Vogel, Eds. (Academic Press, New York, 1965), p. 221.
28. W. Fiedler, in *Primatologia*, H. Hofer, A. H. Schultz, D. Stark, Eds. (Karger, Basle, 1956).
29. This research was supported in part by NSF grant GB 1318, and the experiments reported here comprise part of an M.A. thesis (A.S.H.) submitted to Hunter College of the City University of New York, 1965.

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Virus: Mixed Infection with Herpes Simplex and Simian Virus 40

Abstract. *Mixed infection, the infection of a single cell by two distinguishable viruses, has been demonstrated by electron microscopy in cultures of African green monkey kidney cells after inoculation with simian virus 40 and herpes simplex. Mixed infection occurs rarely when the two viruses are inoculated simultaneously, but if herpes is inoculated 24 hours after SV40 both viruses are found in the same nucleus in about 5 percent of intact cells.*

Mixed infection, the infection of a single cell by two distinguishable viruses, has been demonstrated by electron microscopy with African green monkey kidney (AGMK) cells infected with adenovirus 12 and simian virus 40 (SV40) (*1*). Since the two types of virus particles were intermingled within the same nucleus, the possibility of phenotypic mixing with production of virus particles with altered oncogenic properties was suggested. Subsequently, evidence of phenotypic mixing and "hybrid" formation after mixed infection of monkey kidney cells with several human adenoviruses and SV40 has been presented (*2*).

In order to determine whether mixed infection with SV40 and another type of DNA virus was possible, we have infected AGMK cultures with SV40 and herpes simplex virus (HSV). Tube cultures of the kidney cells were obtained from Microbiological Associates, Inc., Bethesda, Maryland. The strain of SV40 and methods of growth and titration have been described (*1*). The strain No. 11124 of HSV, obtained from W. K. Ashe, was grown and titrated in diploid human Wi-38 fibroblasts (*3*).

Three groups of AGMK tubes were used in each experiment. The first was inoculated with $10^{5.4}$ $TCID_{50}$ (tissue culture infective dose, 50 percent effective) of HSV; the second was infected with $10^{5.4}$ $TCID_{50}$ of HSV and $10^{6.9}$ $TCID_{50}$ of SV40 simultaneously; and the third was infected with $10^{5.4}$ $TCID_{50}$ of HSV and $10^{6.9}$ $TCID_{50}$ of SV40, but the inoculation of HSV was delayed 24, 26, or 30 hours after the initial inoculation of SV40.

Methods for infection of cultures and electron microscopy have been described (*1*). Incubation times and results are listed in Table 1.

Single infection with HSV or simultaneous infection with the two viruses resulted in a herpes-type cytopathic effect within 24 hours. By electron microscopy, after 42, 47, and 72 hours of incubation, herpes particles were found in over 90 percent of intact cells. No cells with SV40 particles only were identified in the cultures infected with SV40 and HSV simultaneously, but a rare cell (less than 1 percent) was found containing both types of particles within the same nucleus.

When SV40 was inoculated 24 hours prior to HSV, the cytopathic effect was still of the herpes type, and, by electron microscopy, approximately 60 percent of the intact cells contained HSV only, 20 percent contained SV40 only, and 5 percent contained both types of virus particles within the same nucleus (Fig. 1). Delay of inoculation with HSV for 26 and 30 hours after SV40 produced a mixed type of cytopathic effect, and, by electron microscopy, approximately half the cells contained HSV only and half contained SV40 only. Mixed infection was seen in less than 1 percent of cells in the group with the 30-hour interval between SV40 and HSV.

Table 1. Mixed infection with herpes simplex virus (HSV) and simian virus 40 (SV40).

