tion in the concentration of sulfur compounds taken with a 2-hour sequential sampler (*9*).

We cannot now state quantitatively how much of the $SO_4^{2-}$ aerosol is natural; however, on the basis of the known source strengths of sulfur compounds in this region, it seems likely that human sources of $SO_2$ dominate. The sources of $SO_2$ (mainly fossil-fuel power plants) are located on a spatial scale of from 10 to 100 km in Missouri and the surrounding states. The removal distance for $SO_2$ and its oxidation products is of the order of 1000 km (*10*), so that any site in the region is continually under the influence of a large number and variety of sources.

It is intriguing to speculate that these compounds, which dominate the light-scattering hazes in eastern Missouri, may also extend to the entire Midwest. Such visible, turbid air is noted in summer from perhaps Topeka, Kansas, to the East Coast and from Chicago, Illinois, to Little Rock, Arkansas (*11*), and only really disappears with massive intrusions of Canadian air in winter. Until more studies of these acid aerosols are available, their real and potential effects can only be matters for speculation. The utility of control strategies for particulate matter in cities [including sulfates, which are alleged to have effects on health (*2*) and which may be mainly regional and not urban] will be even less certain than the preliminary results presented in this report (*12*).

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**References and Notes**

1. C. Junge and G. Scheich, *Atmos. Environ.* **3**, 423 (1969); J. Forrest and L. Newman, *J. Air Pollut. Contr. Ass.* **23**, 761 (1973).
2. C. M. Shy and J. F. Finklea, *Environ. Sci. Technol.* **7**, 204 (1973).
3. D. S. Covert, N. C. Ahlquist, R. J. Charlson, *J. Appl. Meteorol.* **11**, 968 (1972); S. S. Butcher and R. J. Charlson, *An Introduction to Air Chemistry* (Academic Press, New York, 1972), p. 206.
4. In all cases, the aerosol is exposed only to increasing humidity so that hysteresis is not a concern.
5. R. J. Charlson, *J. Colloid Interface Sci.* **39**, 240 (1972).
6. K. T. Whitby, R. B. Husar, B. Y. H. Liu, *ibid.*, p. 177; R. B. Husar, K. T. Whitby, B. Y. H. Liu, *ibid.*, p. 211.
7. T. G. Dzubay and R. K. Stevens, paper No. 8-5 presented at the 2nd Joint Conference on Sensing of Environmental Pollutants, Washington, D.C., 10–12 December 1973 (*Proc. Instrum. Soc. Am.*, in press).
8. A. P. Altshuller, *Environ. Sci. Technol.* **7**, 709 (1973).
9. Dzubay and Stevens (*7*) showed that the 2-hour average sulfur concentrations (expressed as the element but understood likely to be $SO_4^{2-}$) ranged from 2 to 12 $\mu g/m^3$, whereas the titanium concentration ranged from 0.02 to 2 $\mu g/m^3$ and the lead concentration varied from 0.1 to 2.5 $\mu g/m^3$.
10. H. Rodhe, C. Persson, O. Akesson, *Atmos. Environ.* **6**, 675 (1972).
11. E. C. Flowers, R. A. McCormick, K. R. Kurfis, *J. Appl. Meteorol.* **8**, 955 (1969).
12. A more detailed version of this report will appear elsewhere (*Atmos. Environ.*, in press).
13. Supported in part by grant R800665 from the Environmental Protection Agency and by grant GA 27662 from the Atmospheric Sciences Section of the National Science Foundation.

