the compounds are shown in Table 1.

DNA synthesis. The effects of the various mercury compounds on DNA synthesis in intact cells are shown in Fig. 1. While most of the compounds inhibited synthesis effectively, three appeared to be exceptional: pHMB and pHMBS were significantly less potent, and Me_2Hg had no detectable effect.

A subcellular system such as isolated nuclei enables the direct measurement of the effects of an exogenous agent on nucleic acid synthesis by allowing the use of radiolabeled nucleoside triphosphates, the direct precursors of nucleic acids, as substrate. The effects of the various mercury compounds on DNA synthesis in isolated nuclei are shown in Fig. 2. All compounds examined, with the exception of Me_2Hg, showed strong inhibitory activity.

In order to compare the potencies of the various compounds in inhibiting DNA synthesis in intact cells and in isolated nuclei, the concentration which inhibited synthesis by 50% is shown in Table 2. The relative potencies of the compounds in the two systems were reasonably consistent; the major discrepancies occurred with pHMB and pHMBS (see Discussion).

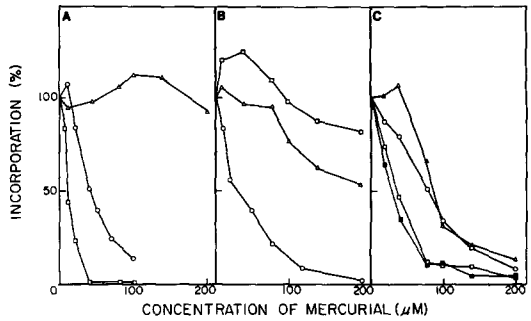

Fig. 1. Effects of mercury compounds on DNA synthesis in intact cells. Data are expressed as a percentage of the incorporation in the absence of added compound. Values for 100% in the various experiments were between 1.2 and 10.2×10^4 cpm. (A) Alkyl compounds: (○) MeHgCl, (□) EtHgCl, and (△) Me_2HgX. (B) Aryl compounds: (○) ØHgAc, (□) pHMB, and (△) pHMBS. (C) Inorganic compounds: (○) $HgSO_4$, (□) $Hg(ClO_4)_2$, (△) $HgCl_2$, and (■) $Hg_2(ClO_2)_2$.

Since a major site of action of mercury compounds is sulfhydryl groups of proteins [22], it was of interest to examine the relative potencies of these compounds in inhibiting DNA polymerase α, the enzyme which is primarily responsible for the synthesis we have measured in intact cells and isolated nuclei [23]. The results of such a study, which are also presented in Table 2, indicate that, in general, the relative potencies of the compounds in inhibiting the enzyme are similar to their relative potencies in inhibiting DNA synthesis in isolated nuclei. Major discrepancies appeared to occur, however, with MeHgCl and EtHgCl, suggesting that these compounds may exert their inhibitory effects on DNA synthesis at a site or sites other than the polymerase.

RNA synthesis. The effects of the various compounds on RNA synthesis in intact cells are shown in Fig. 3. The relative potencies of most of the compounds are similar to those for DNA synthesis (Fig. 1). The most notable exceptions are ØHgAc, pHMBS, $Hg(ClO_4)_2$ and $Hg_2(ClO_4)_2$.

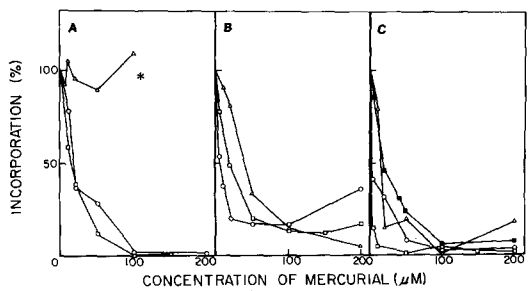

Fig. 2. Effects of mercury compounds on DNA synthesis in isolated nuclei. Values for 100% in the various experiments were between 1.1 and 7.4×10^3 cpm. Symbols are the same as in Fig. 1.