Subgroup	Incubation time (hr)	Cells infected (%)		
		HSV	SV40	Mixed
Group 1, HSV				
a	42	90		
b	47	90		
Group 2, HSV and SV40 simultaneously				
a	42	90		
b	47	90		
c	72	90		<1
Group 3, HSV preceded by SV40				
a*	76	60	20	5
b†	73	50	40	
c‡	72	40	50	<1

*HSV inoculated 24 hours after SV40; †HSV inoculated 26 hours after SV40; ‡HSV inoculated 30 hours after SV40.

Although it was possible to demonstrate mixed infection with SV40 and

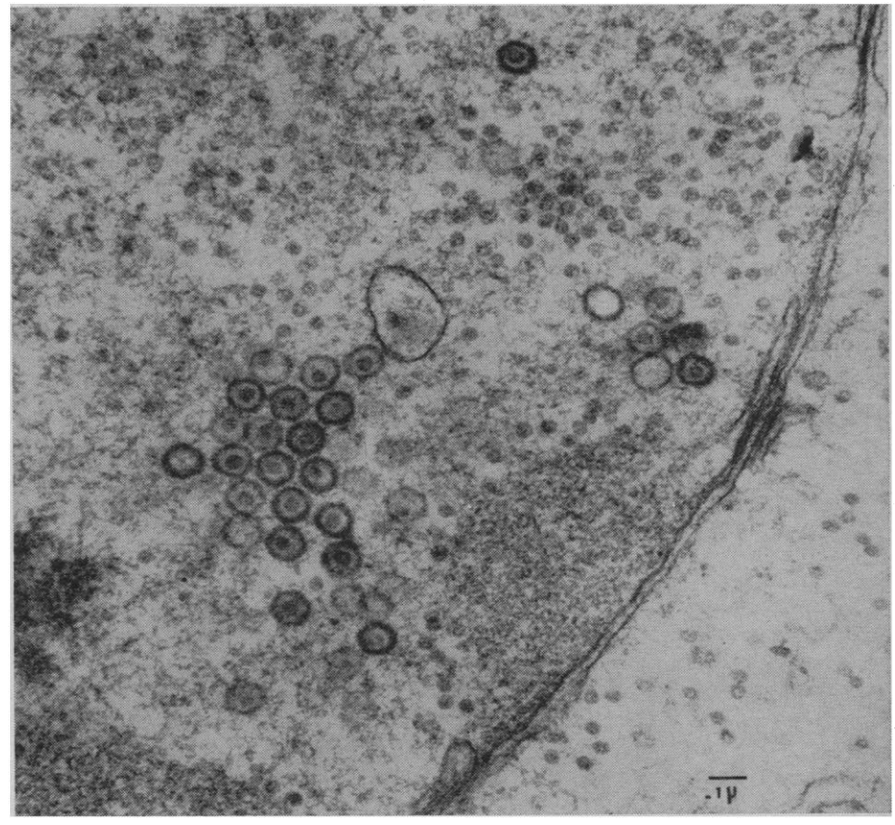

Fig. 1. African green monkey kidney cell with intranuclear clusters of herpes simplex virus (95 to 100 mμ) and SV40 (45 mμ) (× 56,500).

HSV in the monkey kidney cells, the number of cells with both types of virus particles within the same nucleus was smaller than that observed previously in similar cultures infected with SV40 and adenovirus 12 (*1*). Titrations of HSV in the progeny of cultures infected with HSV and SV40 showed no enhancement of HSV similar to the enhancement of adenovirus 12 by SV40 (*4*). Nevertheless, the morphological demonstration of HSV and SV40 in close proximity within the nucleus of the same cell suggests the possibility of phenotypic mixing, with production of virus particles having altered biological properties.

