

*The Brief Notes section of this issue is printed by photolithography.*

### 37RC CELLS RAPIDLY RECOVER THEIR RNA SYNTHESIS AFTER INHIBITION WITH HIGH DOSES OF ACTINOMYCIN D

ARRIGO BENEDETTO and WIKTOR DJACZENKO. From the Centre of Virology,  
Institute of Microbiology, Rome University, 00100, Italy

Actinomycin D (AD) produces in most cultured cell lines a complete and essentially irreversible inhibition of DNA-dependent RNA synthesis (Reich et al., 1961; Girard et al., 1964). Several RNA species are reported to be AD resistant (Paul and Struthers, 1963; Roberts and Newman, 1966; Penman et al., 1968; Perry et al., 1970; Dubin and Montencourt, 1970). On the cellular level, a number of factors may be responsible for the different AD sensitivity of various cell systems (Djaczenko et al., 1970; Schluederberg et al., 1971).

In the present study we demonstrate that in 37RC cells rapid recovery of RNA synthesis takes place after inhibition with high doses of AD, possibly because they retain a relatively small intracellular pool of the antibiotic. We also compare the AD sensitivity of 37RC cells with that of HeLa cells in several experimental conditions.

We used four different strains of the 37RC line (*Cercopithecus* kidney cells) and the S3 strain of the HeLa line. All lines were kept in monolayers in one of the following culture media: (a) Hanks' balanced salt solution (Hanks and Wallace, 1949); (b) minimal essential medium (Eagle, 1959); or (c) Synthetic 199 (Difco Laboratories, Inc., Detroit, Mich.). Each medium was supplemented with 5% hydrolyzed lactalbumin, 5% or 10% inactivated calf serum, and 100 OU/ml of penicillin and 100  $\mu$ g/ml of streptomycin. We used also mycoplasma-free lines of 37RC and HeLa S3 maintained in Synthetic 199 medium with addition of 100  $\mu$ g/ml of kanamycin. This antibiotic was removed from the culture medium 2 hr before the beginning of the experiments. We performed

the test of Barile and Schimke (1963) and applied electron microscopy to confirm the absence of pleuro-pneumonia-like organisms (PPLO) from these cultures. The results obtained with PPLO-contaminated and PPLO-free lines were similar.

AD was a generous gift of Merck, Sharp, & Dohme, Darmstadt, Germany.

In continuous labeling experiments, uridine-<sup>3</sup>H (1  $\mu$ Ci/ml; 5 Ci/mmol) in 4 ml of prewarmed culture medium was added to the cells. At 15-min intervals over the experimental period of 3 hr, the incorporation was stopped by the removal of the radioactive medium and the addition of 1 ml of solution containing 0.5% sodium dodecyl sulfate, 0.01 M Tris (pH 7.4), 0.0015 M MgCl<sub>2</sub>, 0.01 M NaCl, 0.05 M ethylenediaminetetraacetic acid, and 100  $\mu$ g/ml of papain. In pulse-labeling experiments, cells were labeled for 10 min with uridine-<sup>3</sup>H (4  $\mu$ Ci) dissolved in 1 ml of Hanks' balanced salt solution. The incorporation was stopped as described for continuous labeling. After dissolution of labeled cells for 10 min, samples were precipitated with an equal volume of 20% trichloroacetic acid and the precipitates were deposited on Millipore filters and washed. The radioactivity was measured in a Beckman LS 133 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.).

We carried out a detailed analysis of the sensitivity of uridine-<sup>3</sup>H incorporation in 37RC and HeLa S3 cells to AD after pretreatment and during continuous treatment with the antibiotic.

Fig. 1 a shows that for HeLa S3 cells there is no essential difference in the inhibition of uridine incorporation between cells pretreated with, and

