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Differential Inhibitory Effects of Actinomycin D Among Strains of Poliovirus[1]

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ABSTRACT

SCHAFFER, FREDERICK L. (University of California, Berkeley), AND MARJORIE GORDON. Differential inhibitory effects of actinomycin D among strains of poliovirus. J. Bacteriol. 91:2309–2316. 1966.—Actinomycin D exerted a differential effect on the ability of strains of poliovirus to replicate in HeLa cells. LSc-2ab was studied as an example of a strain markedly inhibited by actinomycin; MEF_1 served as a control strain with minimal inhibition. The effect was noted at an actinomycin concentration of 0.1 μg/ml, but 2.5 μg/ml was used for most studies. Variability in the effect was attributed, in part, to physiological factors. Actinomycin was effective when present during the first 2 hr of LSc infection, but had little effect if present at later times. It did not block adsorption or initiation of ecilpse. It did block synthesis of ribonucleic acid in LSc-infected cells. Several possible modes of action are discussed, the most attractive being that actinomycin blocks synthesis of some cell component, the concentration of which is more critical for replication of some poliovirus strains than others.

The antibiotic actinomycin D has been of considerable interest in virus research as well as other areas of biology in the past few years. Actinomycin very efficiently blocks cellular ribonucleic acid (RNA) synthesis. This has been attributed to binding of the drug to the deoxyribonucleic acid (DNA) template, preventing its action with RNA polymerase. In comprehensive reviews on the effects of actinomycin, Reich (14) and Reich and Goldberg (16) cited a number of reports on the effect of the drug on the replication of RNA viruses. Certain viruses, such as influenza, reovirus, and Rous sarcoma, were inhibited, whereas others, including poliovirus and other picornaviruses, were not inhibited by the drug. The latter has been interpreted as independence of DNA in the formation and action of the viral RNA-primed RNA polymerase.

Suppression of cellular RNA synthesis by actinomycin during infection has been a useful tool in virus-cell studies and in preparation of labeled virus. The studies reported here grew out of attempts to use this tool with LSc strain poliovirus in HeLa cells. A marked reduction in yield of LSc virus was found, whereas the MEF_1 strain was only slightly inhibited. The studies were extended by including additional poliovirus strains, and experiments were designed to examine various parameters that might be involved in the differential effect of the drug upon the replication of the strains. Independent studies by P. D. Cooper (*personal communication*) have duplicated some of our findings. Cooper has emphasized different aspects of actinomycin inhibition of poliovirus, and his results, in general, are not in conflict with ours.

[1] A preliminary report of this work was presented at the 65th Annual Meeting of the American Society for Microbiology, Atlantic City, N.J., 25–29 April 1965.

MATERIALS AND METHODS

Cells. The cells employed were a line of HeLa cells obtained from Norman Darby of the Cell Culture Division of the Naval Biological Laboratory. The cells were propagated as monolayers in medium HM (12) supplemented with 10% bovine serum.

Poliovirus. Mahoney, MEF_1, and Saukett strains were received at the University of California Virus Laboratory in 1952 from Jonas Salk. LSc-2ab, Leon $12a_1b$, and Leon strains were received from Albert Sabin in 1957. Commercial oral (Sabin) vaccine was produced by Pfizer Ltd., Sandwich, England. The R30 strain (27) was received from Renato Dulbecco in 1960. Type 1 isolates from an individual at 2 days and 65 days after receiving oral vaccine were those studied by Wasserman and Fox (28), and were provided by Dr. Wasserman. All the virus strains were propagated in HeLa cells, either in Eagle's medium or medium HM, but in the absence of serum.

Plaque assays. The procedure with the use of HeLa

cell monolayers in prescription bottles mounted in racks (23) has been described (21). The overlay medium was modified as follows: freshly autoclaved 1.2% agar was mixed with double-strength medium HM at about 50 C and supplemented with one-twentieth volume calf serum (noninhibitory to poliovirus). When dextran sulfate agar overlay was employed, it was prepared in the same manner, except that sodium dextran sulfate 2000 (Pharmacia Fine Chemicals, Inc., New Market, N.J.) was incorporated at 0.5 mg/ml, and the bicarbonate was reduced to one-third its usual concentration.

Actinomycin. Actinomycin D was kindly supplied by the Merck Institute for Therapeutic Research, West Point, Pa.

