Relative Effects of Interferon on Virus Plaque Formation.* (31346)

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The effect of interferon on virus plaque formation is widely used to measure the antiviral action of interferon. Commonly, the amount of interferon required to inhibit 50% of the potential plaques from developing is referred to as a unit of interferon. Units of interferon activity will vary according to the assay procedure and probably depend to a large extent on the sensitivity of the assay virus to interferon. A standard unit of interferon has not been established in this country to compare the units of interferon in different assay procedures or in different laboratories. The present study relates the units of interferon activity in 3 assay systems that are employed in our laboratory.

Materials and methods. Media and cell cultures. Growth medium for the establishment of chick cell cultures consisted of Gey's balanced salt solution (BSS) with 5% calf serum, 0.1% lactalbumin hydrolysate, 0.1% proteose peptone and 0.0025 M Tris or 0.06% sodium bicarbonate. Maintenance medium used for virus and interferon assays with vaccinia virus consisted of BSS with 0.11% sodium bicarbonate, 0.1% proteose peptone, 0.1% lactalbumin hydrolysate and 0.1% yeast extract. An agar overlay was used for assays employing Semliki Forest or vesicular stomatitis viruses. Agar overlay medium consisted of the same maintenance medium with 5% calf serum and 0.5% "Ionagar" and omitting the yeast extract. Penicillin and streptomycin were added to all media at 375 units and 188 μg per milliliter respectively. Chick embryo fibroblast (CEF) cell cultures were prepared as previously described(1).

Viruses. Vaccinia, chikungunya, Semliki Forest and vesicular stomatitis viruses were employed. Vaccinia virus, strain NY 914, was grown on the chorioallantois of 11-day-old developing chick embryos. Infected membranes were removed 48 hours after inoculation and triturated with maintenance medium. Semliki Forest (SFV) and chikungunya viruses were obtained from Dr. James Porterfield, National Institute for Medical Research, London. Stock chikungunya and Semliki Forest virus preparations were made by inoculating newborn mice intracerebrally with virus and harvesting the brains 36 to 48 hours later. Vesicular stomatitis virus (VSV), Indiana strain, was obtained from Dr. Samuel Baron, National Institutes of Health, Bethesda. Stock preparations of VSV were prepared by inoculating primary cultures of chick embryo fibroblasts and harvesting the supernatant fluids 20 hours later. All virus preparations were centrifuged at 800 *g* for 30 minutes to remove cellular debris and were stored in glass ampules at −70°.

Interferon. Interferon was produced by inoculating chick embryo cell cultures with chikungunya virus(1). The procedure was often modified in that the cultures were inoculated with virus immediately after dispensation of cells. Cultures were incubated at 37° for 20 to 24 hours and the supernatant fluids removed, centrifuged to remove cell debris, and heated at 65° for 30 minutes to inactivate residual virus(2).

Virus and interferon assays. Vaccinia virus and interferon were assayed by the methods described by Lindenmann and Gifford(3,4). Serial dilutions of an interferon preparation were made in maintenance medium and 1.0 ml aliquots of each dilution were added to each of 4 bottles of chick embryo fibroblast cultures which were previously drained of growth medium. Immediately thereafter, approximately 200 PFU of vaccinia virus in 1.0 ml of maintenance medium were added to each bottle. Control bottles received 1.0 ml of maintenance medium in lieu of interferon. All bottles contained a final volume of 2.0 ml. Cultures were incubated for 44 to 48

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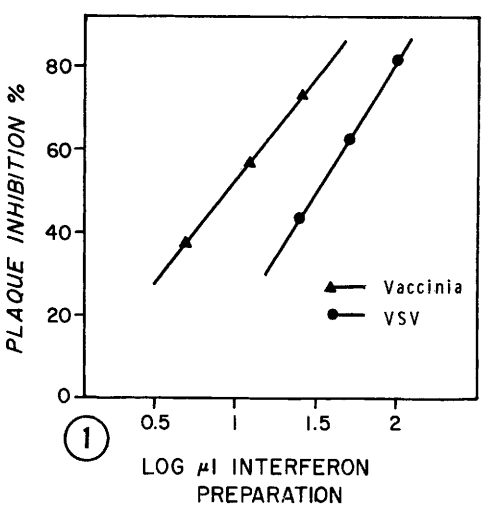

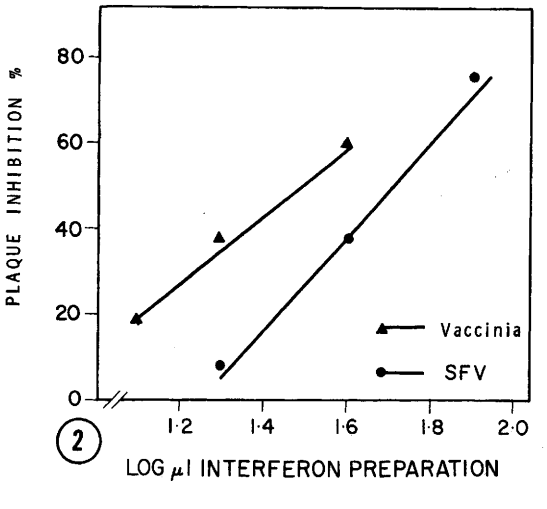

FIG. 1. Dose response lines for an interferon preparation assayed with vaccinia and vesicular stomatitis viruses.

