Inhibition of Moloney Leukaemia Virus Production by
N-methylisatin-β:4'-diethylthiosemicarbazone

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SUMMARY

N-methylisatin-β:4'-diethylthiosemicarbazone (M-IBDET) inhibited the
production of Moloney leukaemia virus (MLV). Virus inhibition was related to
drug concentrations and time of treatment. The effective antiviral drug concen-
trations ranged between 3-4 μM and 34 μM. Virus reverse transcriptase activity even
at concentrations of 34 μM-M-IBDET was not inhibited. At virus inhibitory con-
centrations the drug reduced RNA synthesis only very slightly and did not affect
protein synthesis at all, although growth and DNA synthesis of host cells were
suppressed. The inhibition of cellular DNA synthesis was reversible. Comparison
of M-IBDET with actinomycin D, cycloheximide and α-amanitin in terms of their
inhibitory effect on the release of MLV into the culture medium showed that
M-IBDET was comparable to the other antimetabolites. The inhibition of MLV
production by M-IBDET was confirmed by various parameters of virus assay. It
was concluded from the experimental evidence that M-IBDET specifically inhibits
MLV production.

INTRODUCTION

The activity of thiosemicarbazone compounds against pox viruses has been extensively
studied (Bauer, 1972). N-methylisatin-β-thiosemicarbazone (M-IBT) is one of the few
clinically effective synthetic antiviral drugs acting by inhibiting the replication of DNA-
containing pox viruses. The antiviral activity of dibutyl-substituted M-IBT appears primarily
to be against RNA-containing viruses from the picorna group (Sadler, 1965; Pearson &
Zimmerman, 1969) and unexpectedly against ectromelia, a DNA virus in mice, which
belongs to the pox viruses (O'Sullivan et al. 1963). This paper presents evidence that N-
methylisatin-β:4'-diethylthiosemicarbazone (M-IBDET) inhibits the production of
Moloney leukaemia virus (MLV), an RNA tumour virus.

METHODS

Cells and viruses. 3T3/NIH and XC cells were grown in Eagle's minimal essential medium
supplemented with 10% foetal calf serum. Chronically MLV-infected 3T3/NIH cells (3T3/
MLV) were obtained by infecting 3T3/NIH cells with Moloney strain of murine leukaemia
virus (Teitz et al. 1971). Virus stocks were prepared from spleens of lymphomatous rats
according to the method of Moloney (1960). A 10⁻³ dilution of virus stock induced lymph-
oma within 2 to 3 months in 50% of the BALB/c mice inoculated at birth with the virus.
Subconfluent 24 h 3T3/NIH cultures were inoculated with the virus stock, the cells were incubated and the medium changed at 3-day intervals. The infected cells were subcultured 12 days later when virus infection was well established. The 3T3/MLV cell line has been maintained for 4 years in our laboratory and virus release exceeds levels of 10⁶ TCID₅₀/m. 3T3/NIH and 3T3/MLV cells are seeded to a concentration of 10⁵ cells/ml and usually reach confluency in 3 to 4 days.

Chemical compounds. N-methylisatin-β-thiosemicarbazone (M-IBT), isatin-β-4′:4′-diethylthiosemicarbazone (IBDET), N-methylisatin-β-4′:4′-diethylthiosemicarbazone (M-IBDET) and other thiosemicarbazones of various mono- and bicyclic α-ketolactames were synthesized by us at the Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, Israel (F. Edelstein et al. unpublished data). Stock solutions of 10 mg/ml were made in dimethylsulphoxide (DMSO). Actinomycin D, cycloheximide and α-amanitin were purchased from Sigma.

Radio-isotopes. ³H-leucine (sp. act. 37.8 Ci/mmol), ³H-uridine (sp. act. 22.8 Ci/mmol) and ³H-thymidine (sp. act. 35 Ci/mmol) were purchased from the Nuclear Research Center, Israel. ³H-deoxythymidine 5-triphosphate (³H-TTP; sp. act. 49 Ci/mmol) was obtained from New England Nuclear Corp., Boston, Mass, U.S.A. Poly(rA) and oligo(dT)₁₂₋₁₈ were obtained from Miles Laboratories Inc., Elkhart, Ind., U.S.A.

