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SHORT COMMUNICATION

INHIBITORY EFFECT OF INTERFERON ON THE MULTIPLICATION OF SHOPE FIBROMA VIRUS *IN VITRO*[1]

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Since the discovery of the " inhibitory factor " by NAGANO and KOJIMA (1954) and of " interferon " by ISAACS and LINDENMANN (1957), reports have been made on the inhibition of virus multiplication by these factors *in vitro* as well as *in vivo*. Though the natures of these factors have not yet been elucidated entirely, the name " interferon " (IF) will be used in this paper.

In the preceding paper (KISHIDA *et al.*, in press) the inhibitory effect of IF on tumor formation with Shope fibroma virus (SFV) was reported. In this paper the effect of IF on the multiplication of SFV in cell cultures *in vitro* is reported.

The IF preparation used in these experiments was a gift from Dr. Y. NAGANO, in whose laboratory it was prepared by extracting rabbit skin 4 or 5 days after application of vaccinia virus strain DV to the shaved skin. The preparation had been purified by repeated precipitation with ammonium sulfate and its titer was estimated to be 3125 u/ml (1 u/ml corresponds to a 50 per cent inhibitory dose of IF against infection with 10 ID_{50} of vaccinia virus in rabbit). To obtain a stock suspension of SFV, the primary monolayer culture of rabbit kindey (RK) cells was infected with SFV, strain OA. After 2 to 3 days incubation the infected cells were harvested and treated by sonic oscillation to obtain a suspension of SFV. The virus titer in 50 per cent tumor forming units (TFU_{50}), was estimated at $10^{3.8}$/0.2 ml, determined by an *in vivo* test for tumor forming ability on rabbit skin.

Infection of the cells with pox group viruses, including SFV, produces " B " type inclusion bodies in the cytoplasm, which are claimed to be the site of viral protein and DNA syntheses (KATO *et al.*, 1963 a; KATO *et al.*, 1963 b). In cowpox infected cells, the size of the inclusion bodies represents the extent of the process of viral infection in the cells (KATO *et al.*, 1964). A similar parallelism was observed in SFV infected cells (KATO *et al.*, 1965). In a representative experiment, 0.2 ml of the stock suspension of SFV produced 982 inclusion body bearing cells per 1000 cells after 48 hours when inoculated onto RK cells grown on a

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cover slip in a Leighton type square tube. Throughout the experiments lactalbumin Earle medium supplemented with 10% bovine serum (LE-medium) was used.

To examine the effect of different concentrations of IF, the RK cells on cover slips in a series of culture tubes containing LE-medium were treated for 24 hours with five fold serial dilutions of IF of concentration ranging from 625 to 1 u/ml. Then the culture in each tube was washed three times with phosphate buffered saline to remove the IF. The cells were then challenged with 0.2 ml of SFV stock suspension for two hours and then fresh LE-medium was introduced. After incubation for another 48 hours, the cells on the cover slip were fixed with methanol and stained in the standard way with Giemsa's solution in phosphate buffer, pH 6.8. The number of inclusion body bearing cells per 1000 cells was counted. As shown in Table 1 a decrease in the number of inclusion bodies with increase in the concentration of IF up to 625 u/ml was observed.

TABLE 1 *Effect of the concentration of IF used for pretreatment on the number of inclusion body bearing cells in SFV infection*

IF (u/ml)*	Number of Inclusion Body Bearing Cells**
0	926
1	710
5	550
25	240
125	60
625	32

* Pretreatment for 24 hours before SFV infection.
** Number per 1000 cells.

Treatment of the cells with a constant concentration of 25 u/ml of IF before SFV challenge, showed that inclusion body formation was affected even when the cells were treated for 3 or 6 hours before SFV infection. The maximum effect was observed when the cells were treated for 24 hours before SFV infection and the number of inclusion body bearing cells was estimated as 80 in contrast to the value of 982 for control cells which were not treated with IF (Table 2).

TABLE 2 *Relationship between period of IF pretreatment and number of inclusion body bearing cells*

Period of Pretreatment with IF (25 u/ml) (hours)	Number of Inclusion Body Bearing Cells*
0**	720
3**	380
6	538
24	80
Control	982

* Number per 1000 cells.
** IF treatment continued during SFV infection.