FIG. 2. Dose response lines for an interferon preparation assayed with vaccinia and Semliki Forest viruses.

hours at 37°. The medium was then decanted and the cell sheets stained with crystal violet. Plaques were enumerated after 6-7× magnification of the monolayer.

For VSV and SFV virus assays and for interferon assays using these viruses, the following procedure was employed. The growth medium was decanted from CEF cell cultures and replaced with 2.0 ml of a serial dilution of interferon made in growth medium. Usually 4 cultures were used for each dilution of interferon. The cultures were incubated at 37° for 6 to 12 hours. After removal of the interferon containing medium, the cell sheets were inoculated with approximately 50 to 100 PFU of VSV or SFV virus in 0.2 ml. After one hour adsorption of the virus, with frequent rocking of the bottles to redistribute the virus, the residual unabsorbed virus was removed and molten agar overlay added. When plaques had developed in the control bottles, the agar overlay was removed, the cells were stained with crystal violet, and the plaques were enumerated.

In all assays the per cent inhibition of plaque formation was plotted against the logarithm of the dose of interferon (in microliters). The amount of interferon which inhibited 50% of the potential plaques (PDD_{50} unit) was determined by interpolation.

Results. Different interferon preparations were assayed on chick embryo cells using vaccinia virus and either SFV or VSV as challenge viruses. The details of the 3 assays are presented in *Materials and methods*. The same batch of chick embryo cells was employed for each separate comparative study. Representative dose response curves are shown in Fig. 1 and 2. Analysis of variance showed that the difference in slopes between the VSV and vaccinia dose response lines is not significant. The PDD_{50} units obtained by interpolation from each dose response curve are presented in Table 1. Vaccinia virus was

TABLE I. PDD_{50} Unit of Interferon Activity When Assayed with 3 Different Viruses.

Interferon preparation	PDD_{50} unit of interferon activity — Challenge virus — Vaccinia*	Semliki Forest†	VSV†
1	2.9	—	19.5
2	5.0	—	32.9
3	8.6	—	37.0
4	10.4	—	45.5
5	35.0	49.0	—

* Virus and interferon were added simultaneously.

† Cells were treated for 6 to 7 hr (VSV) or 12 hr (SFV) with interferon, residual interferon was then removed and cells infected with virus. See text for further details.

about 5.5 ± 1.2 times more sensitive than VSV and 1.4 times more sentitive than SFV when the resultant PDD_{50} units were compared.

Discussion. Interferon preparations induced in chick embryo cell cultures by several viruses gave parallel dose response curves allowing estimates to be made of the relative potencies of each interferon preparation(1). When different viruses are employed to assay an interferon preparation the slope of the dose response lines may differ significantly(1). These differences in slope may be due to variations in the assay systems for the various challenge viruses. It is not valid to make a comparison of the sensitivities of two viruses to interferon if the slopes of the dose response lines differ markedly since the differences between the assays will depend upon the definition of the unit of activity which is arbitrarily chosen. In this study a valid comparison could be made since the slopes of the VSV and vaccinia dose response lines were similar. It has been previously shown that the chikungunya assay was 2.9 times more sensitive than the vaccinia assay system as described. The present study extends this observation by indicating that the vaccinia assay is about 5.5 times more sensitive than the VSV assay and 1.4 times more sensitive than the SFV assay system under the conditions imposed. The relative sensitivities of VSV and vaccinia are comparable with that obtained in different laboratories with the same interferon preparation(5).

These comparisons are somewhat different from those reported by Ruiz-Gomez and Isaacs(6) but probably reflect the greater sensitivity of the vaccinia assay used herein whereby the interferon is continuously present during plaque formation.

Summary. Assays of interferon in chick cell cultures were performed using vaccinia, Semliki Forest and vesicular stomatitis viruses as challenge viruses. Vaccinia virus was found to be approximately 5.5 times more sensitive than vesicular stomatitis virus and 1.4 times more sensitive than Semliki Forest virus to the action of interferon under the conditions of the assays.

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Early Effects of SV_{40} on Growth *in vitro* of Hamster and Human Tissue Cells.* (31347)

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To investigate the early events after infection *in vitro* of primary cells by SV_{40}, suspensions of cells from several organs were exposed to the virus and then propagated simultaneously in soft agar and in monolayer tube culture. It was reasoned that by exposing cells to virus under these conditions a more complete representation of the cellular spectrum present in the tissues might be brought into contact with virus, since it is likely that a certain degree of selection occurs when cells are cultivated *in vitro*

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