RNA-dependent DNA polymerase (reverse transcriptase) assay. Reverse transcriptase activity was tested in 0.1 ml of tissue culture fluid or in purified virus samples suspended in tris-NaCl buffer, pH 8.3 (100 mM-tris, 120 mM-NaCl, 20 mM-DTT). Samples were pre-incubated with 10 µl of 2.2 % Nonidet P-40 for 15 min and then mixed with an equal amount of reaction mixture. For exogenous reaction the reaction mixture contained 100 mM-tris-HCl, pH 8.3, 100 mM-NaCl, 2 mM-manganese acetate, 40 mM-dithiothreitol, 20 µM-poly(rA), 5 µM-oligo(dT)₁₂₋₁₈, 20 µM-TTP and 1 µCi ³H-TTP (usually ³H-TTP to yield 220 ct/min/pm0). For endogenous reaction the mixture used was as previously described (Teitz, 1971) except that ³H-TTP was added to yield 2 x 10⁶ ct/min/pmol. Reaction mixtures were incubated for 30 min at 40 °C and the reaction was terminated by chilling the tubes and adding 0.1 ml of 100 mM-sodium pyrophosphate (pH 7.0), 0.1 ml of 200 mM-EDTA, 50 µg yeast RNA and 4 ml cold 10% TCA. The precipitate was collected by filtration through a glass fibre paper, GF/B (25 mm), and washed thoroughly with 5% TCA containing 1 mM-sodium pyrophosphate and then twice with ethanol. The glass fibre papers were dried and radioactivity was determined in a liquid scintillation counter (all values were the average of at least three identical reactions).

Assay of virus production by ³H-uridine labelling of virus RNA. Confluent 3T3/MLV cell cultures were incubated with growth medium containing the appropriate concentration of M-IBDET and ³H-uridine at 10 µCi/ml. The supernatant fluid was harvested and clarified by low speed centrifugation. The virus particles were then pelleted by centrifugation in a Beckman SW27 rotor at 95000 g for 90 min. The sediment was suspended in 0.2 ml citrate-phosphate buffer, pH 7.0, layered on top of a 5 ml 15 to 60% linear sucrose gradient and centrifuged in a SW50.1 rotor at 234000 g for 3 h. The gradients were fractionated into 0.2 ml samples and acid-precipitable radioactivity was measured.

Assay of infectious virus. MLV infectious virus was assayed by the induction of syncytia when co-cultivated with XC cells. Titration of virus was done with the 3T3/NIH mouse cell line in semi-micro cluster plates (Costar Cambridge, Mass., U.S.A.) according to the method of Klement et al. (1969). Cells were fixed with methanol and stained with Giemsa and border lines between two cultures were scored for syncytia. The virus titre is expressed as TCID₅₀/ml (by the Reed–Muench method on four plates).

Assays measuring cell DNA, RNA and protein synthesis. Cell culture monolayers in 2 cm
plastic Petri dishes were incubated with ^3 H-thymidine, ^3 H-uridine or ^3 H-leucine. All labelled precursors were added at 2 \mu Ci/ml. At indicated times triplicate cultures were washed and solubilized with 1 ml of 1% SDS. An equal volume of 20% TCA was added and the precipitates were collected by filtration through glass fibre filters. Acid-precipitable radioactivity was determined in a liquid scintillation counter.

Analysis of radioactively-labelled virus proteins in SDS-PAGE. Virus particles in supernatant fluids of labelled cultures were pelleted and banded in a 15 to 60% linear sucrose gradient. Virus bands at 1·16 to 1·18 g/ml were sedimented at 95 000 g for 90 min and suspended in 40 \mu l dissociation buffer (20 mM-tris-HCl, pH 8·0; 1% SDS; 4 M-urea; 1% mercaptoethanol). Samples were immersed in boiling water for 2 min and 0·001% bromophenol blue and 15% (v/v) sucrose were added. Electrophoresis was performed in 7·5% polyacrylamide gels in the presence of 0·1% sodium dodecyl sulphate using the buffer system described by Laemmli (1970). Gels were frozen and sliced 1 mm apart. For gel solubilization 0·2 ml of 30% \( \mathrm{H}_2\mathrm{O}_2 \) was added to each slice and incubated overnight at 60°C. The amount of radioactivity was detected by adding 2 ml to toluene-based scintillation fluid (Insta-Gel, Packard Instrument Co. Ltd., Ill., U.S.A.). Mol. wt. of labelled virus proteins were determined according to the mobility of standard proteins run in parallel.