It is noteworthy that SFV infection of IF-treated cells resulted not only in a decrease in the number of inclusion body bearing cells, but also in variation in the appearance of these inclusion bodies, which looked incomplete or diffuse. In another series of experiments, using the fluorescent antibody technique which indicates the presence of virus antigens, the inclusion bodies also showed a variety of forms. Consequently, from the point of view of inclusion body formation, the appearance of this variation in the form of the inclusion bodies suggests that the effect of IF on virus multiplication does not necessarily always follow the "all or none principle in IF action" claimed by COOPER *et al.* (1959). (Figs. 1, 2, 3 and 4).

The results reported here clearly demonstrate that IF prepared from cells infected with vaccinia virus inhibits not only tumor formation by SFV in rabbits but also the formation of inclusion bodies *in vitro*, indicating its eventual inhibition of SFV multiplication.

Further studies on the mechanism of inhibition of SFV multiplication by IF as well as on tumor production in rabbits, including studies on the relationship between incomplete formation of inclusion bodies and infectivity, are now in progress.

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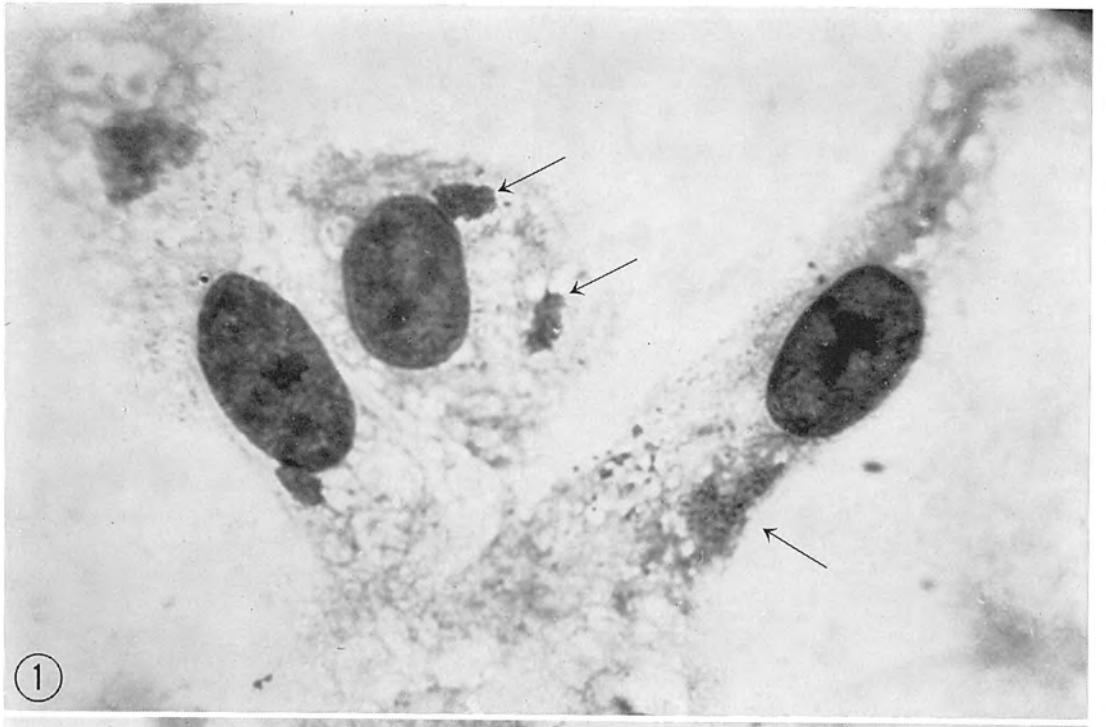
FIGURE 1 Inclusion bodies in RK cells 48 hours after infection with SFV. Not treated with IF. Giemsa staining.

FIGURE 2 Inclusion bodies in RK cells treated with IF (25 u/ml) 24 hours before SFV infection. Giemsa staining.