23 November 1973

## Dexamethasone Stimulation of Murine Mammary Tumor Virus Expression: A Tissue Culture Source of Virus

Abstract. *In mouse cell lines derived from mammary adenocarcinomas, the synthetic steroid dexamethasone stimulates production of murine mammary tumor virus. Viral RNA and antigens are increased as much as 20-fold, and culture fluid supernatants from steroid-treated cells contain type B particles with reverse transcriptase. These cells provide a possible tissue culture source of this virus and a model system for studying the mechanism of action of corticosteroids and the regulation of transcription of integrated viral DNA.*

Studies on the etiology of mammary tumors have relied largely on the mouse model system in which the murine mammary tumor virus (MMTV), an RNA-containing virus with a reverse transcriptase, is an important factor in naturally occurring mammary adenocarcinomas (*1*). Expression of MMTV in mouse tissues other than mammary glands is frequently recognized (*2*), and virus production in vivo is generally associated with epithelial tissues. In an effort to study the virus-cell interaction in greater detail, several cell lines that are derived from spontaneous mouse mammary tumors and contain relatively low levels of MMTV have been established (*3–5*). We found that clonal cell populations from one of these MMTV-positive tumor cell lines (CCL-51) had markedly different amounts of MMTV RNA and virion protein, but comparable amounts of MMTV DNA as measured by nucleic acid association reactions (*6*). These results and the widespread presence of MMTV DNA in different mouse strains (*7, 8*) suggested that although MMTV was present in all murine cells, its expression might be regulated by cellular control mechanisms acting at the level of MMTV RNA transcription from DNA.

In addition to the virus, a second

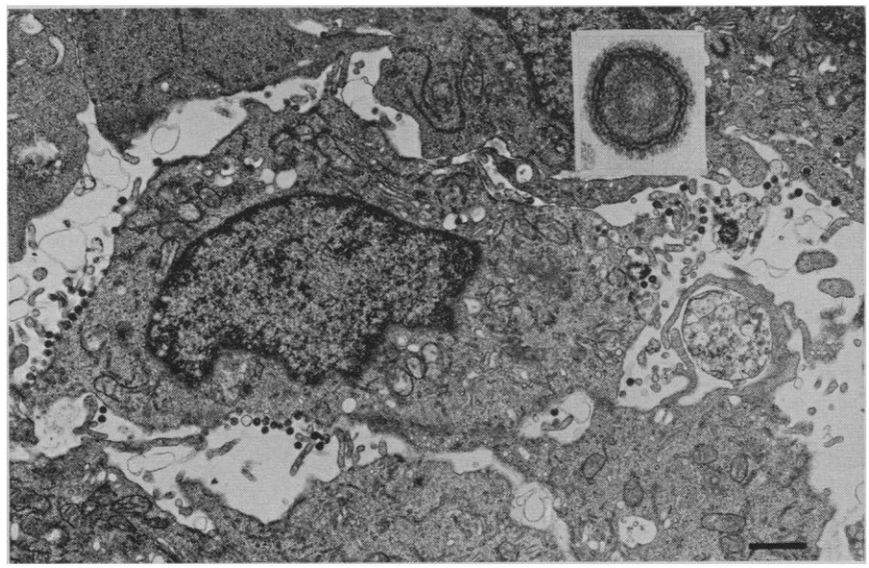

Fig. 1. Electron photomicrographs of dexamethasone-treated L8A Cl 11. Cells treated with dexamethasone for 72 hours were processed as described (*18*). Many type B particles are seen associated with the plasma membrane, apparently having completed the budding process. Intracytoplasmic type A particles were rare. Typical MMTV surface projections are seen on the particle in the inset. Bar, 1 $\mu$m; inset, × 115,000.

major factor in natural murine mammary tumorigenesis is the influence of hormones. Since hormones modify epithelial differentiation, possibly by interacting through cytoplasmic receptors with cellular DNA (*9*), we attempted to stimulate MMTV production from these cell lines with steroids.

The effect of dexamethasone, a potent synthetic corticosteroid, on MMTV particle and antigen production from various murine cell lines is shown in Table 1 and Fig. 1. Four cell lines have been tested: the two clones of CCL-51, of which one (L8A Cl 11) produces relatively high amounts of MMTV proteins and the other (LBA Cl 6) synthesizes only 0.5 to 2 percent as much MMTV RNA or antigen as Cl 11; NBRL 2999, a cell line derived from a C3H mouse mammary tumor by Owens and Hackett (*5, 10*); and KA31, a Kirsten sarcoma virus–transformed nonproducer cell line of BALB/c mouse clone A31 (*11*), which has no detectable MMTV RNA or protein but which does contain MMTV DNA sequences (*8*).