RESULTS

Inhibition of MLV production by M-IBDET

The thiosemicarbazones of various mono- and bicyclic \( \alpha \)-ketolactames were examined for their effect on MLV. The effect of the compounds was studied on MLV production in the chronically infected mouse cell line 3T3/MLV. Virus production was monitored by examining the release of the virus to the culture medium as determined by its reverse transcriptase activity.

Treatment with M-IBT and eight other related compounds (F. Edelstein et al. unpublished data) did not affect MLV production. In contrast, both the dialkyl-substituted compounds M-IBDET and IBDET inhibited virus production. While treatment with IBDET at 17 \mu M showed a c.p.e. after 18 h, no visible c.p.e. was seen with 17 \mu M-M-IBDET.

The effect of different concentrations of M-IBDET on MLV production was examined in order to find the optimal effective dose. Virus release in the drug-treated and untreated cells was followed for a 12 h period. Fig. 1 shows the kinetics of MLV release from the 3T3/MLV cells in the presence of various concentrations of M-IBDET. It can be seen that the amount of the released virus was reduced proportionally to the concentration of the drug. At M-IBDET concentrations ranging between 3·4 and 34 \mu M, virus production was effectively inhibited after 6 to 9 h of treatment.

Cellular (3T3/MLV) inhibition by M-IBT, M-IBDET and IBDET

Tests more quantitative than c.p.e. were applied to the 3T3/MLV cells after treatment with the thiosemicarbazones in order to determine the effect of the drugs on the DNA, RNA and protein synthesis of the host cells.

The incorporation of radioactive precursors was followed during the 6 h incubation period with the drug. As seen in Fig. 2, 17 \mu M-M-IBDET completely suppressed ^3 H-thymidine incorporation after 1 h of treatment. After 3 h, 17 \mu M-M-IBDET caused only about 26% inhibition and 8·5 \mu M had almost no effect on ^3 H-thymidine incorporation. The 17 \mu M-M-IBT did not reduce DNA synthesis during the period examined. ^3 H-uridine incorporation into cellular RNA was affected very little by 17 \mu M-M-IBDET during 6 h of treatment. No effect on cellular protein synthesis was found when examined by ^3 H-leucine incorporation.
Fig. 1. Kinetics of MLV release under different doses of M-IBDET. 3T3/MLV confluent cultures were incubated in the presence of M-IBDET at the following concentrations: 34 μM (□—□), 17 μM (■—■), 8.5 μM (▼—▼), 3.4 μM (▼—▼) and 1.7 μM (○—○). Control cultures (●—●) contained 0.1% DMSO. At the indicated times virus was determined by reverse transcriptase activity (see Methods).

Fig. 2. Effect of M-IBDET, M-IBT and IBDET on DNA synthesis of host cells. 3T3/MLV monolayers were incubated with 3H-thymidine-labelled medium also containing 8.5 μM M-IBDET (○—○), 17 μM each of M-IBDET (O—O), M-IBT (△—△) and IBDET (▲—▲). Control cultures (O—O) contained 0.1% DMSO. 3H-thymidine incorporation into the cells was followed for 6 h (see Methods).

Fig. 3. Reversal of M-IBDET inhibition of cellular DNA after removal of the drug. 3T3/MLV monolayers were treated with 17 μM M-IBDET for 6 h. Drug-treated and untreated cells were washed twice and fresh medium containing 3H-thymidine was added. M-IBDET (17 μM) was added to part of the previously treated cells. 3H-thymidine incorporation was followed for 7 h. ●—●, Control, untreated cells; ○—○, 6 h pretreatment and 7 h post-treatment and △—△, 6 h pretreatment.

