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Received June 1, 1965. P.S.E.B.M., 1965, v120.

Effect of Interferon on Vaccinia Virus Yield and Plaque Formation.* (30519)

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The effects of interferon on virus yield and on plaque formation have been widely used to measure the antiviral action of interferon. The two procedures have not been compared often although dosages of interferon obtained in plaque inhibition assays are employed in other types of studies. The present study relates the effect of various plaque-depressing units of interferon upon vaccinia virus yield and plaque formation in chick embryo fibroblast cultures.

Materials and methods. Interferon. Interferon was produced by inoculation of chick embryo tissue cultures with Chikungunya virus(1). Cultures were incubated at 37° for 24 hours and the supernatant fluids removed, centrifuged to remove cell debris, and treated with 0.02 M zinc acetate at pH 6.0 according to the method of Lampson *et al*(4). The precipitated interferon was collected by centrifugation and solubilized by adding 0.01 M HCl to pH 2.5. The preparations were dialyzed against 20 volumes of 0.9% sodium chloride at pH 6.0 and finally with Gey's balanced salt solution (BSS) at pH 7.2. After dialysis the interferon-containing fluids were heated at 65° for 30 minutes to inactivate any residual virus.

Media and tissue cultures. Growth medium for the establishment of chick cell monolayers consisted of Gey's BSS with 0.0025 M Tris, 5% calf serum, 0.25% lactalbumin hydrolysate and 0.1% proteose peptone. Mainte-

* This investigation was supported by Grants AI-04361 and 5 TI AI-0128 of Nat. Inst. of Allergy & Infect. Dis., N.I.H., USPHS.
† Trainee in Microbiology and Infectious Diseases supported by Training Grant 5 TI AI-0128.

nance medium, used for virus and interferon assays and for virus yield determinations, consisted of BSS with 0.11% sodium bicarbonate, 0.25% lactalbumin hydrolysate, 0.1% yeast extract, and 0.1% proteose peptone. Tissue cultures were prepared from 9- to 10-day-old chick embryos according to the method described by Gifford *et al*(1).

Virus. Vaccinia virus, IHD-E strain, was employed. Stock virus was prepared by inoculating 11-day-old chick chorioallantoic membranes. Infected membranes were removed 48 hours after inoculation, triturated with maintenance medium, and centrifuged at 800 *g* for 30 minutes to remove tissue debris.

Virus and interferon assays. Virus and interferon were assayed by the plaque procedure according to the methods described by Lindenmann and Gifford(5,6). Serial dilutions of interferon preparations were made in maintenance medium and 1 ml aliquots of each dilution were added to 4 bottles of chick fibroblast monolayers at the same time as approximately 200 PFU of vaccinia virus. The total volume in each bottle was adjusted to 2.0 ml by addition of maintenance medium and the cultures were incubated at 37° for 46 hours. Cells were then stained with crystal violet and plaques enumerated after 6-7× magnification of the monolayer. A dose-response curve was prepared by plotting the percentage of plaque inhibition against the logarithm of the interferon dose. A PDD_{50} unit of interferon was determined and is the amount of an interferon preparation which depressed the plaque count to 50% of the control values. For virus yield determinations replicate cultures of chick fibroblasts, with or without interferon pretreatment for 6 to 12 hours, were infected with 1 ml of vaccinia virus for 2 hours at 37°. Residual virus was removed and assayed to determine the multiplicity of infection. Cells were washed 3 times with BSS and 2 ml of maintenance medium added and the cultures incubated at 37°. A multiplicity of about 2 PFU/cell was employed. At 24 or 33 hours after infection, infected cell cultures were removed to —70°. They were subsequently thawed and cells disrupted to release the intracellular

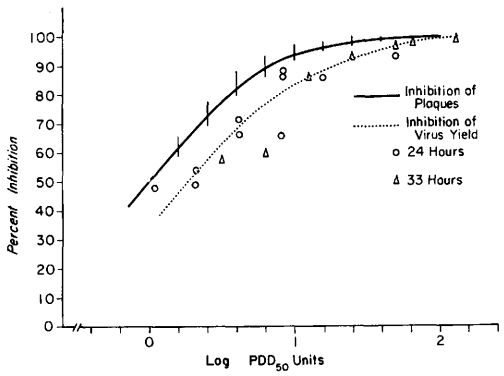

FIG. 1. Effect of interferon on vaccinia virus yield and plaque formation in chick embryo fibroblast cultures.

virus by alternate freezing in a dry ice-acetone bath and thawing at 37°. The process was repeated 6 times for maximum breakage of cells and release of virus. Control cultures yielded approximately 0.8 to 1.1 × 10^7 PFU.

