Effects of Actinomycin D on Avian Myeloblast and BAI Strain A Virus RNA Synthesis in Vitro

ROSEMARIE ZISCHKA, A. J. LANGLOIS, P. R. RAO, R. A. BONAR, AND J. W. BEARD

Department of Surgery, Duke University Medical Center, Durham, North Carolina

Summary

Avian myeloblasts infected with BAI strain A avian tumor virus were cultured in vitro and treated with actinomycin D. The effects of the drug on cell growth and death and the synthesis of cell and virus RNA were examined. Actinomycin D (AD) concentrations of 0.01-0.05 μg/ml caused cell damage and death at proportional rates, and, as shown in earlier work, inhibited cell RNA synthesis up to 60% without depressing virus RNA synthesis or virus particle liberation. In comparison, 0.5 μg AD/ml caused more rapid cell death with parallel inhibition of 70-85% of cell RNA synthesis and 70% of virus RNA synthesis but did not decrease the output of particles having the characteristic virus adenosinetriphosphatase activity. These results are discussed and compared with findings with other virus-host systems.

Introduction

Assembly and elaboration of the BAI strain A avian tumor virus (7) occurs by particle budding at the external cell membrane (39) of myeloblasts from birds with myeloblastic leukemia (15) induced by the agent. Evidence of other aspects of cell involvement in the synthesis and formation of the agent containing RNA, lipid, and protein (12) has been sparse. Tracer studies (35) indicated that synthesis of viral RNA involved poly-nucleotides occurring in the soluble fraction of the cells.

Results with other virus-cell systems suggested that AD, which binds reversibly to DNA (14, 17) and inhibits DNA-dependent RNA synthesis (29) might be used to investigate the possible role of DNA in the synthesis of BAI strain A virus RNA by myeloblasts. Formation of some RNA viruses such as Mengo (16, 27, 28), polio (33, 44), and vesicular stomatitis (2), has been reported to proceed in the presence of AD. Tobacco mosaic virus RNA is synthesized (31), although there may be a small reduction in infectious agent yield in the presence of the antibiotic. Results with Newcastle disease agent have varied with experimental conditions (6, 21, 24, 30, 43) from enhancement of virus synthesis to about 90% inhibition of the process. Elaboration of infectious influenza (6, 21) and fowl plague (4, 5, 30) viruses was inhibited more than that of Newcastle disease virus, but the results were strongly influenced by experimental conditions. Influenza virus-induced RNA synthesis was diminished, although it was less sensitive to the drug than was virus production. In another study (13) with a different strain of influenza and with fowl plague virus, no inhibition of virus-induced RNA synthesis was seen. Differences in antibiotic dose, cell type, and methods of assay all influence the results of the inhibitor studies and make a close comparison of the various results with the myxoviruses difficult. Also of interest was the finding (19) that AD could inhibit the in vitro synthesis of RNA using reovirus RNA as template and an RNA polymerase from Escherichia coli. Reovirus formation appears to be enhanced at low concentrations of AD and inhibited by higher levels of the antibiotic (20, 34).

Closely related to the present work were findings with 2 avian tumor virus-host cell systems. AD sharply depressed synthesis of Rous sarcoma virus RNA (37) and of infectious Rous sarcoma virus (2, 3, 36, 40, 41) and "Rous-associated virus" (3) when added to cultures of chick embryo cells close to the time of infection. Virus elaboration by established Rous cells was much less sensitive (36, 40, 41) to the drug, and the inhibition was reversed on removal of AD. It has been reported briefly (1) that synthesis of BAI strain A virus RNA by infected chick embryo fibroblasts was inhibited by very low concentrations of AD added at the time of infection.

In the present work, we investigated the effects of AD on growth of and RNA synthesis by avian leukemic myeloblasts and on elaboration of BAI strain A virus and virus RNA synthesis by the myeloblasts cultured in vitro. As already reported (45), addition of AD to myeloblast cultures resulted in marked decline in cell RNA synthesis, while, at the concentrations used then, the rate of virus RNA synthesis did not decrease. Work with higher concentrations of AD has shown inhibition of viral RNA synthesis as well as rapid cell death. The present work included studies on virus liberation into culture fluids in relation to cell growth and damage. Morphologic cell changes were examined by light and electron microscopy. The biochemical and virus elaboration studies are considered in this report, and the ultrastructural features of AD-treated myeloblasts will be described separately (22).