Fig. 4. In vitro kinetics of reverse transcriptase activity of MLV in the presence of M-IBDET. Ten μg of sucrose gradient-purified virus samples in 0.1 ml tris-NaCl buffer, pH 8.3, were mixed with equal volumes of exogenous and endogenous reaction mixtures containing 34 μM M-IBDET. Control mixtures contained 0.1% DMSO. At indicated times, 20 μl amounts were withdrawn and incorporation terminated as described. Exogenous reactions: ●—●, control; ○—○, with M-IBDET. Endogenous reactions: ●—●, control; ○—○, with M-IBDET.
Inhibition of MLV by M-IBDET

Table I. Minimal 50% inhibitory dose of different antimetabolites on MLV production*

<table>
<thead>
<tr>
<th>Antimetabolites</th>
<th>MID&lt;sub&gt;50t&lt;/sub&gt;</th>
<th>MID&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>µM</td>
</tr>
<tr>
<td>Actinomycin D</td>
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<td>0.08</td>
</tr>
<tr>
<td>Cycloheximide</td>
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<td>3.5</td>
</tr>
<tr>
<td>α-Amanitin</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>M-IBDET</td>
<td>5</td>
<td>17</td>
</tr>
</tbody>
</table>

* 3T3/MLV confluent cultures were treated with different drug concentrations (0.1 to 100 µg/ml). Virus production was measured 6 h after drug exposure by measuring reverse transcriptase activity.

† The minimal effective dose required to inhibit 50% of enzyme activity.

The reversibility of M-IBDET blockage of cellular DNA was tested by exposing 3T3/MLV cells to 17 µM-M-IBDET for 6 h (Fig. 3). The drug was removed and DNA synthesis subsequently monitored by <sup>3</sup>H-thymidine incorporation. DNA synthesis was seen to recover after a lag and eventually approached the control rate of synthesis.

Comparison of MLV inhibition by M-IBDET, actinomycin D, cycloheximide and α-amanitin

In an attempt to clarify the mechanism of the antiviral activity of M-IBDET, we compared the range of effectivity of several known antimetabolites like actinomycin D, cycloheximide and α-amanitin to M-IBDET in terms of their effect on MLV production. The amount of MLV released into the culture medium within 6 h was determined in the different drug-treated cells at various concentrations and compared to untreated cells. The minimal effective dose required to inhibit 50% of virus production was determined for each drug. Table 1 summarizes the results. We can conclude that the effective concentration range of M-IBDET is within the range of the other antimetabolites tested.

Effect of M-IBDET on reverse transcriptase of MLV

Reverse transcriptase activity of MLV provided a quantitative assay for screening the effect of the different thiosemicarbazones on MLV production. Therefore the effect of M-IBDET on the endogenous and exogenous polymerase reactions within the virus particles was examined in the same reaction mixture used to assay virus yield by its reverse transcriptase activity. Fig. 4 shows the kinetics of the <i>in vitro</i> polymerase reaction. Addition of 34 µM-M-IBDET to the reaction mixture had no effect on the kinetics of <sup>3</sup>H-TMP incorporation. M-IBDET also had no inhibitory effect on the enzymic activity of a partially purified MLV enzymic preparation prepared according to Teitz (1974). Very similar results were obtained with M-IBT and IBDET; at 34 µM neither compound affected the enzymic reaction in our experimental conditions.

Inhibition of infectious virus production by M-IBDET

The inhibitory effect of M-IBDET on MLV production was determined on the basis of the virus reverse transcriptase activity. It was important, therefore, to confirm the drug effect on virus production by assaying virus infectivity. Culture media of drug-treated and untreated MLV cells were assayed for virus infectivity during an 18 h period of drug exposure. Fig. 5 shows that 17 µM suppressed infective virus production after 6 h of drug treatment. Infective virus levels were reduced by 2 logs after 18 h of drug exposure. The inhibitory effect of the drug was more pronounced when virus production was measured by infectivity than by reverse transcriptase activity (see Fig. 2). Therefore the possible effect of M-IBDET on
Fig. 5. Release of infectious viruses in the presence of M-IBDET. 3T3/MLV confluent cell cultures were treated for 18 h with two different doses of M-IBDET, 17 \( \mu \)M (●—●) and 8.5 \( \mu \)M (▲—▲); 0.1% DMSO was added to control cultures (○—○). At indicated times titres of infectious virus in extracellular fluids were determined; the results are represented as log TCID_{50} (see Methods).

