Effect of Actinomycin D on Lactic Dehydrogenase Virus Multiplication in Mouse Macrophages (34618)

H. G. DuBuy and M. L. Johnson
(Introduced by S. Baron)

National Institute of Allergy and Infectious Diseases, Laboratory of Viral Diseases, National Institutes of Health, Bethesda, Maryland 20014

In a previous paper (1) we reported that lactic dehydrogenase virus (LDV) replicated *in vitro* in mouse peritoneal macrophage cultures. Multiplication started within 3 hr after infection, and reached a maximum titer of 10^7 ID_{50}/ml of supernatant within 15 hr, when cells were infected with a multiplicity of at least 100. These results allowed the study of the effect of actinomycin D, which blocks all DNA-directed RNA synthesis of the host and that of DNA viruses but does not affect the multiplication of many RNA viruses. Only one report on the effect of actinomycin D on LDV has appeared: Crispens (2) reported that this antibiotic did not inhibit LDV multiplication in intact mice, at the dosage used (10 μg of actinomycin D/mouse). The effect of actinomycin D on LDV multiplication in tissue culture at a dose which clearly inhibited uridine incorporation in the host cell has been studied and is reported in this paper.

Materials and Methods. Animals. Randomly bred Swiss albino mice were supplied by the NIH animal production section.

Virus. The strain of LDV used was isolated from a naturally infected Ehrlich ascites carcinoma, carried in CDF_1 mice (3).

Media. Medium 199 and Eagle's minimum essential medium, containing 100 units of penicillin and 100 units of streptocmycin/ml were obtained from the NIH media preparation section. Fetal bovine serum (FBS) was obtained from Microbiological Associates, Bethesda, Maryland.

Macrophages. Mouse peritoneal macrophages were obtained as described previously (1), and grown in stoppered 16 × 95-mm Leighton tubes, in medium 199 containing 20% FBS.

Virus titrations. Virus titers were determined as described before (1, 3). The ID_{50}/ml was calculated by the method of Karber (4).

Technical details. Preliminary experiments were performed to determine whether actinomycin D inhibited RNA synthesis in macrophages. 3 sets of 3 Leighton tubes each were seeded with 1–5 × 10^6 cells. The cells were cultivated at 37° for 48 hr. The medium, 1 ml/ tube, was replaced every 24 hr. After 48 hr, the cultures were preincubated at 37° for 1 hr with 3, 1, and 0 μg of actinomycin/-tube. Then they were incubated for another hour after addition of ^{3}H-uridine and the contents were analyzed.

The rate of RNA synthesis was determined by measuring the incorporation of ^{3}H-uridine into the acid insoluble portion of the cells. The original medium was collected, and the cell layers were washed 3 times with cold phosphate-buffered saline (PBS). The cells were scraped into PBS and centrifuged. The pellets were then extracted with 5% cold trichloroacetic acid (TCA), and washed with 0.1% cold TCA. The pellets containing the acid-insoluble RNA was hydrolyzed in 5% TCA at 100° for 25 min, cooled, centrifuged, and the supernatant was collected.

The various fractions were assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer II 3380, using the absolute activity analyzer. The results are presented as dpm per 1 Leighton tube.

The effect of actinomycin D on LDV multiplication in macrophages was determined as follows. One-day-old macrophage cultures were divided into 2 or 3 groups. In one group, the medium was replaced by fresh medium containing 1 or 3 μg of actinomycin

TABLE I. Incorporation of ^{3}H-Uridine in Macrophage Cultures, After 1 hr, in the presence of 0, 1, and 3 μg of Actinomycin D/Leighton Tube (1 ml).[a]

Act. D (μg)	Acid-insol. cell fraction (dpm)		Inhibition (%)	
	Exp. 1[b]	Exp. 2[b]	Exp. 1	Exp. 2
0	2104.2 (2.5)	18,222.5 (1.0)	0	0
1	105.6 (7.5)	2505.0 (2.5)	95	86
3	22.0 (9.5)	552.5 (3.5)	99	97

[a] Tubes contained ca. 5×10^6 cells. The standard deviation (%) is given in parentheses.

[b] 0.1 μCi of ^{3}H-uridine/tube added in Exp. 1, and 0.5 μCi added in Exp. 2.