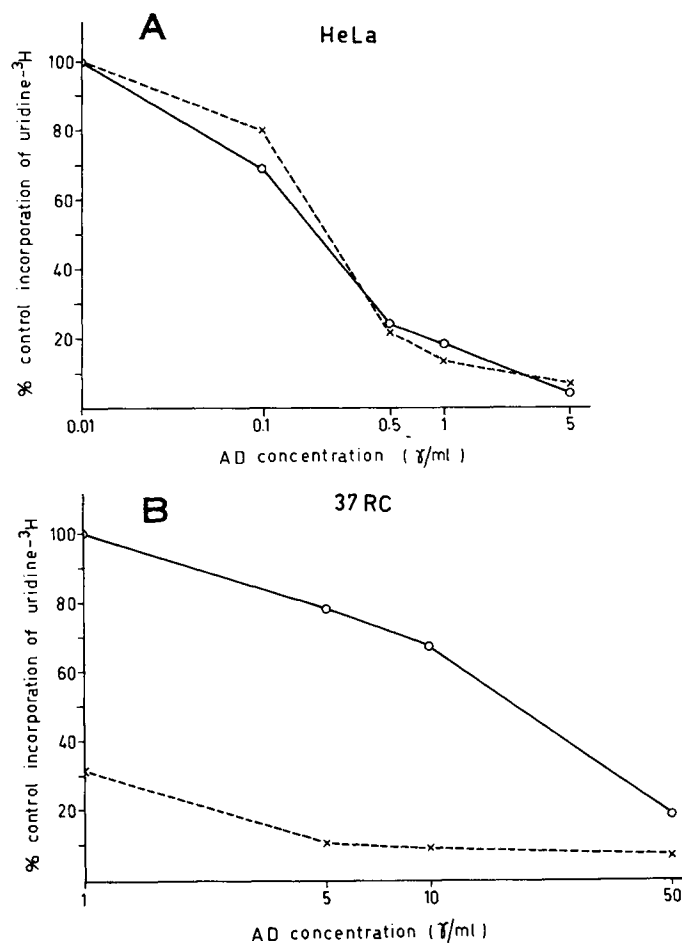


FIGURE 1 Effects of AD on RNA synthesis in HeLa S3 (Fig. 1 a) and in 37RC (Fig. 1 b) cells. One set of cultures was treated at  $-1$  hr with the indicated concentrations of AD. They were repeatedly washed at  $0$  hr with AD-free medium, and then transferred to fresh medium containing  $1 \mu\text{Ci/ml}$  of uridine- $^3\text{H}$ . Samples were taken at 15-min intervals and the rate of incorporation was found to be approximately linear over  $2$  hr. Values of incorporated radioactivity at  $1$  hr are expressed as percentage of the control value without AD, and plotted against the concentrations of AD. To a second set of cultures, AD and  $1 \mu\text{Ci/ml}$  of uridine- $^3\text{H}$  were added simultaneously at  $0$  hr, and the antibiotic was maintained in the culture medium throughout the experimental period. The values of incorporated radioactivity at  $1.5$  hr are expressed as percentage of control values. Each point is calculated from the mean value of three cultures.  $-\circ-\circ-$ , after pretreatment with AD.  $-\times-\times-$ , during continuous treatment with AD.

continuously treated with, AD in the range of concentrations from  $0.01 \mu\text{g/ml}$  to  $5 \mu\text{g/ml}$ . HeLa S3 cells, therefore, show no significant recovery from AD inhibition. In contrast, 37RC cells show a striking ability to recover from AD in pretreatment experiments (Fig. 1 b); even after inhibition by  $50 \mu\text{g/ml}$  of AD, some recovery of RNA synthesis occurs. At lower concentrations of AD, from  $1 \mu\text{g/ml}$  to  $10 \mu\text{g/ml}$ , there is almost complete recovery of RNA synthesis after AD pretreatment.

The level of AD-resistant synthesis of RNA is slightly higher in 37RC cells than in HeLa S3 cells.

The kinetics of inhibition and recovery of RNA synthesis in 37RC cells were studied in pulse-labeling experiments. RNA synthesis declines to about 15% of the control value during the first 10 min after addition of AD. The incorporation of uridine- $^3\text{H}$  is then maintained at the same level until removal of AD; 20 min after removal of AD,

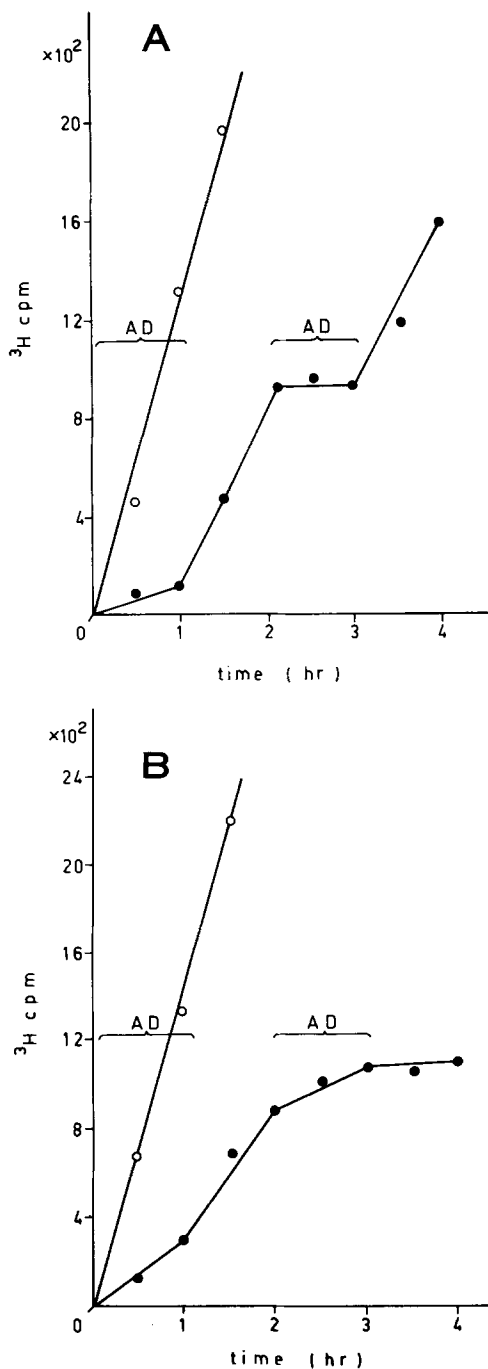


FIGURE 2 Kinetics of uridine- $^3\text{H}$  incorporation into HeLa S3 (Fig. 2 b) and 37RC (Fig. 2 a) cells during intermittent treatment with AD. At 0 hr, AD ( $5 \mu\text{g}/\text{ml}$  for 37RC and  $0.5 \mu\text{g}/\text{ml}$  for HeLa S3 cells) and uridine- $^3\text{H}$  were added simultaneously to a set of cultures. At 1 hr the medium was replaced by medium contain-

the rate of incorporation reaches 35%, and at 1 hr 80% of the control value.