Table 2. Inhibition of DNA synthesis by mercury compounds

Compound	IC_{50}* (μM)		
	Intact cells	Isolated Nuclei	DNA polymerase α
MeHgCl	40	15	2.8
EtHgCl	10	15	5.6
Me_2Hg	NI (200)‡	NI (100)‡	NI (100)‡
ØHgAc	30	5	1.5
pHMB	300†	20	0.7
pHMBS	210†	40	3.1
$HgCl_2$	90	15	0.2
$HgSO_4$	80	5	0.2
$Hg(ClO_4)_2$	35	5	0.6
$Hg_2(ClO_4)_2$	30	20	0.6

* IC_{50} = concentration required to produce 50% inhibition.
† Estimated by extrapolation (see Fig. 1B).
‡ NI = no detectable inhibition at indicated concentration.

In isolated nuclei two types of RNA synthesis can be considered independently, based upon their sensitivity to low concentrations of α-amanitin. The synthesis which is inhibited by α-amanitin (α-amanitin-sensitive) is catalyzed by RNA polymerase II whereas the synthesis which is resistant to α-amanitin is catalyzed by RNA polymerases I and III [24]. Based upon our previous findings with MeHg [21], the mercury compounds might be expected to have quite different effects on the two types of synthesis. As shown in Figs. 4–6, all the compounds, with the sole exception of Me_2Hg, were effective inhibitors of α-amanitin-resistant synthesis and some of the compounds were able to stimulate α-amanitin-sensitive synthesis. The latter effect is the subject of a separate publication [19].

Effects of non-mercurial sulfhydryl reagents. A major site of interaction of mercury compounds with cellular components is at sulfhydryl groups in proteins. It was thus of interest to compare the effects of mercury compounds with those of non-mercurial sulfhydryl-binding reagents. The results of studies on two such compounds, IA and NEM, are shown in Fig. 7 and may be summarized as follows: IA inhibited both DNA and RNA syntheses in intact cells but had very little effect on any type of synthesis in isolated nuclei. NEM also inhibited both DNA and RNA syntheses in intact cells; in nuclei it showed some inhibition of DNA synthesis, no inhibition of α-amanitin-resistant RNA synthesis, and moderate stimulation of α-amanitin-sensitive RNA synthesis. Neither of these patterns of effects resembles the patterns of any of the mercury compounds which we have examined (see Discussion).

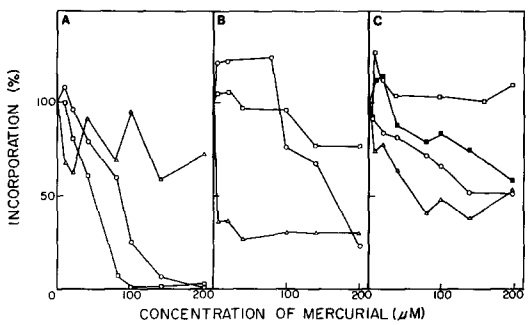

Fig. 3. Effects of mercury compounds on RNA synthesis in intact cells. Values for 100% in the various experiments were between 2.0 and 20 × 10^4 cpm. Symbols are the same as in Fig. 1.

DISCUSSION

The mercury compounds we have examined tend to fall into four groups in terms of relative cytotoxicity (Table 1): high (MeHg, EtHg, ØHg), medium (all the inorganics), low (pHMB, pHMBS), and not detectable (Me_2Hg). These relative potencies are consistent with those reported by Umeda *et al.* [25] for the three compounds of this group which they tested. The relative potencies of the compounds in cytotoxicity are, in general, similar to their relative potencies in the inhibition of DNA synthesis in intact cells. However, it is not clear that inhibition of DNA synthesis is the primary mechanism by which these compounds inhibit cell division.