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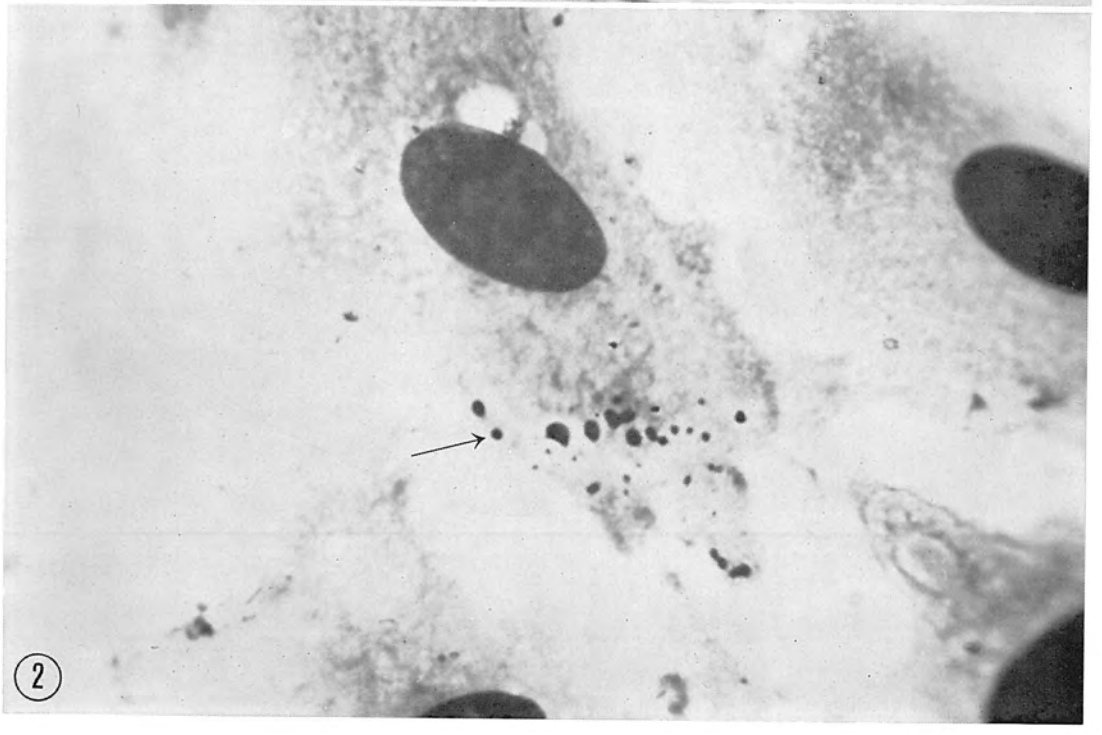

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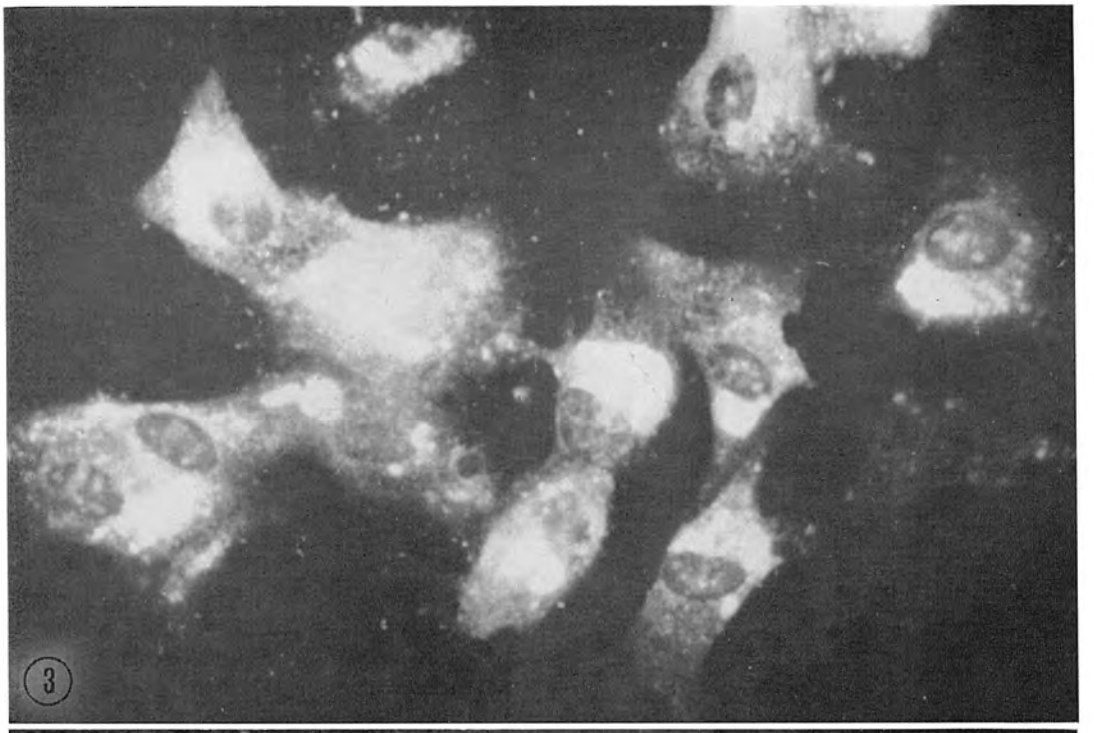
FIGURE 3 Inclusion bodies demonstrated by the fluorescent antibody technique in RK cells infected with SFV 48 hours after infection.