Test of actinomycin effect on poliovirus growth. Because of the nature of the experiments, the procedures were varied in some instances, and the variations will be described in connection with the appropriate experiments. The standard procedure was as follows: monolayers of HeLa cells in 16-oz prescription bottles (or occasionally 2-oz bottles) were washed three times with Dulbecco's buffer and inoculated with sufficient virus to achieve an input multiplicity of greater than one. After adsorption at 37 C for 1 hr, the inoculum was removed with washing. Medium (either Eagle's or HM) with or without actinomycin (at 2.5 μg/ml) was added, and the infected cells were incubated overnight at 37 C. The cells and fluid were harvested together by freezing and thawing.

Infectious RNA. LSc-2ab strain poliovirus stock [approximately 2×10^8 plaque-forming units (PFU)/ml] was extracted twice with phenol at room temperature. A 1-ml amount of the aqueous phase was passed through a short Sephadex G-75 column (packed volume of 8 ml), charged with 0.01 M phosphate, 0.0025 M ethylenediaminetetraacetate (EDTA), *p*H 7.0, and collected in a volume of 2 ml. The titer of the RNA was 2×10^4 PFU/ml by the 2 M $MgSO_4$ technique (8).

RNA synthesis. Tritiated uridine incorporation in HeLa cells was measured by a modification of the cover slip technique of Eggers and Tamm (4). Replicate HeLa cell monolayers on 15-mm circular cover slips, four per 5-cm petri dish, were inoculated with 2 ml of Eagle's medium either with 5×10^7 PFU/ml of LSc or MEF virus, or without virus. After 1 hr at 37 C, the inoculum was removed with washing and replaced with 2 ml of tris(hydroxymethyl)aminomethane (Tris)-buffered Eagle's medium containing 20 μg/ml of thymidine and 2.5 μg/ml of actinomycin D. At 3 hr postinoculation, tritiated uridine was added to a level of 1 μc/ml. Fixed and washed cover slips were immersed (cell side up) in toluene–2,5-diphenyloxazole (PPO)–1,4 - bis[2 - (5 - phenyloxazolyl)]-benzene (POPOP) scintillation fluid at the bottom of vials and counted in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

Effect of actinomycin on poliovirus strains. Table 1 summarizes the results of four experiments which include all strains tested. Mahoney, R30,

TABLE 1. *Effect of actinomycin D (2.5 μg/ml) on replication of various strains of poliovirus in HeLa cells*

Expt	Virus	Type	Titer (PFU/ml)		Per cent of control
			Control	Actinomycin	
1*	Mahoney	1	1.4×10^9	3.4×10^8	24
	LSc-2ab	1	2.9×10^8	2.5×10^6	0.8
	MEF_1	2	7.6×10^8	3.0×10^8	39
2*	R30	1	6.6×10^8	2.1×10^8	32
	LSc-2ab	1	1.8×10^8	1.2×10^7	7
	Saukett	3	5.0×10^8	2.4×10^8	48
3*	LSc-2ab	1	2.6×10^8	6.5×10^5	0.3
	MEF_1	2	2.1×10^8	5.4×10^7	26
	Leon $12a_1b$	3	4.5×10^7	3.7×10^5	0.8
4†	LSc-2ab	1	2.0×10^8	1.1×10^7	6
	Commercial vaccine	1	7.5×10^7	2.4×10^6	3
	2-day isolate	1	1.7×10^8	1.0×10^7	6
	65-day isolate	1	1.6×10^8	5.7×10^7	36
	MEF_1	2	3.4×10^8	2.1×10^8	62
	Leon	3	2.1×10^7	2.5×10^7	120
	Commercial vaccine	3	5.4×10^7	4.5×10^6	8

* The serum-containing medium was removed immediately prior to inoculation of cells, and Eagle's medium was used for virus growth.

† The serum-containing medium was replaced 24 hr before inoculation, and medium HM was present during virus growth.

Saukett, and Leon viruses were tested only once; all other strains were tested in additional experiments with essentially the same results. Two strains, namely, MEF_1 and LSc-2ab, were included in most of the experiments in this study. There was considerable variability in the magnitude of the response to actinomycin, but the LSc strain was always more sensitive than MEF_1 in any given experiment. A partial explanation for the variability will be found in investigations of various parameters reported below.

