in which the male sterility factor was transmitted by grafting from *Petunia* through stems of a tobacco plant to *Petunia* and the failure of the factor to induce male sterility in tobacco itself.

The asexual transmission of the factor responsible for cytoplasmic male sterility in *Petunia* to other species would be useful in the fields of genetics and plant breeding where male sterility is widely used in the convenient production of hybrid seed. Transmission of this factor from *Petunia* through the stem of tobacco is a step in this direction. Sand[4] failed to demonstrate asexual transmission of a type of cytoplasmic male sterility derived from an interspecific cross between *Nicotiana debneyi* and *N. tabacum*. He concluded that graft transmission of cytoplasmic male sterility does not occur readily in plants and that in tobacco cytoplasmic male sterility or fertility is under autonomous control. Negative results were also obtained by Ohta[5] for graft transmission of cytoplasmic male sterility in red pepper (*Capsicum annuum*).

Six cytoplasmic male sterile *Petunia* plants (*P*–300–58) were grown and decapitated before flowering. Six young tobacco (*Nicotiana tabacum* var. Turkish) plants were cleft-grafted on to the cytoplasmic male sterile *Petunia* stocks. Both *Petunia* stocks and tobacco scions grew well and both flowered. All the *Petunia* flowers were cytoplasmic sterile and all the tobacco flowers were fertile. The tobacco flowers set seed, which were saved to plant subsequent generations. After the tobacco seed was collected, three of the grafted tobacco plants were decapitated and a normal fertile *Petunia* (*P*–5–58, maintainer) was cleft-grafted on to each tobacco scion. Thus, the plant now consisted of a cytoplasmic male sterile root stock, an inter-graft *N. tabacum* stem (12 in. long) and a normal *Petunia* top. One of the normal *Petunias* flowered without evidence of altered fertility, similar to that reported for the graft generation of *Petunia* on *Petunia*[3]. Three of the *Petunia* flowers were selfed and seed from two of these flowers were planted. At the same time, seed from the tobacco plants were planted. Controls consisted of normal (*P*–5–58) *Petunia* grafted on normal (*P*–5–58). No evidence of altered male fertility was detected in the graft or subsequent generations. Two thousand flowers on the tobacco plants grown from this seed were examined; all were fertile. Thus, there was no evidence for movement of the cytoplasmic sterility factor from *Petunia* to tobacco in this generation. However, subsequent generations of this tobacco may express some degree of altered male fertility. Nine *Petunia* plants, grown from seed produced by the two flowers mentioned here, comprising the first generation after grafting, produced some sterile flowers. A total of 104 flowers on the nine plants were classified; 90 were fertile, 7 were sterile and 7 were partially sterile.

Table 1. CLASSIFICATION OF FLOWERS OBTAINED FROM *Petunia* PLANTS TWO GENERATIONS AFTER INTER-GRAFTING WITH *Nicotiana tabacum*

First generation parents	No. of plants	Classification		
		Fertile	Sterile	Partially sterile
Fert. selfed*	11	124	0	0
Fert. selfed	7	47	5	0
Fert. selfed	60	566	2	0
Fert. selfed	22	220	0	0
Fert. selfed	12	146	6	1
Fert. selfed	12	119	18	1
Fert. selfed	51	510	0	0
Fert. selfed	17	170	0	0
Pt. st.† selfed	20	200	0	0
Pt. st. selfed	1	13	0	0
Pt. st. selfed	23	320	28	14
Pt. st. selfed	3	30	0	0
Pt. st. × pt. st.	89	977	0	4
Pt. st. × fert.	8	80	0	0
St.‡ × fert.	82	820	0	0
St. × fert.	31	300	111	62
St. × fert.	2	20	0	0
Total	451	4,662	170	82

* Fert., normal pollen fertility.
† Pt. st., partially cytoplasmic male sterile.
‡ St., cytoplasmic male sterile.

To obtain seed for the second generation after grafting in *Petunia*, fertile flowers were selfed, sterile flowers were crossed with *P*–5–58 normal pollen, and partially sterile flowers were either selfed or crossed. From these selfs and crosses, 17 populations were obtained in which 451 plants grew to maturity. In these populations, a total of 4,914 flowers were examined, of which 4,662 (94·9 per cent) were classed fertile, 82 (1·7 per cent) partially sterile, and 170 (3·4 per cent) sterile (Table 1). Not all selfed fertile flowers produced exclusively fertile offspring, nor did all sterile flowers when crossed produce only sterile individuals, thus indicating that some of the ovules contained the cytoplasmic sterility factor, and some did not.