FIGURE 4 Inclusion bodies demonstrated by the fluorescent antibody technique in RK cells treated with IF (25 u/ml) 24 hours before SFV infection.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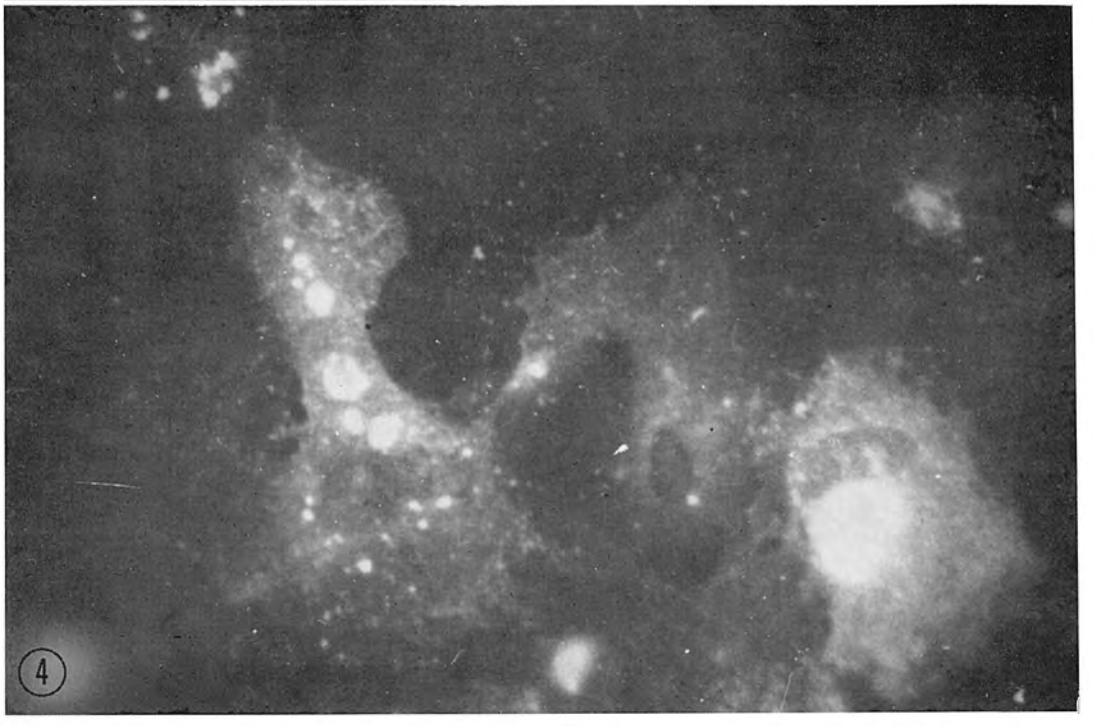
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