It is of interest that the types 1 and 3 attenuated strains of the Sabin oral vaccine, LSc-2ab and Leon $12a_1b$, respectively, were relatively sensitive to actinomycin, whereas the virulent strains, including those of Salk killed virus vaccine, were relatively unaffected by the drug. Since the LSc-2ab and Leon $12a_1b$ viruses initially studied were "laboratory strains," viruses were isolated from contemporary commercial types 1 and 3 vaccines. Their sensitivities to the drug confirmed the findings with the laboratory strains. The decrease in sensitivity of the 65-day isolate relative to the 2-day isolate from an oral vaccine recipient parallels the observation that the 2-day isolate was antigenically similar to the parent LSc-2ab vaccine, whereas the 65-day isolate was more like virulent type 1 viruses (28).

Vogt and co-workers (27) observed that R30 and LSc-2ab strains possessed common characteristics of the d marker and lack of neuropathogenicity for monkeys. The relative insensitivity of R30 virus to actinomycin shows that a generalization cannot be made regarding a relation between lack of virulence and sensitivity to actinomycin.

Effect of actinomycin concentration on LSc-2ab. Preliminary experiments indicated that the replication of LSc virus was significantly inhibited by concentrations of actinomycin as low as 0.1 μg/ml. The results of one experiment with various concentrations of the drug are presented in Fig. 1. The upper curve shows the effect of concentration when titrated under regular agar overlay. Takemoto and Liebhaber (24) found that under dextran sulfate agar overlay the majority of the population in an LSc stock appeared as "inhibited" plaques of pinpoint size, whereas variants uninhibited by the acid polysaccharide appeared as "enhanced" plaques of normal or larger size. The lower curve of Fig. 1 shows the effect of actinomycin concentration on such enhanced plaques. The parallel nature of the curves indicates that actinomycin exerted no selective influence upon the relative proportion of the enhanced plaque variants.

One-step growth curve of LSc-2ab in actinomycin. Figure 2 clearly shows the reduced yield of

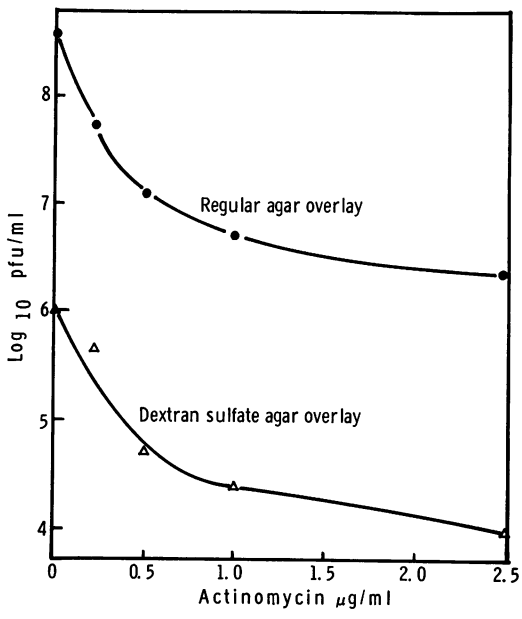

FIG. 1. *Effect of actinomycin concentration on LSc-2ab virus yield. Results of assays under standard and dextran sulfate agar overlays are shown in upper and lower curves, respectively.*

LSc virus in the presence of 2.5 μg/ml of actinomycin. With respect to time, there appears to be a slight delay in initiation of maturation relative to normal virus growth. Because of the reduced yield, the actual initiation of maturation may be obscured by residual uneclipsed inoculum, and the apparent delay may not be real. There also appears to be a delay in reaching maximal yield, the significance of which was not further investigated.

Effect of temperature. The experiment shown in Table 2 demonstrates that, at a reduced temperature of 30 C, actinomycin had less effect on both LSc and MEF_1 replication. However, the differential effect between the two viruses was maintained.

Effect of visible light. Since actinomycin is highly colored and the structure of the phenoxozone ring system is similar to those of photosensitizing dyes, the possible effect of visible light was determined. There was no significant difference in the yield of LSc virus grown in actinomycin in the dark and assayed in either the light or dark (21) as compared with virus grown and assayed with normal room illumination. This showed that the actinomycin effect was not due to photodynamic action either during virus replication or by dye bound to mature virus.