Materials and Methods

MYELOBLAST CULTURES. White Leghorn chicks of Line 15 (Regional Poultry Research Laboratory, East Lansing, Mich.) (42) were inoculated with BAI strain A virus at 3-5 days of...
age. Birds developing high concentrations of leukemic myeloblasts in the peripheral circulation determined by daily blood smears and high content of virus in the plasma estimated by adenosinetriphosphatase (ATPase) activity (11) 18–32 days after inoculation were bled by heart puncture. Myeloblasts collected by centrifugation of the heparinized blood were washed in Gey’s saline solution and suspended for culture by the procedures previously described (11) in a mixture of equal parts of chicken serum and Medium 199 with penicillin, streptomycin, and added folic acid. All cultures were incubated at 37°C on a rotatory oscillator.

In some experiments, cell growth and virus release were studied in the usual (11) 5-ml cultures containing 2.5–5 × 10⁷ cells/ml incubated in 50-ml Erlenmeyer flasks. Medium was changed and cultures were divided at appropriate intervals (11), 2–3 days in growing cultures. When larger amounts of material were required, similar cultures were of 30- or 60-ml volumes in 500-ml flasks. Cultures containing about 10⁸ cells/ml were used in some short-term studies. Fresh cultures were maintained for 7–15 days before study to permit attainment of the “equilibrium” state (10) of exponential cell growth and constant rate of virus liberation. Attributes of cell growth and virus formation vary (11) with cells from different donors, and cultures were selected for suitable behavior in these respects. Viable cells were counted in a hemocytometer by distinction from “nonviable” elements staining with trypan blue (10). Numbers of virus particles were estimated by ATPase activity measured in reference to a standard of virus content determined by electron microscopic particle enumeration (32). Virus amount was measured in some studies by both enzyme activity and direct particle count. In some experiments, quantitative relationships between cell growth and virus release were analyzed as previously described (10).

Treatment with AD (obtained from Merck, Sharp and Dohme, West Point, Pa.) was effected by diluting fresh aqueous antibiotic solution in desired concentrations in whole culture medium. The number of viable cells in a culture was determined, and the cells recovered by centrifugation were resuspended in the medium containing AD. Cell exposure to antibiotic was continuous in the periods of study, and necessary changes of culture fluid were made with medium containing AD in order to provide for the additional DNA formed in the cultures still growing in low AD concentrations.

Incorporation of tritium-labeled uridine, specific radioactivity 3 c/mmole (New England Nuclear Corporation, Boston, Mass.), was studied by addition of 1 or 5 μc of uridine-³H/ml of culture.

RNA extraction. For studies on cell RNA synthesis, myeloblasts were washed 3 times with Gey’s saline solution after exposure to uridine-³H and broken with a Wig-L-Bug (Crescent Dental Manufacturing Co., Chicago, Ill.) for 1 min using the 2-ball pestles. All cells were broken in this period, and the results were uniformly reproducible with small amounts of material. The nucleic acids were extracted with hot 10% NaCl and precipitated with ethyl alcohol, and RNA was separated from DNA by alkaline hydrolysis as described (35). The RNA was determined by absorption at 260 μm. The ultraviolet absorption spectrums indicated a high degree of purity. RNA solutions were evaporated to dryness, and the residues were transferred to vials for radioactivity measurement in a liquid scintillation counter (Packard Instruments Company, Inc., La Grange, Ill.) as described (35).

Virus isolation. Virus was purified by 4 cycles of alternate low and high speed centrifugation (12) of culture fluids. After the last centrifugation, virus was resuspended in 0.2 ml water plus 1 ml Hyamine (10X) and transferred to counting vials for radioactivity determination. In the earlier part of these studies, some of the virus suspensions were treated with ribonuclease and cold uridine as described before (35) to test for possible contamination by cell RNA. No difference was found between treated and untreated preparations, and the treatment was omitted in later