The prompt recovery of RNA synthesis in 37RC cells after pretreatment with high doses of AD suggests that the cells retain only small quantities of AD in the intracellular pool. This possibility is supported by experiments in which 37RC cells were treated intermittently with  $5 \mu\text{g}/\text{ml}$  of AD (Fig. 2 a). The removal of the antibiotic after 1 hr allows the recovery of RNA synthesis almost to the rate in control cells. Upon a second addition of the antibiotic, the incorporation of uridine- $^3\text{H}$  levels off again, and again recovers almost completely on removal of AD. Similar experiments with HeLa S3 cells show a striking difference (Fig. 2 b). A level of AD ( $0.5 \mu\text{g}/\text{ml}$ ) was chosen which allows appreciable recovery of RNA synthesis. After treatment with AD for 1 hr, RNA synthesis recovers to about 28% of the control value; but after a second treatment between 2 hr and 3 hr, no further recovery occurs. This progressive inhibition may be the result of a progressive accumulation of AD in the intracellular pool.

We have demonstrated that the AD sensitivity of 37RC cells is considerably less than that of HeLa S3 cells. In pretreatment experiments, RNA synthesis in 37RC cells still recovers after application of levels of AD 30 times higher than those which provoke irreversible inhibition in HeLa S3 cells. We conclude that the passage of AD into and out of 37RC cells is probably rapid, its direction depending on the concentration of the antibiotic in the culture medium; and that the low retention of AD in 37RC cells is responsible for the rapid availability of DNA templates for RNA synthesis after removal of the antibiotic. The results of intermittent treatment demonstrate, moreover, that AD even at high levels does not provoke severe secondary toxic effects in 37RC cells.

Because they have the properties described in this note, 37RC cells are an excellent model for the study of the molecular mechanisms of inhibition and recovery of the synthesis of a particular RNA species and for the study of regulatory

ing only uridine- $^3\text{H}$  ( $1 \mu\text{Ci}/\text{ml}$ ). At 2 hr, medium with AD ( $5 \mu\text{g}/\text{ml}$  or  $0.5 \mu\text{g}/\text{ml}$ ) and uridine- $^3\text{H}$  ( $1 \mu\text{Ci}/\text{ml}$ ) was added to the cells. At 3 hr, the medium was again replaced by medium containing only uridine- $^3\text{H}$  ( $1 \mu\text{Ci}/\text{ml}$ ). Samples were taken at 30-min intervals. —●—, incorporation of uridine- $^3\text{H}$  into cells intermittently treated with AD. —○— incorporation of uridine- $^3\text{H}$  into control cells.

mechanisms which operate via transcription of DNA.

The authors wish to express their thanks to Dr. Ronald Hancock for valuable suggestions and discussions of problems connected with this study.

Received for publication 14 July 1971.

#### REFERENCES

1. BARILE, M. F., and R. T. SCHIMKE. 1963. *Proc. Soc. Exp. Biol. Med.* 114:676.
2. DJACZENKO, W., A. BENEDETTO, R. PEZZI, and A. CASSONE. 1970. VIIth International Congress of Electron Microscopy, Grenoble. 173.
3. DUBIN, D. T., and B. S. MONTENECOURT. 1970. *J. Mol. Biol.* 48:279.
4. EAGLE, H. 1959. *Science (Washington)*. 130:432.
5. GIRARD, M., S. PENMAN, and J. E. DARNELL. 1964. *Proc. Nat. Acad. Sci. U.S.A.* 51:205.
6. HANKS, J. H., and R. E. WALLACE. 1949. *Proc. Soc. Exp. Biol. Med.* 71:196.
7. PAUL, J., and M. G. STRUTHERS. 1963. *Biochem. Biophys. Res. Commun.* 11:135.
8. PENMAN, S., C. VESCO, and M. PENMAN. 1968. *J. Mol. Biol.* 34:49.
9. PERRY, R. P., J. R. GREENBERG, and K. D. TARTOF. 1970. *Cold Spring Harbor Symp. Quant. Biol.* 35:577.
10. REICH, E., R. M. FRANKLIN, A. J. SHATKIN, and E. L. TATUM. 1961. *Science (Washington)*. 134:556.
11. ROBERTS, W. K., and J. F. E. NEWMAN. 1966. *J. Mol. Biol.* 20:63.
12. SCHLUEDERBERG, A., R. C. HENDEL, and S. CHAVANCH. 1971. *Science (Washington)*. 172:577.