Effect of cells. The initial observations of the inhibitory effect of actinomycin with LSc polio-

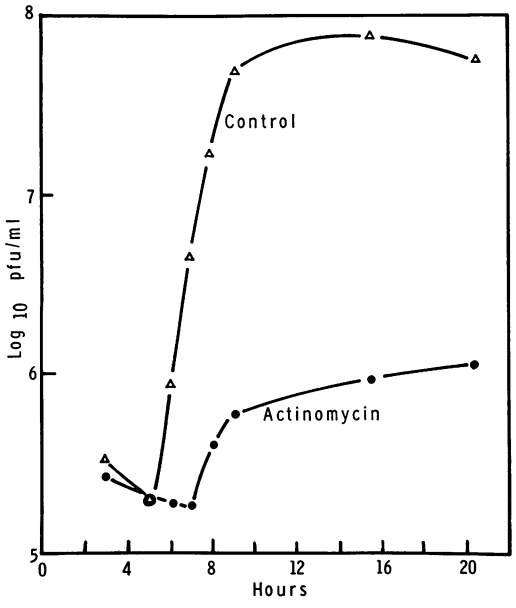

FIG. 2. *One-step growth curves of LSc-2ab virus without and with actinomycin at 2.5 μg/ml. Virus was harvested by freezing and thawing at the times indicated.*

TABLE 2. *Effect of temperature on growth of LSc-2ab and MEF$_1$ viruses in 2.5 μg/ml of actinomycin D**

Virus	Temp	Titer (PFU/ml)		Per cent of control
		Control	Actinomycin	
	C			
LSc-2ab	37	1.3×10^8	1.2×10^6	1
	30	1.3×10^8	9.9×10^6	8
MEF$_1$	37	4.8×10^8	7.9×10^7	16
	30	2.3×10^8	1.9×10^8	83

* Replicate bottles of HeLa cells were placed in separate incubators at 37 and 30 C and harvested after 30 hr of incubation.

virus were in HeLa cells found to be contaminated with pleuropneumonia-like organisms (PPLO). The use of such cells was discontinued, and the results presented in this report were obtained with a line of HeLa cells determined to be free from PPLO by microbiological and enzymatic tests. There was, however, no significant difference in the effect with the two HeLa lines, indicating that PPLO contamination was not a contributing factor. On the other hand, preliminary tests with primary monkey kidney cells suggested that differences in cells may be important in the magnitude of the effect with actinomycin. In monkey kidney cells, the effect appeared to be more marked than in HeLa cells.

Effect of medium. Actinomycin was incorporated in Eagle's medium in some of these experiments, and in medium HM in others. When tested in the same experiment, there was no difference attributable to the medium present during virus growth. The effect of actinomycin was not related to differences in *p*H of the medium. Cooper (*personal communication*) has recently found that preinfection presence of certain sera (or a component thereof, presumably insulin) markedly affected the ability of poliovirus to replicate in the presence of actinomycin. Removal of the serum (pooled ox serum) from the medium 1 day prior to infection was tested in our system. This procedure appeared to reduce the variability from experiment to experiment, and to enhance the differential between LSc and MEF$_1$ viruses. Accordingly, it has been incorporated in the most recent experiments, for example experiment 4 of Table 1.

Time of effect of actinomycin. The results of an experiment in which actinomycin was present only in 2-hr pulses prior to and during LSc virus growth are presented in Fig. 3. Actinomycin had relatively little effect if added at 2 hr postinfection or later, but exerted a maximal effect if present during the first 2 hr of infection. The low yield resulting from the presence of actinomycin during the 2-hr preinoculation period can probably be attributed to carryover, because of the failure of simple washing to remove actinomycin from cells.