---

**TABLE 1**

<table>
<thead>
<tr>
<th>ACTINOMYCIN D (μg/ml)</th>
<th>Viable cells</th>
<th>Virus particles/ml × 10⁻¹⁰ (24 hr)</th>
<th>ATPase</th>
<th>Direct count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
<td>4 hr</td>
<td>24 hr</td>
<td>0 hr</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td>9.7</td>
</tr>
<tr>
<td>0.1</td>
<td>10.1</td>
<td>10.8</td>
<td>11.2</td>
<td>96</td>
</tr>
<tr>
<td>0.2</td>
<td>10.9</td>
<td>12.2</td>
<td>7.7</td>
<td>96</td>
</tr>
<tr>
<td>0.5</td>
<td>10.4</td>
<td>10.6</td>
<td>3.2</td>
<td>98</td>
</tr>
</tbody>
</table>

---

**Chart 1.** Cell growth and virus release in 5-ml myeloblast cultures containing different concentrations of AD. X—-X indicates virus particle number; and O and • represent viable cell counts. The solid line was fitted (10) on the assumption of logarithmic cell growth, and the dash line on the assumption that the rate of virus particle release was constant/cell/hr. All values were the average of estimates on triplicate cultures. The arrows indicate the first cell change to medium containing AD, and •'s indicate change to fresh medium containing the antibiotic. Cell concentration was maintained at 2-5 × 10⁷/ml.
Aclinomycin D and RXA Synthesis

CONTROL ACTINOMYCIN 0

HOURS

GHAUT 2. Cell growth and death and virus release over 24 hr in myeloblast cultures with and without 0.5 µg Al/ml added at 0 time. The lines were fitted (10) to the total cell count by assuming logarithmic cell growth for 18 hr and to the virus particle count by assuming constant rates of virus production/cell (total)/hr for 18 hr. Dashed portions of the lines indicate extrapolations.

2.5-5 × 10^7 myeloblasts/ml, the cells grow exponentially for indefinite periods and liberate virus into the culture fluid at constant rates/cell/hr (11). The agent released can be estimated quantitatively in terms of numbers of physical virus particles by ATPase assay standardized by electron microscopic particle enumeration, as already shown (9) in earlier tissue culture experiments. Detection of departures from the usual pattern of cell growth and virus release can be facilitated by mathematical description of the data (10).

Chart 1 shows that continued cell exposure to 0.001 µg AD/ml in such cultures did not influence either cell growth or rate of virus release. The small transient decrease in rate of virus output in this experiment was not significantly different from that frequently observed in myeloblast cultures (11). In contrast, higher antibiotic concentrations caused cell damage and death after decreasing periods of exposure, and with 0.1 µg/ml, essentially all of the cells were nonviable within 48 hr.

It is notable, however, that virus output, as indicated by this method of analysis, did not decline appreciably with onset of decline in the number of viable cells. This was particularly noticeable in the experiment with 0.05 µg of antibiotic in Chart 1.

For determination of effects on cell and virus RNA synthesis, it was desirable to employ antibiotic concentrations sufficient to exert specific influence without death of the cells within the period of biochemical study. Since 0.05 µg AD/ml (Chart 1) had no inhibitory effect on virus RNA synthesis (45), the time course of effects of higher concentrations was examined. Table 1 gives the data obtained in a 24-hr experiment to determine the effects of antibiotic concentrations of 0.1, 0.2, and 0.5 µg/ml on cell growth and viability and virus output. Single control and treated 5-ml equilibrium cultures but containing about 10^6 myeloblasts/ml in this experiment were used for each concentration. Little effect

Chart 3. Effect of different concentrations of AlD on incorporation of uridine-3H into myeloblast cell RNA. Cells at concentrations of 3-5 × 10^7/ml were exposed to AD for 3 hr, then uridine-3H was added for 3 or 6 hr, and the RNA was extracted.

Recovered virus was determined by ATPase activity of the agent obtained in the 3rd sedimentation cycle.

Cell Examination. Cells from various cultures were examined by phase contrast microscopy, and samples were taken for ultrastructural study described in another report (22).

Results

Preliminary studies were made to determine the influence of different concentrations of AD on myeloblasts as usually maintained in vitro. Under the conditions of 5-ml volumes containing 2.5-5 × 10^7 myeloblasts/ml, the cells grow exponentially for indefinite periods and liberate virus into the culture fluid at constant rates/cell/hr (11). The agent released can be estimated quantitatively in terms of numbers of physical virus particles by ATPase assay standardized by electron microscopic particle enumeration, as already shown (9) in earlier tissue culture experiments. Detection of departures from the usual pattern of cell growth and virus release can be facilitated by mathematical description of the data (10).

