EFFECT OF CORTISONE ON ACTIVATION OF LYSOSOMAL ENZYMES RESULTING FROM MENGOVIRUS INFECTION OF L-929 CELLS

B. Hotham-Iglewski[1,2] and E. H. Ludwig

Virus Laboratory
Department of Microbiology
Pennsylvania State University, University Park, Pennsylvania

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The work of Allison et. al. (1963) and Wolff and Bubel (1964) has shown that virus infection results in the release of lysosomal enzymes into the cytoplasm. These workers (Allison et. al., 1963; Wolff and Bubel, 1964) have associated the activation of lysosomal enzymes with the onset of viral cytopathogenic effects. DeDuve (1963) reports that lysosomes are stabilized by vitamin A and corticosteroids. Furthermore, cortisone has been reported to inhibit virus production in certain virus-cell systems (Kelbourne, 1957; Erichsen, 1963). It was, therefore, of interest to examine the effect of cortisone on virus production and release and on the virus induced activation of lysosomal enzymes.

We have obtained experimental evidence to show that cortisone reduced the amount of virus released while having little effect

1. Postdoctoral Trainee, Research Training Grant 5T1-GM-512. Institute of General Medical Sciences, NIH

2. Present address: Department of Pathology, University of Colorado Medical Center, Denver, Colorado.

on the total production of virus. We have also found that the activation of lysosomal enzymes during virus infection is reduced in the presence of cortisone.

L-929 cells were infected with mengovirus at a multiplicity of 5 PFU/cell. Half of the cultures were infected with a virus inoculum containing no cortisone. The virus was adsorbed at 36°C for 1 hour. Following adsorption, the monolayers were washed twice with Hanks balanced salt solution. The cultures inoculated with the cortisone-containing virus suspension received Melnick's medium (1955) containing 10% bovine serum to which 0.01 mg/ml of cortisone had been added. The remaining cultures received Melnick's medium with 10% bovine serum but without cortisone.

At intervals, beginning 2 hours after infection of the cells, a 1 ml sample of medium was removed from each of several cortisone-containing cultures and from control cultures lacking cortisone. These samples were subsequently titrated for virus released from the cells during the virus production cycle. After withdrawal of the medium sample, each culture, with its remaining medium, was alternately quick-frozen and thawed 3 times to release virus from the cells. The resulting suspensions were subsequently titrated for total virus produced, that is virus released to the medium plus cell-associated virus.

Virus titers were determined by the plaquing method of Holland and McLaren (1959) except that the overlay contained Medium 199 with 10% bovine serum instead of yeast extract medium. The overlay medium contained 0.3% washed Difco Nobel Agar. (Table 1) While total virus production during the 8 hours fol-

Table 1

Effect of Cortisone on Production and Release of Mengovirus by L-929 Cells.

Cortisone in Medium		Hours after Infection of Cells 2	4	6	7	8
None	Released Virus	2.0×10^2*	7.0×10^4	7.1×10^5	3.7×10^7	4.2×10^8
	Total Virus	2.7×10^5	3.8×10^5	1.5×10^8	6.1×10^8	6.8×10^8
0.01 mg/ml	Released Virus	1.9×10^2	7.2×10^2	3.9×10^4	2.0×10^5	4.1×10^6
	Total Virus	2.8×10^5	3.4×10^5	3.6×10^7	2.0×10^8	4.3×10^8

*PFU/ml Each value is the average of at least 3 samples.

lowing infection of the cells was only slightly inhibited by cortisone, the amount of virus released from cortisone-treated cells was 5% or less of the amount released from control cells from the fourth hour of the virus production cycle until its end at 8 hours (Brownstein and Graham, 1961).

The effect of cortisone on the activation of lysosomal enzymes in Mengovirus-infected cells was determined by measuring the concentrations of acid phosphatase and beta glucuronidase released by the lysosomes according to the method of Allison et. al. (1963). Cortisone-treated L-929 cell cultures, and control cultures without cortisone were prepared as described above. All cultures

were inoculated with Mengovirus at a multiplicity of 5 PFU/cell with 0.01 mg cortisone/ml present in the virus inocula for the cortisone treated cultures. Immediately following inoculation of the cells with virus (0 time) and at 2 hour intervals thereafter for 8 hours, the cells were removed from the culture bottles by gently rolling glass beads over the surface of the monolayer. The cells were sedimented by centrifugation at 900 x g for 20 minutes, washed 3 times with 0.25M sucrose, resuspended in 0.25M sucrose, and homogenized for 5 seconds in a Bronwill cell homogenizer (Bronwill Scientific Company). The homogenate was centrifuged at 2000 x g at 4°C for 25 minutes, and the supernatant fluid was removed for enzyme assay. Enzymes measured in the supernatant fluid were considered to be those released by the lysosomes into the cytoplasm. The sedimented pellet was suspended in 0.2% Triton x-100 in distilled water to release the lysosomal bound enzymes (deDuve et al., 1955).

Table 2 shows that Mengovirus infection of L-929 cells resulted in a release of lysosomal enzymes to the cytoplasm. The accumulation of acid phosphatase and beta glucuronidase in the cytoplasmic fraction appeared shortly after the initiation of release of virus, 4 to 6 hours after infection. The activation of lysosomal enzymes continued to increase throughout the 8 hour virus production cycle. Cells which were infected with a Mengovirus inoculum containing 0.01 mg cortisone/ml and overlayed with medium containing 0.01 mg cortisone/ml following the virus adsorption period, exhibited a reduced level of solubilization of lysosomal enzymes. As is seen in Table 2, 8 hours after infection

Table 2

Effect of Cortisone on Distribution of Lysosomal Enzyme Activity in Lysosomal (L) and Cytoplasmic (C) Fractions of Mengovirus-Infected L-929 Cells.

Cortisone in Medium	Hours after Infection	Beta Glucuronidase (L)	Beta Glucuronidase (C)	Acid Phosphatase (L)	Acid Phosphatase (C)
None	0	.23*	.06	.36	.12
"	2	.20	.08	.34	.14
"	4	.16	.10	.30	.18
"	6	.12	.14	.20	.30
"	8	.06	.22	.12	.34
0.01mg/ml	0	.24	.06	.38	.12
"	2	.22	.06	.38	.12
"	4	.24	.07	.36	.14
"	6	.18	.08	.36	.14
"	8	.20	.08	.35	.15

*Activities are expressed in optical density units as described by Allison et. al. (1963).

the concentration of lysosomal enzymes in the cytoplasmic fraction of the cortisone treated cells was less than half that in the untreated virus-infected cells.

DeDuve (1963) reports that cortisone stabilizes lysosomes thereby reducing the release of lysosomal enzymes to the cytoplasm. The results of this study indicate that cortisone greatly reduced the release of lysosomal enzymes to the cytoplasm during virus

infection. While cortisone did not significantly inhibit the total amount of Mengovirus produced in L-929 cells, it did reduce the amount of virus released into the medium. Thus, it appears that the activation of lysosomal enzymes may be involved in the release of the virus from the host cell.

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