Establishment of eclipse phase. Because the action of actinomycin was primarily during the early phases of infection, the possibility existed that the block was at the stage of uncoating the incoming virus. (The block was not at the adsorption stage, since the drug was effective when added after adsorption and washing at 1 hr postinoculation.) The question of uncoating and establishment of eclipse was approached in two ways (Table 3). The detergent, sodium dodecyl sulfate (SDS), is effective in extraction of uneclipsed virus (13). There was no significant difference between the amount of residual inoculum extracted from actinomycin-treated and control cells, indicating that the drug did not block uncoating of the virus. Infection of cells with viral RNA bypasses the removal of the protein coat by the cells. Inhibition of viral yield in actinomycin-treated cells after inoculation with LSc-2ab RNA also showed that the block was at some point other than uncoating. (The relatively low yield from the RNA-inoculated control, as compared with the virus in $MgSO_4$ control, may be due to partial inactivation of the

RNA during frozen storage and handling. The RNA was not titered at the same time as inoculation of the cells in Table 3, but the nominal input was based upon an initial titer at the time of preparation of the RNA and a repeat titer after the first of two periods of storage at −70 C. The relatively high efficiency of infection following inoculation of intact virus in 2 M $MgSO_4$ may be attributed to the technique whereby the $MgSO_4$ was diluted with HM medium and allowed to stand long enough for virus adsorption to occur.)

Effect on RNA synthesis. A comparison of incorporation of tritiated uridine in uninfected, LSc-infected, and MEF-infected actinomycin-treated HeLa cells is presented in Fig. 4. (Preliminary experiments established the adequacy of the cover slip technique and confirmed the well-known block of RNA synthesis in uninfected

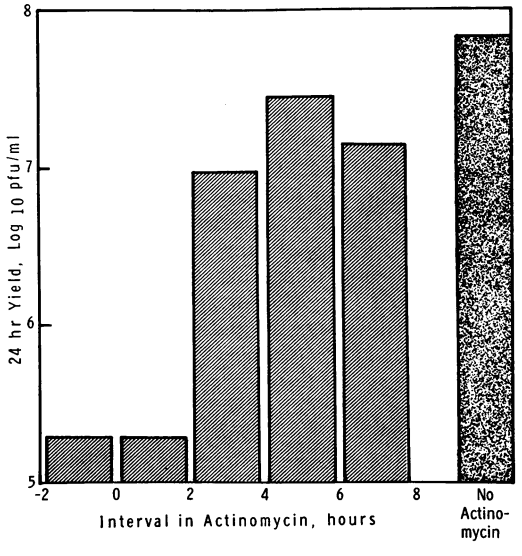

FIG. 3. *Effect of pulses of actinomycin on yield of LSc-2ab. Six replicate HeLa monolayers in 16-oz bottles were washed and 10 ml of Eagle's medium was added to each; the medium in one bottle (−2 to 0 hr) contained 2.5 μg/ml of actinomycin. After 2 hr of incubation at 37 C, the medium was removed and each bottle was chilled and inoculated with 10^8 PFU/ml of LSc virus. After 1.5 hr of adsorption at 0 to 4 C, the inoculum was removed with washing. (The time necessary for adsorption in the cold was not included as part of the time of incubation.) The bottles were warmed to 37 C and 10 ml of medium was added to each, with 2.5 μg/ml actinomycin present in the 0- to 2-hr bottle. At the end of 2, 4, 6, and 8 hr, the medium was changed in each bottle, with actinomycin being present for appropriate 2-hr intervals. Virus was harvested at 24 hr by freezing and thawing. (Titration of the supernatant fluids at 6 or 8 hr showed that no more than 10^4 to 10^5 PFU/ml were lost due to virus release.)*

TABLE 3. *Effect of actinomycin on adsorption and initiation of eclipse of LSc virus**

Prepn	PFU/ml	
	Actinomycin	Control
RNA-inoculated,† 9-hr yield.....	$<10^1$	8.0×10^2
Virus-$MgSO_4$-inoculated,† 9-hr yield.....	$<10^1$	6.1×10^8
Unadsorbed inoculum.....	5.9×10^1	7.8×10^1
Virus-inoculated,‡ 9-hr yield.....	1.3×10^3	1.7×10^6
Unadsorbed inoculum.....	8.9×10^2	1.0×10^4
SDS treatment,‡ 1-hr extract.....	1.0×10^4	8.2×10^3
Unadsorbed inoculum.....	9.2×10^2	8.8×10^3

* Replicate HeLa monolayers in 2-oz bottles were incubated with medium HM for 24 hr, and then for 2 hr with 2.5 μg/ml of actinomycin or with HM alone. Titers are all for 4 ml per bottle except for SDS extracts which were 1 ml.

† Inoculum = 2×10^2 PFU (based upon previous titration) in 0.1 ml of 2 M $MgSO_4$, diluted after 12 min at room temperature with 4 ml of medium HM; inoculum was removed after additional 33 min.