Chart 1 shows that continued cell exposure to 0.001 µg AD/ml in such cultures did not influence either cell growth or rate of virus release. The small transient decrease in rate of virus output in this experiment was not significantly different from that frequently observed in myeloblast cultures (11). In contrast, higher antibiotic concentrations caused cell damage and death after decreasing periods of exposure, and with 0.1 µg/ml, essentially all of the cells were nonviable within 48 hr.

It is notable, however, that virus output, as indicated by this method of analysis, did not decline appreciably with onset of decline in the number of viable cells. This was particularly noticeable in the experiment with 0.05 µg of antibiotic in Chart 1.

For determination of effects on cell and virus RNA synthesis, it was desirable to employ antibiotic concentrations sufficient to exert specific influence without death of the cells within the period of biochemical study. Since 0.05 µg AD/ml (Chart 1) had no inhibitory effect on virus RNA synthesis (45), the time course of effects of higher concentrations was examined. Table 1 gives the data obtained in a 24-hr experiment to determine the effects of antibiotic concentrations of 0.1, 0.2, and 0.5 µg/ml on cell growth and viability and virus output. Single control and treated 5-ml equilibrium cultures but containing about 10^6 myeloblasts/ml in this experiment were used for each concentration. Little effect

SEPTEMBER 1966

1841

Downloaded from cancerres.aacrjournals.org on April 12, 2017. © 1966 American Association for Cancer Research.
TABLE 2

EFFECT OF ACTINOMYCIN D ON URIDINE-3H UPTAKE INTO RNA OF CELLS AND VIRUS
IN AVIAN MYELOBLASTOSIS

Each value is the mean of 3 separate flasks. Values for inhibition of viral RNA synthesis were calculated from total radioactivity to avoid the errors in estimating the small numbers of virus particles produced.

<table>
<thead>
<tr>
<th></th>
<th>Virus</th>
<th>Cell RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No actinomycin (0.5 µg/ml)</td>
<td>No actinomycin (0.5 µg/ml)</td>
</tr>
<tr>
<td>5-hr experiment*</td>
<td>Total radioactivity (cpm)</td>
<td>Total radioactivity (cpm)</td>
</tr>
<tr>
<td></td>
<td>5410</td>
<td>5410</td>
</tr>
<tr>
<td></td>
<td>2090</td>
<td>2090</td>
</tr>
<tr>
<td></td>
<td>37.1 X 10^4</td>
<td>37.1 X 10^4</td>
</tr>
<tr>
<td></td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>475</td>
<td>475</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>10-hr experiment*</td>
<td>Total radioactivity (cpm)</td>
<td>Total radioactivity (cpm)</td>
</tr>
<tr>
<td></td>
<td>881</td>
<td>881</td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>16.7 X 10^4</td>
<td>16.7 X 10^4</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>556</td>
<td>556</td>
</tr>
<tr>
<td></td>
<td>188</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>66</td>
</tr>
</tbody>
</table>

* For 2 hr, 9 X 10^6 cells/ml were exposed to 0.5 µg actinomycin D/ml, and uridine-3H (5 µc/ml) was then added for 3 hr.
* For 3 hr, 1.4 X 10^7 cells/ml were exposed to 0.5 µg actinomycin D/ml, and uridine-3H (5 µc/ml) was then added for 7 hr.

The proportion of nonviable cells in the control culture remained at approximately the 5% level, and the total number of cells in the control thus reflected principally the number remaining viable. It is probable that decline in the cell growth in the control culture after 18 hr was related to the high concentration of myeloblasts and consequent depletion of the culture medium.

In contrast, increase in nonviable cells in the treated culture began at about 6 hr, and the number of viable cells declined progressively. At the end of 24 hr, the numbers of viable and nonviable cells were approximately equal. That cell growth continued despite the lethal action of antibiotic on some myeloblasts, however, was shown by the continued increase in total cells in the treated culture until the 21-hr period.