When varying concentrations of dexamethasone were added to the three mouse cell lines of mammary gland origin, there was pronounced stimulation of several indicators of MMTV production. The percentages of L8A Cl 6 cells positive for the MMTV cell surface antigen (*12*) increased from 6 percent before treatment to 65 percent after 7 days of treatment with dexamethasone (0.1 $\mu$g/ml). In the case of L8A Cl 11, cell surface antigen was detectable in 20 to 30 percent of untreated cells and more than 80 percent of treated cells; for NBRL 2999, the corresponding values were 72 percent before treatment and almost 100 percent afterward. Levels of MMTV gp52(s1) (*13*) also increased 10- to 20-fold in treated L8A cells. Electron microscopy indicated that production of type B particles became abundant (Fig. 1), and the extracellular type B particles had surface projections typical of MMTV in L8A Cl 11. In contrast to those in earlier reports, the particles were free in intercellular spaces rather than in the process of maturation (budding) or associated with the plasma membrane (*3, 6*). The supernatant culture fluids from dexamethasone-treated L8A Cl 11 contained a sedimentable DNA polymerase activity with biochemical and chromatographic characteristics of MMTV; this activity was 20- to 50-fold higher than that in untreated cultures (data not shown). Untreated KA31 cells, in contrast to L8A Cl 6, L8A Cl 11, and NBRL 2999, do not have detectable MMTV RNA or antigens; dexamethasone treatment of KA31 cells did not result in detectable amounts of MMTV antigens or particles. Thus, dexamethasone stimulation appears, so far, to be specific for murine cells of mammary origin and to be an enhancement of basal levels of MMTV production. By comparable radioimmunoassays, no increase in the p30(gs) antigen of mouse type C virus was detected in any of these dexamethasone-treated cell extracts (*14*).

In addition to these immunologic, electron microscopic, and enzymatic studies, dexamethasone effects on MMTV-specific RNA in L8A Cl 6 cells were measured by [$^3$H]DNA·RNA hybridization with [$^3$H]DNA synthesized from MMTV RNA by using reverse transcriptase (*6, 7, 15*). Viral RNA in untreated L8A Cl 6 was present in significantly lower amounts than in L8A Cl 11, the relatively high MMTV expressor (Fig. 2). In L8A Cl 6 cells treated with dexamethasone for 72 hours, viral RNA levels approached those in the untreated L8A Cl 11 cells. These results indicate that dexamethasone stimulation of MMTV antigen production in L8A Cl 6 is accompanied by an increase in MMTV RNA. It is

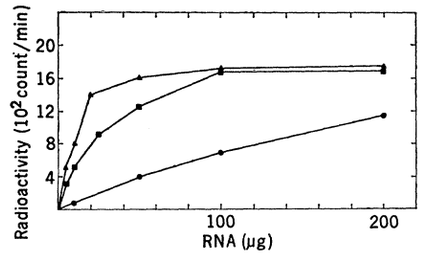


Fig. 2. Hybridization with RNA of control and dexamethasone-treated L8A Cl 6. Cells were seeded in 500-ml Bellco roller flasks ($\sim 1 \times 10^7$ cells per flask) in Dulbecco's modification of Eagle's medium containing 10 percent fetal calf serum and grown at 37°C. When the cells had covered approximately 75 percent of the area of the bottle (3 to 4 days), they were placed in fresh medium with or without dexamethasone (10 $\mu$g/ml) and grown for an additional 72 hours. Cells were then scraped into PBS and washed three times in PBS at 4°C by centrifugation at 800 rev/min for 5 minutes (Damon/IEC model PR6000). Total cellular RNA was extracted (*19*); recoveries from the two lines were comparable. [$^3$H]DNA • RNA hybridizations were analyzed (*6, 19*) with the use of *Neurospora* $S_1$ nuclease, which degrades single-stranded DNA but not DNA in RNA • DNA hybrids. Each reaction contained single-stranded [$^3$H]DNA (2500 count/min of trichloroacetic acid–precipitable material) prepared from MMTV harvested from RIII mouse milk (*6*). Radioimmunoassay of MMTV GP52(sl) in this experiment indicated an eightfold increase of antigen in the dexamethasone-treated L8A Cl 6, somewhat less than that reported in Table 1. Symbols: L8A Cl 6 (●); L8A Cl 6 plus dexamethasone (■); and L8A Cl 11 (▲).