Fig. 6. Sucrose gradient profiles of virus particles radioactively labelled in RNA in the presence of M-IBDET. \( ^{3} \)H-uridine (10 \( \mu \)Ci/ml) labelled medium supplemented with 17 \( \mu \)M-M-IBDET was added to 3T3/MLV confluent cell cultures. Control cultures contained 0.1% DMSO. Radioactively-labelled virus was collected 6 h later and analysed on sucrose gradients (see Methods). ●—●, Curve A, control; ○—○, curve B, M-IBDET-treated.

Table 2. Contact effect of M-IBDET and M-IBT on MLV*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time of exposure (h)</th>
<th>Reverse transcriptase†</th>
<th>Infectivity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-IBDET</td>
<td>3</td>
<td>3.8</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3.8</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.2</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>2.1</td>
<td>3.5</td>
</tr>
<tr>
<td>M-IBT</td>
<td>3</td>
<td>4.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.0</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>2.2</td>
<td>4.2</td>
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<tr>
<td>Control</td>
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<tr>
<td></td>
<td>6</td>
<td>3.8</td>
<td>5.5</td>
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<td></td>
<td>12</td>
<td>3.0</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1.9</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* One ml samples of 3T3/MLV culture medium (10^{6.5} TCID_{50}/o.I ml infectious virus and 4 \( \times \)10^{8} \( ^{3} \)H-TMP/30/o.I ml active enzyme) were incubated at 37 °C in the presence of 17 \( \mu \)M-M-IBDET or M-IBT. Control samples contained 0.1% DMSO. At the indicated intervals o.l ml amounts were assayed for reverse transcriptase activity and virus infectivity (see Methods).
† Reverse transcriptase activity expressed as pmol \( ^{3} \)H-TMP \( \times \)10^{-9} incorporated in standard reaction.
‡ Log infectious virus titre.

Virus infectivity by mere contact inactivation at similar experimental conditions was examined. MLV exposure to 17 \( \mu \)M-M-IBDET in culture media caused no reduction in its reverse transcriptase activity during the 18 h incubation period. Relative virus infectivity was also not reduced after 3 and 6 h while after 18 h exposure of the virus to the drug 0.5 log decrease in virus infectivity was noted. Under the same conditions M-IBT did not inactivate virus reverse transcriptase activity or infectivity (see Table 2).
Inhibition of MLV by M-IBDET

Effect of M-IBDET on the release of radioactively-labelled RNA virus particles

Inhibition of virus production by M-IBDET was also confirmed by reduction in the yield of virus particles radioactively labelled in their RNA. Curve A in Fig. 6 shows a single peak of \(^{3}H\) radioactivity in a sucrose density gradient purification of \(^{3}H\)-uridine-labelled virus from culture fluid of untreated 3T3/MLV cells. The peak had its maximum radioactivity at 1.16 to 1.18 g/ml, the characteristic density of type C particles. Curve B in Fig. 6 had an identical density peak and corresponds to a sucrose density gradient purification of culture medium from the 17 \(\mu\)M-M-IBDET-treated cells. Thus, a 50% reduction in labelled virus RNA yield was obtained.

Effect of M-IBDET on virus structural proteins

M-IBDET did not seem to affect the protein synthesis of the host cells and, therefore, its effect on the various virus structural proteins was examined to ascertain a possible preferential inhibition of specific virus structural proteins. Fig. 7 illustrates the virus structural protein profile of MLV on SDS acrylamide gel. Five distinct peaks corresponding to the different virus proteins can be detected. In the particular conditions used for electrophoresis the low mol. wt. proteins migrate close together. In the M-IBDET-treated cell cultures the amount of labelled virus proteins was reduced by about 50%, while the relative proportions of the different virus structural proteins remained the same. Thus, the main peak of p30 contains about 41% of the labelled virus proteins, the glycoprotein peak gp69/71 contains 18%, and gp45 contains about 6% of the labelled virus proteins. Among the lower mol. wt. proteins p15 is about 12%; p12 and p10 are about 22%.
DISCUSSION