Results. Different batches of interferon induced in chick embryos or in chick fibroblast cultures by the same or different viruses gave parallel dose-response curves(6,1). In a large number of assays the average slope of the dose-response curve was found to be 55.5 when plaque inhibitions between 20 and 80% were considered. Slopes represent the change in percentage plaque inhibition for each unit increase of the dose (in logarithms) of interferon preparation. When extended beyond 80% inhibition, it has been shown that the dose-response curve was sigmoidal and that 100% inhibition was only approached asymptotically. Furthermore, after transformation of inhibition percentages into probits, an excellent straight line relationship was obtained (6). In this experiment probit transformation was employed to construct a line with a slope of 55.5 over the range of 20 to 80% inhibition and extrapolated beyond 80% inhibition. The smooth curve of Fig. 1 has been constructed by transforming probits back into percentages. The vertical lines on this curve show the limits when dose-response curves having slopes of 51 and 68 were plotted in a similar manner. These slopes represent the limits that are usually found. For comparative purposes, log PDD_{50} units have been employed on the abscissa. When different

PDD_{50} doses of interferon were added to the cultures which received a multiplicity of vaccinia virus of about 2 PFU/cell and the per cent inhibition of virus yield plotted, a parallel curve was obtained (Fig. 1). Thus, the dose-response curve relating virus yield also seemed to be sigmoidal. For each dose of interferon employed, the per cent inhibition of virus yield was less than the corresponding inhibition of plaque formation.

Discussion. Gifford, Toy, and Lindenmann (2) have suggested that the total plaque area is a measure of the total amount of virus and found a relationship between total plaque area and interferon concentration. The log total plaque area decreased linearly with increasing log interferon concentration. The inhibition of total plaque area was a more sensitive measure of interferon than the depression of plaque count. However, the present study indicates that virus yield is less sensitive than plaque numbers to inhibition by interferon. This might be related to the findings of Ho(3) that viral inhibition caused by interferon could be overcome to a certain extent by increasing multiplicities of infection. In the studies reported here, approximately 2 PFU/cell were employed. However, Lockart (personal communication) does not find a difference in viral yield when different multiplicities of Western equine encephalomyelitis (WEE) virus are employed. These differences may be due to differences inherent in the virus-host cell system. For example, interferon decreases virus yield and increases the latent period in vaccinia and poliovirus-infected chick cells(2,3). In WEE infection, only virus yield seems to be affected(8). Moreover, Lockart, Sreevalsan and Horne(7) found the effect of interferon on virus yield to be more sensitive than its effect on plaque inhibition. These differences serve to emphasize how units of interferon activity may vary according to the assay procedure.

A recognition of the sigmoidal nature of the dose-response curves is important because it indicates that complete inhibition of virus synthesis is difficult to achieve since 100% inhibition is approached asymptotically.

Summary. The effects of interferon on vaccinia virus yield and on plaque formation were compared in chick embryo tissue cultures and the latter procedure was found to be more sensitive. The upper portion of the dose-response curve relating inhibition of virus yield to interferon dose seemed to be sigmoidal.

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Received June 10, 1965. P.S.E.B.M., 1965, v120.

Immunoelectrophoretic Analysis of Serum Proteins in Agar and in Agarose. (30520)

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Brishammar(1) using agarose prepared by Hjerten(2) presented one comparison of the immunoelectrophoretic behavior of enolase in this medium and in agar. It was stated that the lines of precipitation in agarose were clearer, with no halo effects and no false lines of precipitation. Sullmann(3), on the other hand, prepared agarose by the method