A comparison of the effects of the compounds on intact cells with those on isolated nuclei is informative. There are three compounds which have a significantly greater inhibitory effect in isolated nuclei than in intact cells. Two of them, pHMB and pHMBS,

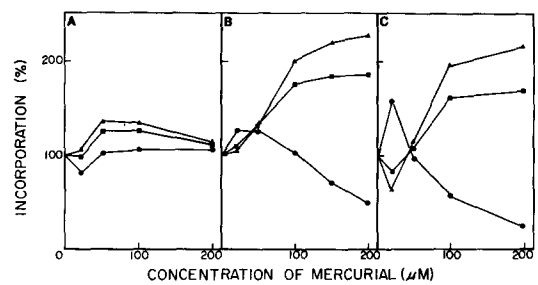

Fig. 4. Effects of alkyl organic mercury compounds on RNA synthesis in isolated nuclei. Values for 100% in the various experiments in this figure and in Figs. 5 and 6 were between 5.8 and 20.7 × 10^3 cpm for α-amanitin-sensitive synthesis and between 3.4 and 8.8 × 10^3 cpm for α-amanitin-resistant synthesis. (A) Me_2Hg. (B) MeHgCl. (C) EtHgCl. Key: (■) total synthesis, (●) α-amanitin-resistant synthesis, and (▲) α-amanitin-sensitive synthesis (total minus resistant).

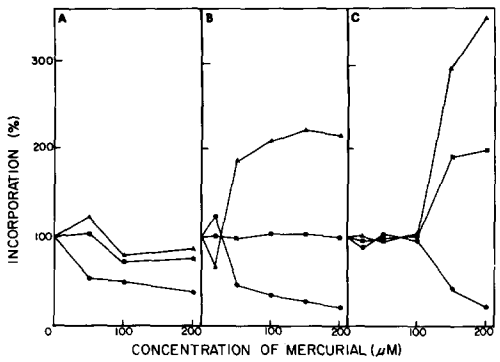

Fig. 5. Effects of aryl organic mercury compounds on RNA synthesis in isolated nuclei. (A) ØHgAc. (B) pHMB. (C) pHMBS. Symbols are the same as in Fig. 4.

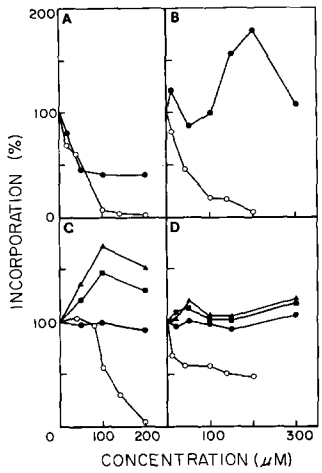

Fig. 7. Effects of NEM (A and C) and IA (B and D) on nucleic acid synthesis. Values for 100% were within the ranges given for the corresponding type of synthesis in the previous figures. (A and B) DNA synthesis in (○) intact cells or (●) isolated nuclei. (C and D) RNA synthesis in (○) intact cells or RNA synthesis in isolated nuclei: (■) total, (●) α-amanitin-resistant, and (▲) α-amanitin-sensitive.

inhibited DNA synthesis strongly in nuclei but relatively poorly in intact cells (Table 2). This difference is most easily explained by lack of permeability of the plasma membrane to these compounds, a factor which would also explain the relatively low toxicity of these compounds (Table 1). The third compound, $Hg(ClO_4)_2$, inhibited RNA synthesis (both α-amanitin-sensitive and -resistant) in nuclei (Fig. 6C) but had no effect on RNA synthesis in intact cells (Fig. 3). In comparing the results with various mercury compounds, it should be kept in mind that the actual molecular form of the Hg in solution depends upon the dissociation constant of the individual compound, as well as on the various anions which are present. Furthermore, in the case of $Hg_2(ClO_4)_2$, the disproportionation reaction $Hg_2^{2+} \rightarrow Hg + Hg^{2+}$ may occur. However, in our systems it is likely to be occurring to only a very limited extent, as suggested by the differing results we have obtained for $Hg(ClO_4)_2$ and $Hg_2(ClO_4)_2$ in some cases in both isolated nuclei and intact cells (Figs. 2C and 3C).