This work presents the first report on the effect of N-methylisatin-β-4'-4'-diethylthiosemicarbazone (M-IBDET) on MLV. Nine synthesized compounds, all thiosemicarbazones of various mono- and bicyclic α-ketolactames were examined with the result that only M-IBDET inhibited virus production at concentrations that caused no visible c.p.e. Bauer and his collaborators found that N-methyl and N-ethyl derivatives of IBT were active against vaccinia virus. The drug M-IBT has been used against smallpox. The addition of dialkyl groups to the terminal nitrogen atom of isatin-β-thiosemicarbazone (IBT) seems to confer a wider antiviral spectrum embracing both DNA and RNA-containing animal viruses. The basis for the extended range of activity appears to be that dialkyl-substituted compounds are capable of inhibiting both cellular and virus DNA synthesis as well as virus RNA-dependent RNA synthesis (Pearson & Zimmerman, 1969). Substitution in the side chain of M-IBT with diethyl eliminates the anti-vaccinal action, but endows high activity against ectromelia (O'Sullivan et al. 1963), as well as against MLV (Edelstein et al. 1978).

Virus production in the presence of different concentrations of M-IBDET showed that 34 μM inhibited virus production almost completely after 3 h of treatment. No increase of virus yield was noted 6 h after treatment with 17 μM and after 9 h treatment with 3.4 μM.

Inhibition of virus production was confirmed by various parameters. The most sensitive proved to be virus infectivity. The small contact inactivation of virus infectivity observed after 18 h exposure to M-IBDET could only partially account for this difference.

M-IBT did not inhibit MLV production. The drug was reported to cause a contact inactivation of Moloney sarcoma virus (MSV) and other viruses (Levy et al. 1976; Fox et al. 1977). In the case of M-IBDET, in additional experiments showing inhibition of MLV production, contact inactivation was eliminated by monitoring the virus in fresh culture media free of the drug.

Reverse transcriptase activity in vitro was not inhibited by 34 μM-M-IBDET. M-IBT was shown to inhibit reverse transcriptase activity in reaction mixtures free of sulphydryl compounds (Levinson et al. 1973). Such inhibition of the enzymic activity by M-IBT or M-IBDET in the absence of dithiothreitol (DTT) was noticed by us only when virus particles were pre-incubated with the drugs at 37 °C and not at 4 °C.

It has previously been shown that isatin-β-thiosemicarbazone (IBT) compounds varied in their effect on the growth and DNA synthesis of animal cells (Sadler, 1965). We found that treatment with 17 μM-IBDET suppressed DNA synthesis after 1 h, while 17 μM-M-IBDET caused 50% inhibition only after 6 h. Some of the isatin-β-thiosemicarbazone derivatives are reversible inhibitors of cellular DNA synthesis. M-IBDET inhibition of cellular DNA was also found to be reversible (Fig. 2). It should be mentioned that MLV replication in the chronic infection of the rat cells, RT43M, is independent of cellular DNA synthesis for short periods, intervals of about 6 h. It has been shown that a 6 h exposure of chronically infected cells to inhibitors of DNA synthesis, such as cytosine arabinoside or 5-fluorodeoxyuridine, does not affect the release of MLV particles into the media (Teitz et al. 1971). It should be emphasized that 17 μM, the antiviral concentration of M-IBDET, did not affect RNA and protein synthesis of the 3T3/MLV cells for the first 6 h after treatment.

The mechanism of action of thiosemicarbazones should be clarified with regard to each specific virus. A specific inhibition of the virus RNA-dependent RNA polymerase accounted for the inhibition of polio virus by N-methylisatin-β-dibutylthiosemicarbazone (M-IBDBT; Pearson & Zimmerman, 1969). M-IBT activity against smallpox and vaccinia is due to a specific inhibition at a late step during virus maturation (Katz & Felix, 1977). Further studies should be aimed at clarifying the mode of the inhibitory action of M-IBDET on MLV replication.
Inhibition of MLV by M-IBDET

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