This transfer of cytoplasmic male sterility factors from *Petunia* through the stem of an apparently 'immune' species is similar to the transmission of potato virus *X* through the stem of the immune potato seedling U.S. Dept. Agric. 41,956 (ref. 6); or the transmission of tobacco mosaic virus through the tissues of dodder (*Cuscuta campestris*) in which it does not multiply[7]; or the transmission of the curling vine virus through resistant varieties of grapes[8].

Interspecific grafts were also made between cytoplasmic male sterile plants of *Petunia* (*P*–266–58) and plants of tomato (*Lycopersicon esculentum*) and of *Nicotiana undulata* without any evidence of transmission to the tomato or tobacco.

Although interspecific graft transmission of the factor responsible for cytoplasmic male sterility did not occur, the factor was transferred through an apparently immune plant to the susceptible *Petunia* where it was expressed in subsequent generations. It may be that only *Petunia*, of the plants tested, is susceptible and that a different type of cytoplasmic sterility is involved in other genera, or that the genotype of the scion does not allow the expression of sterility although cytoplasmic sterility factors are present.

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Role of Interferon in Viral Interference

ACTINOMYCIN *D* inhibits the production of interferon[1]. Since the exact role of interferon in the phenomenon of viral interference has not been fully established, the effect of actinomycin *D* on viral interference was tested. The present results show that when actinomycin *D* inhibited interferon production by a heat-inactivated arbovirus, it also reversed viral interference by the virus.

Mouse brain pools of Semliki forest virus (SFV) and Chikungunya virus and a chick allantoic fluid pool of Newcastle disease virus (NDV) were used. Interferon was prepared by infecting tissue culture plates made from 10-day-old chick eggs with Chikungunya virus. Fluids were collected after 24 h, heated to 65° C for 0·5 h in order to inactivate virus, and stored at −10° C. The interfering virus was made from a mouse brain pool of Chikungunya virus which had been diluted 1/100 and then heated at 37° C for 23 h[2]. Interferon was titrated in chick

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cells utilizing a standard vaccinia virus assay[3]. The number of units of interferon in a preparation was the reciprocal of the highest dilution showing approximately 50 per cent plaque reduction as compared with controls.

Chick embryo fibroblast cultures, which had been prepared from 10-day-old eggs, were incubated at 37° C for 4 h with 0·06 μg/ml. or 0·12 μg/ml. of actinomycin *D*, washed with Gey's buffer, and 0·5 ml. of heat inactivated Chikungunya virus or buffer was then added. At the dilution of the Chikungunya virus pool used, the virus plaque forming unit to cell multiplicity was approximately 1 : 1. After incubation at 37° C for 1 or 3 h the cells were again washed and either 2 ml. of buffer was added and the plates incubated at 37° C for 14 h (for interferon yields), or the cells were challenged with 0·5 ml. of an appropriate dilution of NDV, SFV, or Chikungunya virus, and the virus allowed to adsorb at 37° C for 1 h. The cell sheets were then overlayed with 0·9 per cent agar and plaque medium and incubated at 39° C for NDV, at 35° C for Chikungunya, or at 37° C for SFV. In all cases plaques could easily be counted after 24 h of incubation. Final counts were made after 40 h.

Several experiments were performed. The results of a typical single experiment are shown in Table 1. In plates challenged with Chikungunya virus, plaques were completely inhibited by treatment with the heated virus. Pretreatment with 0·12 μg/ml. of actinomycin *D* neither increased the yield of plaques in controls, nor reversed the inhibition of plaques in plates treated with heated virus.

Table 1. REVERSAL OF VIRAL INTERFERENCE BY ACTINOMYCIN *D*

Challenge virus	Actinomycin *D* (μg/ml.)	Interfering virus*	Units of interferon formed	Plaque count at 40 h	Effect found
Chikungunya	0	–	–	22, 24	—
Chikungunya	0	+	20	0, 0	100% inhibition
Chikungunya	0·12	–	–	20, 22	None
Chikungunya	0·12	+	<4	0, 0	100% inhibition
SFV	0	–	–	215, 225	—
SFV	0	+	20	12, 13	92% inhibition
SFV	0·12	–	–	322, 332	50% enhancement
SFV	0·12	+	<4	284, 304	35% enhancement
NDV	0	–	—	39, 51	—
NDV	0	+	20	10, 15	67% inhibition
NDV	0·06	–	–	55, 66	35% enhancement
NDV	0·06	+	4	50, 58	20% enhancement

SFV = Semliki forest virus. NDV = Newcastle disease virus.

