Fowlpox Virus: Interferon Production and Sensitivity (35013)

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A rapid plaque assay for fowlpox virus was recently developed in which maximal plaque numbers appeared within 78 hr after infection (1). The addition of chymotrypsin to the fluid overlay had an effect on the rate of virus replication since virus yields were obtained earlier and plaques grew at a faster rate. We considered the possibility that the enhancement of plaque formation by chymotrypsin was due to destruction of interferon by the enzyme. Thus, we studied the sensitivity of fowlpox virus to interferon action and its ability to induce interferon. Comparisons were made with vaccinia virus, another poxvirus which can also be enhanced with proteolytic enzymes (2).

Materials and Methods. Cell cultures. Primary cell cultures were established from eviscerated and decapitated 10- to 11-day-old chick embryos by using techniques previously described (3).

Media. The growth medium for the establishment of cell cultures consisted of Gey's balanced salt solution (BSS) supplemented with 0.6% sodium bicarbonate, 5% calf serum, and 0.1% each of lactalbumin hydrolysate and proteose peptone. Maintenance medium was similar to the growth medium except that 0.1% yeast extract replaced the calf serum.

Viruses. Fowlpox virus was a vaccine strain (list no. 5179) obtained from the Amdal Company, Agricultural Division of Abbott Laboratories, North Chicago, Illinois. Virus stocks were grown on the chorioallantois of developing chick embryos or in chick embryo cell cultures in maintenance medium with 3 μg/ml of chymotrypsin. Chick embryo cell cultures were incubated at 37° for 4 days, after which fluid and cells were pooled, frozen and thawed twice, homogenized, and then centrifuged to remove cellular debris. The titer of the stock virus, assayed as previously described (1) with the addition of 5 μg/ml of chymotrypsin to a maintenance medium overlay was found to be approximately 1.3×10^7 PFU/ml. Vaccinia virus, strain NY 914, was prepared on the chorioallantois and assayed as previously described (3).

Chymotrypsin. Purified, crystalline α-chymotrypsin (Worthington Biochemical Corporation, Freehold, N.J.) was employed. The enzyme was reconstituted in sterile distilled water to a concentration of 1 mg/ml (4000 BTEE units/mg), dispensed in aliquots of 1 ml, and stored at −20°. Dilutions were made in maintenance medium as needed.

Interferon. Unless otherwise noted, interferon was induced in chick cell cultures with Semliki Forest virus. A PDD_{50} unit in our vaccinia assay system is equal to 1.4 units of the International Research Reference Standard.

Results and Discussion. Sensitivity of fowlpox virus to interferon. The addition of proteolytic enzymes to cells, after they have been incubated with interferon at 37° for an appropriate time, does not have any effect on interferon action (4). However, before testing the sensitivity of fowlpox virus to interferon, it was desirable to determine if the chymotrypsin required for rapid plaque formation had any effect on any interferon assay. We employed vaccinia virus since plaque formation by this virus is also enhanced by chymotrypsin (2) and it has known sensitivity to interferon. Chick embryo cell cultures were treated overnight with sufficient interferon to give about 90% inhibition of plaque formation. Cultures were then washed and infected with vaccinia virus in the presence or absence of 5 μg/ml of chymotrypsin. Although chymotrypsin en-

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hanced the number of plaques in control bottles by 148%, the resultant inhibition of plaque formation by interferon pretreatment was 91% in the presence of chymotrypsin and 94% in its absence. Thus, chymotrypsin in the overlay did not seem to significantly interfere with interferon action.

The sensitivity of fowlpox virus to interferon was then compared with that of vaccinia virus. Dilutions of interferon were added to chick cell cultures which were then incubated overnight at 37°. Cell sheets were then drained, washed once with growth medium, challenged with 2 ml of fowlpox or vaccinia virus suspension containing 5 μg/ml of chymotrypsin and incubated at 37°. Cultures containing vaccinia virus were removed from the incubator 46 hr later, stained with 0.1% crystal violet, and the plaques were enumerated. Cultures with fowlpox virus were removed after 72 hr of incubation and similarly treated. The percentage inhibition for each dose of interferon was calculated and plotted against the $\log_{10}$ dose of interferon. The results are shown in Fig. 1. The dose of interferon required to inhibit 50% plaque formation was 50 μl with vaccinia virus and 2000 μl with fowlpox. Thus, fowlpox virus was about 40 times less sensitive to interferon than was vaccinia virus.