An impressive feature of the results was the apparently concomitant elaboration of virus particles, as measured by ATPase activity in cultures containing a high proportion of cells which were nonviable as judged by staining with trypan blue (Charts 1 and 2) and which showed marked morphologic change (22). To determine whether the ATPase activity was associated with particulate material, supernatant fluids from control and AD-treated cultures were centrifuged at 3000 X g for 15 min and 30,000 X g for 45 min, and the ATPase activity in pellets and supernatants was determined. The sedimentation behavior of the enzyme activity was that expected of BAI strain A virus in fluid from both treated and untreated cultures; activity was found almost entirely in the supernatant fluid after low-speed centrifugation or in the pellet obtained with the higher speed. Myeloblasts exhibit ATPase activity at the cell surface (39) and, consequently, there was the possibility that the ATPase activity in the culture fluid might have been due to membrane fragments of disintegrating cells. That this was unlikely in the first 18 hr of the experiment is indicated in Chart 2 by the close correspondence with the expected log phase.
Actinomycin D and RNA Synthesis

Chart 4. Cell growth and death and virus formation measured hourly in control cultures and in cultures from the same stock treated with 0.5 μg AD/ml at 0 time. Uridine-3H was added at 3 hr, and cells and virus were collected at 10 hr, extracted, and analyzed to provide the data on RNA synthesis shown in Table 2. Each point represents the mean of 3 culture flasks. The lines were fitted as in Chart 2 with use of the 0-10 hr values for the control and the 0-6 hr values for the AD-treated culture.

Chart 5. Viable and nonviable cells and virus particles in control cultures and in cultures treated with 0.5 μg AD/ml at 0 hr. Uridine-3H was added at 2 hr, and cells and virus were collected at 5 hr and extracted and analyzed to provide the data on uridine-3H incorporation into RNA shown in Table 2. Each point represents the mean value of 3 culture flasks.

between control and treated cultures in particle elaboration/cell and the apparent absence of cell disintegration on electron microscopic examination. The release of particles having ATPase activity by both control and treated cultures appeared to be parallel. It may be seen in Chart 2, however, that when a decline in the number of total cells occurred at 21 or 24 hr, indicating disintegration of cells, the ATPase activity rose, deviating from the normal relation of virus output to cell number. This rise in ATPase activity at the end of the experiment might be explained as being due to cell debris or, possibly, to liberation of intracellular or partially formed virus particles coincidental with cell breakdown. Such increase in ATPase activity corresponding to the time of cell breakdown and decrease in number of total cells was observed, also, in other experiments (Chart 4).

AD effect on myeloblast cell and virus RNA synthesis. The effects of various concentrations of AD on the uptake of uridine-3H into myeloblast cell RNA are shown in Chart 3 for cell concentrations of 3-5 × 10⁶/ml. The degree of inhibition with a given level of AD was somewhat less with higher cell concentrations reflecting the larger amount of DNA available to bind the drug. With 0.5 μg AD and 1 × 10⁶ cells/ml, for example, uptake of uridine-3H into cell RNA was inhibited about 70% (Table 2) as compared with 86% inhibition in cells cultured at the lower cell concentration.

In an earlier study (43) it was found that low concentrations of AD (0.01 and 0.05 μg/ml) did not inhibit uptake of uridine-3H into viral RNA even though a concentration of 0.05 μg/ml was sufficient to cause about 60% inhibition of cell RNA synthesis (43) as well as characteristic nucleolar reorganization (2) and, in time, cell death (Chart 1). It was desirable to extend the studies to higher concentration of AD, if it was possible to do so with intact cells. The results shown in Table 1 and Chart 2 indicated that the effect of 0.5 μg AD/ml on RNA synthesis could be studied for time periods of approximately 6 hr without extensive cell death and degeneration.

The results of 2 experiments with 0.5 μg AD/ml in cultures containing about 10⁶ cells/ml are given in Charts 4 and 5 and Table 2. In the experiment of Chart 4, progressive decrease in the number of viable cells began at 6 hr. In the total period of 10 hr in this experiment, the uridine-3H uptake measured in the interval of 3-10 hr exposure to AD indicated inhibition of both cell and virus RNA synthesis (Table 2).