D/tube; in the control group the medium was replaced by fresh medium only. After 30 min at 37° the supernatants were removed, and replaced with the appropriate medium (with or without actinomycin D) containing 10^8 ID_{50} of LDV/ml. Since each Leighton tube contained ca. 5×10^6 cells, the multiplicity of infection was ca. 20 ID_{50}/cell. The cultures were incubated for 1 hr, washed 5 times to remove nonabsorbed virus, and then reincubated in the appropriate medium. At each time period the fractions of 3 Leighton tubes were pooled, and the LDV titer was determined.

Results. The results of the preliminary experiments, in which the inhibition of RNA synthesis in macrophages by actinomycin D was studied are presented in Table I. They demonstrate that 1 μg of actinomycin D/ml reduced the ^{3}H-uridine incorporation into the pellet to 5–14% of that in the nontreated cells; 3 μg of actinomycin D/ml reduced the incorporation to 1–3%.

The results of the effect of actinomycin D on LDV multiplication in macrophages are presented in Table II. As shown, the titers of the supernatants of the 8-hr samples, containing 1 μg of actinomycin D/tube, are only somewhat lower than those of the controls, although the uridine incorporation of the host cells in the presence of the same amount of actinomycin D was reduced to 5–14% of the controls (Table I). The titers of the supernatants of the 8-hr samples, containing 3 μg of actinomycin D were ca. 1 log lower than those of the controls, although in this case the incorporation of uridine in the host cells was reduced to 1–3% of the controls (Table I).

Microscopic observation of the respective cultures 4 hr after infection, showed that the cells from the actinomycin D series were noticeably more rounded up than those of the controls, particularly in the cultures containing 3 μg of actinomycin/tube. This damage is probably reflected in the progressive decrease in LDV titer of the actinomycin D series.

Discussion. The present results show that LDV can multiply in host cells in which the DNA-directed RNA synthesis was 84–99% inhibited. This indicates that LDV, an RNA virus (5), directs its replication in the cytoplasm of the host cell, not requiring a host DNA function. This conclusion is supported by electron microscopic studies of the LDV which did not reveal any virus present in the host nuclei (6) and by studies demonstrating

TABLE II. Effect of Actinomycin D on LDV Multiplication in Mouse Macrophage Cultures.

Act. D (μg/tube)	1 hr			4 hr		8 hr	
	Sup.	Cells	Fifth wash	Sup.	Cells	Sup.	Cells
0	—	—	1.9[a]	2.3	—	5.9	—
1	—	—	2.1	1.3	—	5.3	—
0	8.3	4.5	3.5	4.9	3.7	6.9	6.1
1	7.7	4.7	3.5	3.9	3.5	6.3	5.1
0	—	4.7	3.5	3.7	—	6.7	—
1	—	4.5	3.7	4.5	—	6.3	—
3	—	3.3	3.5	4.1	—	5.5	—

[a] Each number represents the LDV-titer of the pooled fractions from 3 Leighton tubes.

the absence in host nuclei of LDV antigens, which stain with fluorescent antibodies (7). These results place LDV in the group of RNA viruses which multiply in the host cytoplasm, and are independent of DNA-directed RNA synthesis.

Summary. The mouse macrophage tissue culture technique was used to show that LDV multiplication is independent of DNA-dependent RNA synthesis since concentrations of actinomycin D which inhibited host RNA synthesis did not inhibit LDV multiplication.

The assistance of Dr. S. Margolis in the determination of RNA synthesis is gratefully acknowledged.

1. DuBuy, H. G., and Johnson, M. L., Proc. Soc. Exp. Biol. Med. **128**, 1210 (1968).

2. Crispens, C. G., Experientia **22**, 823 (1966).

3. DuBuy, H. G., and Johnson, M. L., J. Exp. Med. **122**, 587 (1965).

4. Lennette, E. H., *in* "Diagnostic Procedures for Virus and Rickettsial Diseases," p. 48. Amer. Pub. Health Ass. New York (1964).

5. Notkins, A. L., and Scheele, C., Virology **20**, 640 (1963).

6. DuBuy, H. G., and Johnson, M. L., J. Exp. Med. **123**, 985 (1966).

7. Porter, D. D., Porter, H. G., and Deerhake, B. B., J. Immunol. **102**, 430 (1969).

Received Sept. 22, 1969. P.S.E.B.M., 1970, Vol 133.