Induction of interferon by fowlpox virus. Fowlpox virus was added to chick embryo cell cultures at an input multiplicity of 1, 0.1, and 0.0004 PFU/cell. The low multiplicities of virus were included since it has been shown that interferon synthesis is optimal under those conditions in some systems [*e.g.*, see (5)]. All cultures were then incubated at 37° for 30 hr except those with the lowest multiplicity which were incubated for 3 days. At the end of the incubation period, the supernatant fluids were removed, centrifuged to remove cellular debris, and residual virus was destroyed by dialysis against pH 2 buffer for 24 hr. The pH was then adjusted to neutrality by dialysis against Gey's BSS. In

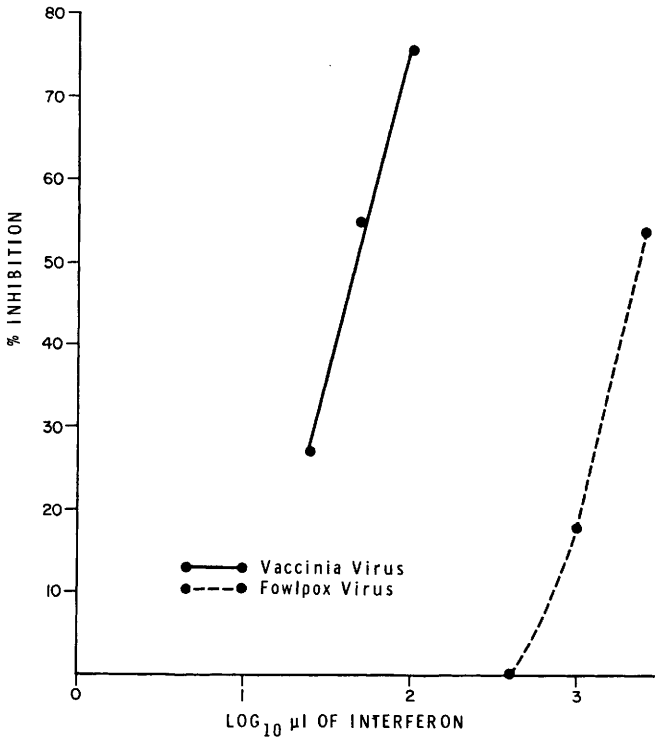

FIG. 1. Comparison of inhibition by interferon of fowlpox and vaccinia virus plaque formation. Fowlpox virus is shown to be about 40 times less sensitive to inhibition by interferon than vaccinia virus.

TABLE I. Production of Interferon by Fowlpox and Vaccinia in Chick Embryo Cells.

Exp. no.	Virus	Input multiplicity	Incubation period (hr)	Yield of interferon (PDD_{50} units/culture)[a]
1	Fowlpox	0.0004	72	≤1
		0.1	30	≤1
		1.0	30	≤1
2	Fowlpox	1.0	48	≤1
		1.0	96	2.5
3	Vaccinia	1.0	24	≤1
		1.0	48	10

[a] Cultures usually contained about 4×10^6 cells at time of inoculation. A PDD_{50} unit of interferon is that amount of interferon which will depress plaque formation by 50% as compared to control.

addition, the supernatants were heated at 65° for 30 min. Interferon assays were performed using vaccinia virus as the challenge. No significant inhibition of vaccinia virus was found even when 500 μl of the fowlpox supernatant fluid was assayed.

Since growth of fowlpox virus is slow (1, 6) in chick cells, we repeated the experiment but harvested supernatant fluids for interferon assays at 48 and 96 hr after infection. A small amount of interferon was detected at 96 hr but not at 48 hr. A comparison was made with vaccinia virus interferon induction and the results are also shown in Table I.

The results show that fowlpox virus is relatively insensitive to interferon action and that both vaccinia and fowlpox viruses are poor and late inducers of interferon in the chick cell system employed. The slow growth of these viruses in chick cell cultures is thus apparently not due to the production of endogenous interferon. This lack of interferon production, coupled with the minimal effect of chymotrypsin on interferon assays, force one to conclude that the enhancement of virus plaque formation by chymotrypsin is probably not due to the destruction of interferon.

Summary. Fowlpox virus was found to be relatively insensitive to interferon in chick embryo cell cultures. Compared to vaccinia virus, 40 times more interferon was required to inhibit fowlpox virus plaque formation. Fowlpox virus was found to be a poor and late inducer of interferon. Interferon was not found at 48 hr after infection but small amounts were found near the end of the replicative cycle (96 hr). The destruction of interferon by chymotrypsin is unlikely to be responsible for the enhancing effect of the enzyme.

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