‡ Inoculum = 3×10^4 PFU of LSc virus. Inoculum was diluted with 4 ml of HM after 1 hr at 37 C and removed. SDS treated cells were extracted with 1 ml of 0.2% SDS per bottle.

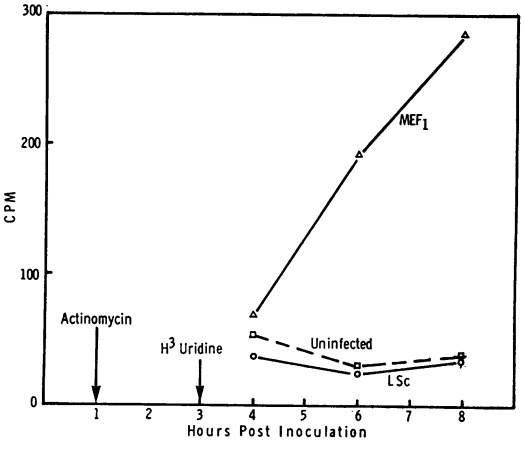

FIG. 4. *Effect of actinomycin on RNA synthesis in cover slip cultures of uninfected and LSc- or MEF-infected HeLa cells. At 4, 6, and 8 hr post-inoculation, duplicate cover slips were removed for radioassay.*

cells. Under conditions comparable to those in Fig. 4, untreated and uninfected cover slips had uptakes of about 1,000 to 1,200 counts per min at 4 hr after addition of H^3-uridine.) Actinomycin was effective in preventing synthesis of RNA in LSc-infected but not in MEF_1-infected cells. To demonstrate the relation between RNA synthesis and virus production, the yield at 8 hr was measured after freezing and thawing additional cover slips from the same dishes. The virus yield was

1.3×10^6 and 7.5×10^4 PFU per cover slip for MEF_1 and LSc, respectively.

DISCUSSION

Ever since the first studies with actinomycin and poliovirus (15), it has been generally accepted that the antibiotic has no effect upon replication of this virus. Recent studies have indicated, however, that variability or reduced yields do occur (P. D. Cooper, *personal communication*; Holowczak, Bacteriol. Proc., p. 119, 1965). Variable or reduced yields in the presence of actinomycin have been observed with other RNA viruses, such as vesicular exanthema of swine virus in pig kidney cells (A. S. Oglesby, Ph. D. Thesis, Univ. of California, 1964) and vesicular stomatitis virus in chick embryo cells (A. J. Hacket, *unpublished data*) in this laboratory, Maus-Elberfeld (ME) virus (7), mengovirus (9), and Newcastle disease virus (W. Robinson and P. Duesberg, *personal communication*). The present report indicates that, at least with poliovirus, the strain of virus is of primary importance in determining the magnitude of the effect of the drug, and that cells and physiological factors may play a secondary role. Recent studies by others have stressed roles of physiological factors such as serum and insulin (Cooper, *personal communication*) and hydrocortisone (Holowczak, Bacteriol. Proc., p. 119, 1965) in preconditioning the cells.

Visible light in the presence of actinomycin inactivates Venezuelan equine encephalitis virus according to a recent report (30). Rott and Scholtissek (19) found that both fowl plague and Newcastle disease viruses were photodynamically inactivated with actinomycin. However, light played no role in the inhibition by actinomycin of fowl plague replication. Exposure to visible light was not the cause of inhibition of poliovirus by actinomycin in our study or that of Cooper (*personal communication*). Exposure of polioviruses to high concentrations of actinomycin and prolonged intense illumination was not tested, and such a procedure might reveal photodynamic inactivation. It should be pointed out, on the other hand, that myxoviruses and arboviruses possess lipoprotein envelopes which might be more effective in binding actinomycin than the lipid-free picornaviruses.

The sensitivity of LSc-2ab poliovirus to the action of actinomycin depended upon the presence of the drug during (or prior to) the first 2 hr of infection. Actinomycin added during the period of active synthesis of viral components and maturation had very little effect on viral yield, but synthesis of viral as well as cellular RNA was blocked when the drug was added shortly after infection. Possible effects of actinomycin on adsorption and initiation of eclipse were ruled out. It thus appears that actinomycin acts upon some early step during viral eclipse, but the experiments do not indicate whether the drug's action is directly involved with some fundamental step in viral replication or is indirectly involved by affecting some necessary cellular system.