Only the non-mercurials NEM and IA displayed the opposite type of pattern, i.e. both of these compounds inhibited RNA synthesis in intact cells but had little effect in isolated nuclei. The simplest explanation for this pattern is that NEM and IA affect nucleoside transport and/or phosphorylation, but not nucleic acid synthesis *per se*. Although the work of Schuster and Hare [26] suggests that some mercury compounds have such an effect, in fact none of the mercury compounds which we have examined shows this pattern; our results with isolated nuclei are thus supportive of the idea that mercury compounds do exert a direct effect on nucleic acid synthesis in cells. These results illustrate the value of the isolated nuclei system in the study of the effects of exogenous agents on nucleic acid synthesis.

Another interesting feature which emerges from our results is illustrated by the effect of pHMB on total RNA synthesis in isolated nuclei (Fig. 5B). At a concentration of 200 μM, for example, the level of synthesis appears to be unaffected. Only when α-amanitin-sensitive and -resistant syntheses are distinguished are the true effects of the compound appar-

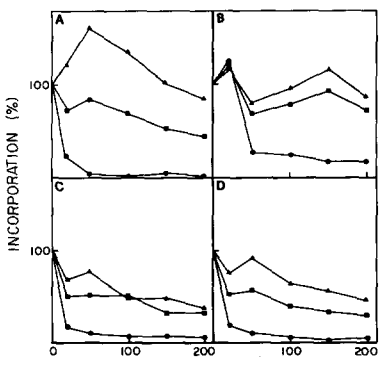

Fig. 6. Effects of inorganic mercury compounds on RNA synthesis in isolated nuclei. (A) $HgSO_4$. (B) $HgCl_2$. (C) $Hg(ClO_4)_2$. (D) $Hg_2(ClO_4)_2$. Symbols are the same as in Fig. 4.

Table 3. Effects of mercury compounds, NEM and IA on the percentage of the RNA synthesis in isolated nuclei which is α-amanitin-resistant

Compound*	% α-Amanitin-resistant
None	30–50
MeHgCl	6
EtHgCl	3
ØHgAc	7
pHMB	13
pHMBS	4
$HgCl_2$	6
$HgSO_4$	9
$Hg(ClO_4)_2$	9
$Hg_2(ClO_4)_2$	9
NEM	20
IA	33

* All compounds at 200 μM.

ent. Furthermore, the different effects on the two types of synthesis result in a change in the composition of the total synthesis which is measured. For example, in the absence of pHMB, 30–50% of the RNA synthesis was α-amanitin-resistant, whereas in its presence only about 13% was (Table 3). This table also shows the percentage of the total synthesis which was α-amanitin-resistant in the presence of the other mercury compounds. A significant change in the composition of the RNA synthesis occurred even with compounds which did not actually stimulate the α-amanitin-sensitive synthesis, such as ØHgAc and $HgCl_2$ and even where both types of synthesis were inhibited, as with $Hg(ClO_4)_2$ and $Hg_2(ClO_4)_2$. In the latter case, this change resulted from the greater sensitivity of α-amanitin-resistant synthesis to inhibition by the mercurials. It is also of interest that this effect did not occur to a significant extent with either of the non-mercurial sulfhydryl reagents. These findings illustrate the importance of considering the two types of RNA synthesis separately and are of particular significance in studies aimed at detecting possible interactive effects of other agents with mercury compounds. If, for example, an agent is added to the system in the presence of a mercury compound, it might appear to have no effect on RNA synthesis. However, it could be that the agent actually does affect α-amanitin-resistant synthesis, but this effect is not detected because in the presence of mercury none of the RNA synthesis is α-amanitin-resistant.

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