* –, Heat inactivated virus not added; +, 0·5 ml. of heat inactivated Chikungunya virus added for 1 h for SFV and Chikungunya, and for 3 h for NDV.

In the case of plates challenged with SFV, heated Chikungunya virus reduced the plaque count by 92 per cent as compared to untreated controls. Pre-incubation with 0·12 μg/ml. of actinomycin *D* raised the plaque count by 50 per cent, and by 35 per cent in cells also treated with heated Chikungunya virus, thus completely reversing the interfering action of the latter. In the plates treated with heated Chikungunya virus, the yield of interferon was reduced from 20 units in controls to less than 4 units in plates treated with 0·12 μg/ml. of actinomycin *D*.

In the case of NDV, preliminary experiments had shown that pretreatment with 0·12 μg/ml. of actinomycin *D* decreased the yield of plaques. Also, no viral interference was detected unless the interval between the addition of the heated virus and the NDV challenge was increased to at least 3 h. When a 3-h interval was used, plaque counts were reduced by 67 per cent. When cells were pretreated with actinomycin *D* (0·06 μg/ml.), plaque counts were raised by 35 per cent and by 20 per cent in plates also treated with heated Chikungunya virus. In the case of the latter, however, interferon production was not completely inhibited by 0·06 μg/ml. actinomycin *D*, and 4 units of interferon were found compared with 20 units in the controls.

Since the ease with which the reversal of viral interference by a standard dose of heated virus varied with the different viruses tested, it was of interest to determine the sensitivity of each of the 3 viruses used to a standard preparation of interferon. The inhibitory effect of a 1/20 and a 1/100 dilution of interferon was determined (Table 2). For SFV, a 1/20 dilution inhibited 50 per cent of the plaques, while for Chikungunya virus a 1/100 dilution sufficed; however, for NDV, no significant inhibition was found at a 1/20 dilution.

Table 2. DILUTION OF STANDARD INTERFERON PREPARATION INHIBITING 50 PER CENT OF VIRUS PLAQUES

Test virus	Dilution of interferon reducing plaque yields to 50 per cent of controls
Chikungunya	1/100
Semliki forest virus	1/20
Newcastle disease virus	<1/20

Listed in order of either increasing sensitivity to interferon (Table 2), or to interference by a standard dose of heated Chikungunya virus (Table 1), the viruses were NDV, SFV, and Chikungunya virus. This is also the order when the ease with which viral interference could be reversed by actinomycin *D* is considered. With NDV, interference was reversed by a concentration of actinomycin *D* which did not completely suppress interferon production. In the case of SFV, a concentration of actinomycin *D* which inhibited the production of interferon reversed the viral interference. For Chikungunya virus, however, interference could not be reversed by a concentration of actinomycin *D* which inhibited interferon production; however, the titres of interferon shown in Table 1 were determined by a vaccinia virus assay system, and Chikungunya virus is 2–3 times more sensitive to the antiviral action of interferon than is vaccinia virus[4]. Thus, a level of interferon which might be undetectable with the assay system used might completely inhibit Chikungunya virus plaque production.

The results are also of interest in that they confirm previously published observations that actinomycin *D* enhances plaque yields of some RNA viruses and depresses production of interferon[1]; however, we were unable to confirm the finding that the yield of Chikungunya virus plaques was increased by actinomycin *D* (ref. 1).

The findings reported here suggest that the effect of actinomycin *D* in reversing viral interference is related to its effect on interferon production. Steroids, which inhibit interferon production[5], have also been shown to reverse viral interference[6]. Both these observations indicate that many examples of viral interference may be very closely related to interferon production.

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Isolation of a Virus resembling Encephalomyocarditis from a Red Squirrel

BRITISH red squirrels have suffered from epidemic outbreaks of unidentified disease at various times in the past, sometimes with mortality rates exceeding 90 per cent[1]. At Thetford Chase (Norfolk) sick and dying squirrels were seen recently with symptoms including paralysis of the hind legs. No grey squirrels are known in this area, so it was decided to attempt isolation of virus material from the red squirrel population to initiate a study of virus diseases affecting squirrels in Britain.

An apparently healthy adult female red squirrel was shot in a locality where several affected animals had been

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