Three possible explanations may be offered for actinomycin inhibition of LSc poliovirus replication: (i) obligatory involvement of DNA in the virus replication scheme, (ii) action at a site other than DNA-primed RNA polymerase, or (iii) a critical concentration of some cellular constituent, the synthesis of which depends upon a messenger RNA coded by the cell genome (more than one constituent and messenger could be involved). Alternative (i) is not ruled out by our experiments, but it appears highly unlikely that some strains of poliovirus would require participation of DNA in replication while other strains would not. Alternative (ii) is somewhat more attractive, but there is no direct evidence pointing to a site of action other than a DNA-primed polymerase. There are two effects of actinomycin in which, according to the authors, DNA-primed RNA polymerases are not involved. The first is fragility of polyribosomes upon mechanical disruption of rat liver (17), and the other involves inhibition of maturation, presumably steric hindrance of packaging, of a DNA bacteriophage (11). Neither of these seems to be pertinent here, but some other activity not linked to DNA might be involved in the differential effect with poliovirus. Gomatos et al. (5) suggested, on the basis of actinomycin inhibition of priming of bacterial RNA polymerase by double-stranded reovirus RNA, that double-stranded intermediate forms of viral RNA might be the site of actinomycin action in various cases of inhibition of RNA virus replication. That such a scheme would operate with some poliovirus strains and not with others appears rather unlikely unless there were a differential binding of actinomycin among the various double-stranded RNA molecules. Rott and co-workers (20) suggested that breakdown of fowl plague RNA or its template by actinomycin may explain the inhibition of replication of that virus. If such a mechanism is operative in the present case, differential stability of the RNA of the various strains would explain the results. The finding of Honig and Rabinovitz (10) may be pertinent to this discussion. They observed an inhibition by actino-

mycin of protein synthesis in ascites cells which was reversible by changes in physiological conditions. This inhibition could not be attributed to instability of template RNA.

The third alternative, a limiting supply of some cellular component, would explain the differential effects with poliovirus strains if the concentration of the component were more critical for those strains readily inhibited, such as LSc-2ab. This alternative is also compatible with the observations of effects of physiological factors in actinomycin inhibition of poliovirus replication and is essentially the same as the explanation offered by Cooper (*personal communication*).

The differential effect between LSc and MEF_1 poliovirus may not be limited to actinomycin inhibition. Preliminary experiments in our laboratory suggest that LSc is more sensitive to inhibition by mitomycin C than is MEF_1. The difference was not as marked as that with actinomycin, but served to point out further the possible importance of virus strains in studies of poliovirus replication where inhibitors or limiting physiological conditions are employed. Possible strain differences should not be overlooked in studies with other viruses as well.

It becomes apparent from studies such as the one presented here that generalized statements on modes of replication of RNA viruses should not be based solely upon inhibition by actinomycin. It was recently found by Shatkin (22) that reovirus, which was previously reported to be actinomycin-sensitive, could multiply in the presence of actinomycin at a concentration that blocked 90% of cell RNA synthesis. With influenza group viruses, recent studies lead to confusing and sometimes conflicting interpretations of the effects of actinomycin (3, 19, 29). Actinomycin studies suggesting involvement of DNA in the Rous sarcoma virus system are strengthened by other lines of evidence (1, 2, 25, 26). On the other hand, recent experiments by Robinson (18) show that limited production of viral RNA can occur in the presence of actinomycin, but that the RNA is not incorporated into mature Rous virus. Other members of the avian leukosis group viruses may or may not be inhibited by actinomycin, depending upon the conditions employed (31; Allen and Zamecnik, Federation Proc. **24**:287, 1965).

After completion of this manuscript, it was reported by Grado and co-workers (6) that replication of Brunhilde strain poliovirus in HEp-2 cells was inhibited by actinomycin. Their findings are in agreement with ours with respect to a one-step growth curve and to the action of actinomycin early in the growth cycle.

ACKNOWLEDGMENTS

P. D. Cooper and W. S. Robinson kindly provided preliminary information and manuscripts prior to publication. A. J. Hackett offered helpful suggestions, and Grant Holloway provided valuable technical assistance.

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