| Dexamethasone (mg/ml) | Cells IF-positive (%) | gp52(s1) (ng/mg) | Electron microscopy |
|---|---|---|---|
| | | *L8A Cl 6* | |
| None | 6 | 61 | − |
| 0.1 | 65 | * | +++ |
| 1.0 | 59 | 2,050 | — |
| | | *L8A Cl 11* | |
| None | 22 | 1,950 | + |
| 0.1 | 80 | * | +++ |
| 1.0 | 82 | 15,600 | +++ |
| | | *NBRL 2999* | |
| None | 72 | * | +++ |
| 0.1 | 94 | * | +++ |
| 1.0 | 96 | * | +++ |
| | | *KA 31* | |
| None | 4 | <10 | − |
| 1.0 | 4 | <10 | − |
| 10.0 | 5 | <10 | − |

* Not tested.

Table 1. Effect of dexamethasone on MMTV production measured by immunoassay and electron microscopy. The murine cell lines were plated at 200,000 cells per 60-mm petri dish in Dulbecco's modification of Eagle's medium with 10 percent fetal bovine calf serum. Four hours after attachment, medium containing dexamethasone (Sigma) at the listed concentrations was added, and incubation was continued at 37° for 6 days. Cells were removed by using 0.25 percent trypsin; washed three times at 4°C with 0.15*M* sodium chloride buffered with 0.01*M* sodium phosphate, *p*H 7.2 (PBS); stained with rabbit antiserum against MMTV for 30 minutes; washed three times with PBS; reacted with fluorescein-conjugated antiserum against rabbit immunoglobulin G (Meloy Labs, Springfield, Virginia) containing a 1 : 30 dilution of rhodamine contrast stain in PBS; and examined with a Zeiss fluorescence microscope. At least 500 cells per slide were examined and scored for MMTV cell surface reactivity (*20*). Normal rabbit serum, included as a control in each assay, stained less than 0.5 percent of viable cells. Cells showing uptake of the dye were considered nonviable and were not counted. The identity of this antigen as MMTV-associated and the serum specificity have been described (*20*). The MMTV glycoprotein gp52(s1) (*11*) was measured by a radioimmunoassay procedure (*6, 21*); antigen is expressed as nanograms per milligram of tissue protein. No increase in type C viral antigens was detected by radioimmunoassay procedures described for murine leukemia virus antigen p30(gs) (*22*). Electron microscopy was performed as described (*18*) and rated as follows: −, no type B particles in at least ten grid squares; +, occassional particle in one to five squares; and +++, at least one type B particle in approximately 80 percent of cells; IF, immunofluorescence reaction.

not known whether synthesis of RNA precedes the increase in antigen.

These results suggest that dexamethasone has a significant effect on MMTV expression in tissue culture cell lines of mammary origin. Viral RNA and antigens were increased as much as 20-fold over basal levels. In addition, a DNA polymerase similar to the type B viral reverse transcriptase was detected in culture fluid supernatants from a permanent cell line. If these particles contain 60*S* to 70*S* MMTV RNA, then a practical in vitro source of MMTV for immunological, biochemical, and biological application may be possible.

Halogenated pyrimidines induce type C viruses from a variety of murine cells (*16*), and dexamethasone was reported to stimulate production of the type C viruses induced by halogenated pyrimidines (*17*). Dexamethasone stimulation of type B particle production from the cell lines described here does not require treatment of cells with halogenated pyrimidines; these substances seem to have little effect with or without dexamethasone on type B particle production in the cell lines thus far examined. These results strengthen our observations that the expression of type B and type C viruses appear to be regulated independently in murine cells (*6*). Thus, in the type B virus system, with the use of a more natural inducer such as corticosteroids, it may be possible to study the natural mechanisms of cellular control of MMTV expression. If these controls involve transcriptional regulation, then this is an ideal system also for studying the mechanism of corticosteroid action by using [$^3$H]DNA • RNA hybridization to follow hormonal effects on RNA transcription from defined genes.