In order to restrict the observations only to viable cells, the study of Chart 5 was limited to a 5-hr period during which there was no increase in nonviable myeloblasts. Nevertheless, in this
experiment, also, there was marked inhibition of both cell and virus RNA synthesis, Table 2.

It is notable, particularly, in Chart 4, that, as in Chart 2, ATPase-positive material increased despite cell damage and decline in virus RNA synthesis. In Chart 4, there was, indeed, a substantial and progressive increase in output of particulate material with ATPase activity above that in the control cultures. Liberation of such particles in the AD-treated cultures of Chart 5 paralleled that of the controls.

Discussion

The myeloblast-BAI strain A virus culture system exhibits some features different from those of most other RNA virus-cell relationships. Each myeloblast from the leukemic bird is presumably infected with virus, and the association results in an enduring process of exponential cell growth and virus synthesis and liberation at constant rates/cell/hr. Cells growing in suspension are constantly available for repeated sampling of the same preparation, and virus production can be measured in terms of physical particle number by enzyme activity or direct electron microscopic count. The myeloblasts investigated were derivatives of cells infected for periods of weeks, and thus the processes requisite for cell growth and virus formation were fully established. Uninfected myeloblasts do not survive in vitro under the conditions employed (8).

Effects of AD on the myeloblasts were exerted on cell growth and on cell RNA synthesis in proportion to the concentration of AD in relation to cell concentration. AD in 0.001 μg/ml concentration did not affect cell growth for 15 days, but higher concentrations caused progressive damage resulting in the onset of cell death after decreasing intervals of exposure (Table 1; Charts 1, 2, 4). Sensitivity to the antibiotic varied with cells from different donor birds and differed greatly with individual elements of a single population as indicated by progressive cell death and by variations in ultrastructural alterations in the later stages of exposure to AD (22). Events occurring with 0.5 μg AD/ml were rather uniform in the intervals of 5-6 hr treatment (Charts 2, 4) during which time discernible ultrastructural alterations were limited to changes in the nucleus.

Cell RNA synthesis was likewise affected by the same factors, but inhibition did not closely parallel changes in cell behavior expressed as growth or survival. Exposure of 3–5 × 10⁷ myeloblasts/ml to AD in concentrations of 0.01, 0.05 (45), and 0.5 μg/ml resulted in inhibition of uridine-3H incorporation into cell RNA of about 30, 58, and 86%, respectively. At 0.01 μg/ml, however, growth continued for 6 days (Chart 1); at 0.05 μg/ml for 48 hr; and at 0.5 μg/ml with about 10⁷ cells for 6 hr (Chart 4) or, occasionally, to 21 hr (Chart 2). Thus, damage to the cells evidenced both by inhibition of RNA synthesis and by ultrastructural alterations did not suffice to suppress growth of all cells in the periods indicated. Nevertheless, exposure to 0.5 μg/ml leads afterward to progressive cell death with inhibition of cell RNA synthesis at levels of 60 to 70% which were not greatly different from the 86% value found with 0.05 μg/ml at the lower cell concentration (45).

A considerable disparity was observed on comparison of cell behavior with synthesis of virus RNA and liberation of the agent. As reported (45), virus RNA synthesis was not inhibited by 0.05 μg AD/ml despite a decrease of about 60% in cell RNA synthesis. Instead, the data suggested an increase in virus RNA synthesis. However, treatment with 0.5 μg/ml resulted in comparable inhibitions of virus and cell RNA syntheses. It is notable that the most significant measurements (Table 2) were made in the period (Chart 5) before onset of cell death as detected by staining with trypan blue and ultrastructural alterations. This indicated that inhibition of virus RNA synthesis occurred in cells which were viable by the criteria applied.

In contrast to the inhibition of cell and virus RNA syntheses was the lack of evidence of concurrent suppression of virus particle formation as estimated by ATPase activity. On the contrary, it was consistently seen that the rate of virus particle liberation either remained constant or increased (Charts 2, 4, 5) even after onset of cell death and disintegration.

The findings were explicit in demonstrating inhibition of virus as well as cell RNA syntheses by AD in sufficient concentration. It was further evident, however, that the processes of virus RNA synthesis were less sensitive to AD than those of cell RNA synthesis, being affected only at higher drug concentrations. An apparently somewhat similar situation has been reported in the case of reovirus in L cells (34) in which cell RNA synthesis could be inhibited as much as 90% without diminishing virus formation. Differential sensitivities of synthesis of various cell RNA's have likewise been reported (25, 29).

