Interferon Production with Different Multiplicities of Semliki Forest Virus.* (32570)

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Gifford(1) found that chikungunya virus best induced interferon production when the input multiplicity of virus was between 0.01 and 0.1 plaque forming units (PFU) per chick embryo cell. In a comparative study of the production of interferon by different viruses, Gifford *et al*(2) showed that chikungunya virus produced 2.6 times more interferon than did Semliki Forest virus when an input multiplicity of 0.06 PFU per cell was employed for the 2 viruses. Since it had not been established that this multiplicity of Semliki Forest virus resulted in maximum production of interferon, the comparison of the efficiency of the 2 viruses may not be valid under all conditions. Therefore, the effect of multiplicity of Semliki Forest virus on interferon yield was studied.

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 0.06% 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 and 0.1% yeast extract. An agar overlay was used for assays employing Semliki Forest virus. Agar medium overlay consisted of the growth medium with 0.5% "Ionagar." Chick embryo cell cultures were prepared as previously described(2).

Viruses. Vaccinia and Semliki Forest 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 virus stock preparations were made by inoculating newborn mice intracerebrally with virus and harvesting the brains 36 to 48 hours later. All virus preparations were centrifuged at 800 *g* for 30 minutes to remove cellular debris and were stored in glass ampules at —60°.

Virus and interferon assays. Vaccinia virus and interferon were assayed by the methods described by Lindenmann and Gifford(3,4). Semliki Forest virus was assayed as described by Riley *et al*(5).

Results. Replicate chick embryo cell cultures were infected with different amounts of Semliki Forest virus diluted in maintenance medium. Cultures were allowed to incubate for 24 hours, the supernatant fluid removed, centrifuged to remove cellular debris, and heated at 65° for 30 minutes to inactivate residual virus. The resultant interferon titers were obtained as described using the same batch of cells for all assays. The results (Table I) show that the 24-hour yield of interferon was greatest when the input multiplicity of Semliki Forest virus was approximately 0.2 PFU/cell. Marked inhibition of

TABLE I. Interferon Production with Different Multiplicities of Semliki Forest Virus.

Exp No.	Input multiplicity of infection (PFU/cell)	PDD_{50} units* of interferon produced per 10^6 cells in 24 hr
1	10	4.9
	1	20.0
	0.1	15.7
	0.01	12.8
	0.001	7.2
2	2	17.4
	1	18.2
	0.8	25.5
	0.4	26.7
	0.2	35.2
	0.1	13.1

* PDD_{50} unit is that amount of interferon needed to reduce the number of vaccinia virus plaques to 50% of the number found on control cultures.

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interferon synthesis occurred when higher multiplicities of virus were employed.

Discussion. The amount of interferon produced by chick embryo cell cultures infected with Semliki Forest virus was dependent upon the multiplicity of infection as previously shown for chikungunya virus in the same type of cell system(1). Optimal yield of interferon was obtained with a different cell-virus ratio than previously found for chikungunya virus(1). It is possible that the "ideal" multiplicity of infection might differ from 1 preparation to another of the same virus. Studies which compare the yield of interferon by different viruses should take this possibility into consideration. The inhibition of interferon synthesis at high multiplicities of virus was more marked with Semliki Forest virus than for chikungunya virus. A similar phenomenon has been shown for rat tumor cells infected with Sindbis virus(6) and dog-kidney cells infected with herpes simplex virus(7). Contrariwise, Burke and Isaacs(8), using ultraviolet-inactivated influenza virus and chick chorioallantoic membranes, did not find any inhibition of interferon production when high doses of non-replicating virus were employed. Furthermore, chikungunya virus preparations usually induced less interferon in chick cell cultures than did the same dilution of the virus preparation in which the plaque forming ability had been lost by preincubation at 35° for 23 hours(9). These findings suggested that active plaque forming virus inhibited the production of interferon and may be related to the "inverse interference" phenomenon described by Lindenmann (10). It is likely that those viruses which rapidly shut off host RNA and protein synthesis would not be able to induce interferon synthesis in such a system as suggested by Aurelian and Roizman(7), Taylor(11), and by Wagner and Huang(12). Quantitative differences in the ability of viruses to induce interferon synthesis may be related to the rapidity and extent of this inhibition of cellular macromolecular synthesis and also to whether the "inactive" virus can also accomplish this cellular inhibition.

Summary. The yield of interferon in chick embryo cell cultures infected with Semliki Forest virus is dependent upon the multiplicity of virus employed. Optimal yields of interferon were found when a multiplicity of about 0.2 was used. Higher multiplicities of virus resulted in decreasing yields of interferon.

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Suppression of Plasma Immunoreactive Insulin by Epinephrine in Obese Subjects.* (32571)

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Hyperinsulinism is one of the metabolic characteristics of obesity as evidenced by increased plasma insulin concentrations in obese

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