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### References and Notes

1. J. J. Bittner, *Science* **84**, 162 (19[illegible] → *ibid.* **95**, 462 (1942); M. B. Visscher, R. C. Green, J. J. Bittner, *Proc. Soc. Exp. Biol. Med.* **49**, 94 (1942).
2. P. Bentvelzen and J. H. Daams, *Eur. J. Cancer* **6**, 273 (1970); W. E. Heston and G. Vlahakis, *J. Natl. Cancer Inst.* **40**, 1161 (1968); G. P. Murphy and W. J. Hrushesky, *ibid.* **50**, 1013 (1973); B. Fulluga, A. Claude, E. Mrena, *ibid.* **43**, 319 (1969).
3. J. A. Sykes, J. Whitescarver, L. Briggs, *J. Natl. Cancer Inst.* **41**, 1315 (1968).
4. E. Y. Lasfargues, B. Kramarsky, N. H. Sarker, J. C. Lasfargues, D. H. Moore, *Proc. Soc. Exp. Biol. Med.* **139**, 242 (1972).
5. R. B. Owens and A. J. Hackett, *J. Natl. Cancer Inst.* **49**, 1321 (1972).
6. W. P. Parks and E. M. Scolnick, *Virology* **55**, 163 (1973).
7. H. E. Varmus, J. M. Bishop, R. C. Nowinski, N. H. Sarker, *Nat. New Biol.* **238**, 189 (1972).
8. E. M. Scolnick, W. P. Parks, T. Kawakami, D. Kohne, H. Okabe, R. V. Gilden, M. Hatanaka, *J. Virol.* **13**, 363 (1974).
9. D. Feldman, J. W. Funder, I. S. Edelman, *Am. J. Med.* **53**, 545 (1972); S. J. Higgins, G. G. Rousseau, J. D. Baxter, G. M. Tomkins, *J. Biol. Chem.* **248**, 5873 (1973); E. V. Jensen and E. R. DeSombre, *Science* **182**, 126 (1973); A. Munck, *Perspect. Biol. Med.* **14**, 265 (1971); E. V. Jensen, M. Numata, P. I. Brecher, E. R. DeSombre, in *The Biochemistry of Steroid Hormone Action*, R. M. S. Saxillie, Ed. (Academic Press, London, 1971), pp. 133–159.
10. D. Fine has independently observed stimulation of MMTV production in this cell line using hormones and iododeoxyuridine (personal communication).
11. S. A. Aaronson and C. A. Weaver, *J. Gen. Virol.* **13**, 245 (1971).
12. J. H. Daams, J. Calafat, E. Y. Lasfargues, B. Kramarsky, P. Bentvelzen, *Virology* **41**, 184 (1970).
13. Designation of antigens is based on informal agreement among immunologists working with RNA tumor viruses to assign antigenic reactivities to specific virion polypeptides and to designate these polypeptides as gp if the protein is a glycoprotein followed by the molecular weight (as determined by electrophoresis in sodium dodecyl sulfate–polyacrylamide gel at *p*H 7.2) and in parentheses any additional terminology. Hence, MMTV gp52(s1) is the 52,000-dalton glycoprotein from MMTV originally designated s1 [R. C. Nowinski, L. J. Old, D. H. Moore, G. Gering, E. A. Boyse, *Virology* **31**, 1 (1967)].
14. In other studies, effective concentrations of dexamethasone (0.1 to 10 μg/ml) did not measurably affect DNA synthesis as measured by [$^3$H]thymidine uptake or colony-forming efficiency in comparison with untreated cells. Hydrocortisone (1 μg/ml) substituted for dexamethasone could stimulate MMTV in cell culture, but maximal stimulation was obtained with dexamethasone. Hormones such as progesterone, testosterone, and aldosterone were ineffective for MMTV stimulation over a wide dose range (0.1 to 10 μg/ml) in the four cell lines examined. In contrast to results in primary mammary gland or tumor cultures, dexamethasone stimulation in these lines does not require added exogenous insulin. Initial dose-response curves suggest that maximal dexamethasone stimulation of MMTV antigen occurs with steroid concentration of ~ 1 μg/ml ($2.8 \times 10^{-6}$ *M*) and requires 24 to 48 hours (unpublished observations).
15. J. Schlom, R. Michalides, D. Kufe, R. Hehlmann, S. Spiegelman, P. Bentvelzen, P. Hageman, *J. Natl. Cancer Inst.* **51**, 541 (1973).
16. D. R. Lowy, W. P. Rowe, N. Teich, J. W. Hartley, *Science* **174**, 155 (19[illegible] → S. A. Aaronson, G. J. Todaro, E. M. Scolnick, *ibid.*, p. 157.
17. M. Paran, R. C. Gallo, L. S. Richardson, A. Wu, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2391 (1973)
18. T. Aoki, R. B. Herberman, P. A. Johnson, *J. Virol.* **10**, 1208 (1972).
19. R. Benveniste and E. M. Scolnick, *Virology* **51**, 370 (1973).
20. R. Gillette and K. Blackman, in preparation.
21. W. P. Parks, R. C. Howk, E. M. Scolnick, R. V. Gilden, S. Oroszlan, in preparation.
22. E. M. Scolnick, W. P. Parks, D. M. Livingston, *J. Immunol.* **109**, 570 (1972).
23. Partly supported by contracts from the Virus Cancer Program of the National Cancer Institute to Meloy Laboratories, Springfield, Virginia.