In some respects, AD inhibition of BAI A virus RNA synthesis by myeloblasts was analogous to inhibition of Rous virus elaboration by chick embryo fibroblasts (2, 36, 37, 40, 41). Most of these latter studies (36, 37, 40) were made under conditions conducive to severe cell damage but, more recently, similar results were obtained (2) with cells not showing morphologic alterations. The experiments with the myeloblast-BAI A virus system showed that inhibition could occur in cultures of viable and growing cells which, nevertheless, all showed nucleolar damage (22). Myeloblasts appeared to be less sensitive to AD than chick embryo fibroblasts. This may account for the reported inhibition of BAI A virus RNA synthesis by fibroblasts (1) treated with AD in 0.005 μg/ml concentration in comparison with no inhibition of BAI A virus RNA synthesis by myeloblasts exposed to 0.05 μg AD/ml.

The basis for continued increase in the number of ATPase-positive particles despite cell damage and partial suppression of virus RNA synthesis is not clear from the data thus far obtained. The particles representing increase might have been derived by fragmentation of the plasma membranes of disintegrating myeloblasts which, like the virus, contain ATPase (39). Nevertheless, several aspects of the data: (a) close correspondence between control and treated cultures in the rates of formation of the ATPase-positive particles (Chart 2); (b) the agreement between ATPase determination and direct particle count (Table 1); and (c) the similarity of sedimentation properties of enzyme activity in the fluids of the control and treated cultures suggested that the particles liberated by the treated cells were similar to the virus. Formation of the agent involves budding of the cell membrane (26) stimulated by thus far unknown mechanisms. Even with inhibition of viral RNA synthesis, continued elaboration of virus par-

1844 CANCER RESEARCH VOL. 26

Downloaded from cancerres.aacrjournals.org on April 12, 2017. © 1966 American Association for Cancer Research.
particles might be possible, due to the presence of small precursor polynucleotides accumulated in the infected myeloblasts (35). Further work is necessary to determine whether such particles contain RNA and exhibit other properties of the virus.

AD inhibition of RSV formation by chick embryo cells has been interpreted to indicate that DNA plays a role as template for virus RNA synthesis (2, 36, 37, 40, 41). This view was supported by the observation of homology between RSV RNA and polynucleotides accumulated in the infected myeloblasts (35). The latter studies were not feasible with the BA I A-myeloblast system because of the lack of a suitable uninfected cell preparation.

Despite the similarity of some features of AD effects on RSV and BA I A virus RNA syntheses, it seems clear, particularly from recent work with myxoviruses (4, 5, 12, 21), that the results of studies using AD inhibition as an indicator of DNA-directed viral RNA synthesis must be interpreted with considerable caution. This is emphasized by a variety of observations: (a) there is a very large difference in sensitivity to AD of fowl plague virus formation measured in 2 cell types (100 times more sensitive in chick embryo cells than in allantoic cells) (5); (b) influenza virus-induced RNA synthesis may be less strongly inhibited than formation of infectious particles or hemagglutinin (13, 21); and (c) influenza virus, the elaboration of which is relatively sensitive to AD treatment (6, 21), apparently induces formation of a new ribonuclease-sensitive RNA synthetase (18) in calf kidney cells. These findings all suggest that while DNA function is involved in all cellular activity, its role may well be other than as template for viral RNA synthesis. The observation that an RNA-synthesizing system using reovirus RNA as template is AD sensitive (19) adds a further dimension to the problem of interpretation, as does the report of other effects of AD (23) apparently not directly related to RNA synthesis. When viral RNA synthesis is unaffected by AD, it appears probable that the synthesis does not require a DNA template, but the converse cannot be assumed.

References


SEPTEMBER 1966

1845


Effects of Actinomycin D on Avian Myeloblast and BAI Strain A Virus RNA Synthesis *in Vitro*

Rosemarie Zischka, A. J. Langlois, P. R. Rao, et al.

*Cancer Res* 1966;26:1839-1846.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/26/9_Part_1/1839

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.