16 October 1973; revised 3 December 1973 ■

## Metabolic Differences between Normal and Neoplastic Cells: Effects of Aminonucleoside on Cytoplasmic Messenger RNA

Abstract. *Treatment of cultured normal human fibroblasts with an adenosine analog (aminonucleoside of puromycin) rapidly inhibits the appearance of cytoplasmic messenger RNA, identified by its polyadenylate sequence. Similar treatment of SV40-transformed fibroblasts does not lead to such an inhibition. Cordycepin, another analog of adenosine, inhibits polyadenylate-containing cytoplasmic RNA in both types of cell.*

When exposed to the adenosine analog aminonucleoside of puromycin, cultured human embryonic lung fibroblasts, WI38 cells (*1*), are arrested in the G1 (before DNA synthesis) and G2 (before mitosis) phases of the cell cycle. However, derivatives of these cells transformed by the oncogenic virus SV40 (WI38-VA13 cells) (*2*), and HeLa cells (a line originally cultured from a spontaneously arising carcinoma of the cervix) can traverse the cell cycle in the presence of this inhibitor (*3*). We have shown that treatment of normal WI38 cells with aminonucleoside inhibits the serum-induced stimulation of the synthesis of some classes of small-molecular-weight nonhistone nuclear proteins and the subsequent stimulation of DNA synthesis, while these processes in transformed WI38-VA13 cells are unaffected by aminonucleoside (*4*). In view of the evidence that nonhistone nuclear proteins are mediators of the stimulus to cell proliferation (*5*), the difference in the sensitivity to aminonucleoside between the growth of normal and transformed cells may lie in the lack of effect of this antimetabolite on the synthesis of small nonhistone nuclear proteins in the transformed cells.

Aminonucleoside has no direct inhibitory effect on protein synthesis as measured by the incorporation of [$^3$H]leucine into total cellular proteins (*3*). It inhibits RNA synthesis, principally ribosomal RNA synthesis, but this effect was evident in both normal and neoplastic cells (*6*). Therefore we have now studied the question of whether the appearance of messenger RNA (mRNA) in the cytoplasm is inhibited by aminonucleoside in normal human fibroblasts, but not in SV40-transformed fibroblasts. The effect of cordycepin (3′-deoxyadenosine), which is equally inhibitory to the growth of