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References

1. G. T. O'Conor, A. S. Rabson, I. K. Berezesky, F. J. Paul, *J. Nat. Cancer Inst.* **31**, 903 (1964).
2. R. J. Huebner, R. M. Chanock, B. A. Rubin, M. J. Casey, *Proc. Nat. Acad. Sci. U.S.* **52**, 1333 (1964); W. P. Rowe and S. G. Baum, *ibid.*, p. 1340; F. Rapp, J. L. Melnick, J. S. Butel, T. Kitahara, *ibid.*, p. 1348; J. M. Easton and C. W. Hiatt, *Proc. Nat. Acad. Sci. U.S.* **54**, 1100 (1965).
3. W. K. Ashe and H. W. Scherp, *J. Immunol.* **91**, 658 (1963).
4. A. S. Rabson, G. T. O'Conor, I. K. Berezesky, F. J. Paul, *Proc. Soc. Exp. Biol. Med.* **116**, 187 (1964).

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Population Flushing with Sexually Sterile Insects

Abstract. *In theory, populations of animals can be displaced by overloading a resource with introduced sterile animals. The theory was tested on natural populations of the Queensland fruitfly* Dacus tryoni *Frogg. Three of four treated populations declined sharply within 2 days after sterile flies had been introduced. This procedure may be useful both as a tool in experimental ecology and as a means of controlling pests.*

The idea of using artificially reared sterile animals to drive out and replace the fertile members of a natural population ("population flushing") was earlier proposed (*1*). The model predicted that fertile animals could be killed or forced to leave a population by introduction of enough sterile animals to overload resources, such as food or living space; repetition could lead to complete extinction of the fertile population. This model has since been tested by single releases of sterile animals into populations of the Queensland fruit fly *Dacus tryoni* Frogg (Diptera, Trypetidae).

The most direct method of testing is to release sterile animals into wild populations whose absolute numbers have been estimated, and then to look for sharp decreases in the treated populations in comparison with untreated control populations. Wintering populations of *Dacus tryoni* near Sydney, Australia, were chosen because they had the following characteristics.

1) Apparently discrete, small populations spend the winter in isolated groups of citrus trees. Considerable proportions of such populations may readily be taken for estimates of population by marking, release, and recapture; the variance of such estimates of population is thereby minimized.

2) Earlier observations had suggested that such populations were stable because the birth rate was zero in winter, the death rate was low, and movement was inhibited by isolation and low temperatures. These facts reduced the possibility that accidental fluctuations in numbers might mask the changes effected by introduction of sterile animals.

During the winter (May–August) of 1963 four of seven suitable populations were treated, while three served as controls. Bailey's method of two-point sampling (*2*) was used. Flies were captured with hand nets and marked on the thorax with quick-drying colored paint (*3*); each fly caught received a mark corresponding to the day of capture. Thus was kept a complete record of the dates of capture for all flies caught. After marking, the flies were released into their home trees.

The sterile flies were reared at 25°C and sterilized with 6000 rad of gamma-rays (^{60}Co) on the 8th day of pupal life. After emergence they were held for four days, fed for two days on sucrose containing $^{32}P(NaH_2P^*O_4)$ at a concentration of 100 μc per gram of sugar, and then released into the treated populations. On recapture they were detected by means of a portable counter (*4*); recaptured sterile flies, marked with paint and released like wild flies, were distinguishable by their radioactivity.

The size of each wild population was estimated for a few days before and after the sterile flies were introduced. For accuracy the number of released flies was set at about three to five times the estimated number of wild flies, because a higher ratio might have overdiluted the wild population and prevented us from recovering enough wild flies for the later estimates.

Table 1. Populations of colonies before and after release of sterile flies; standard error, in parentheses, follows each number. Numbers and SE estimated by Bailey's two-point sampling method.

Colony	Population (No.)	
	Before release	After release
C_1	108(51.7)	115 (46.8)
C_2	149(42.1)	95 (29.3)
C_3	88(30.1)	233(130.0)
T_1	193(67.4)	15 (6.1)
T_3	113(46.8)	31 (5.6)
T_4	85(16.8)	27 (9.3)

The results are shown in Fig. 1. The three control populations (C_1, C_2, C_3) were used to determine whether the processes of capturing, marking, and releasing would of themselves lead to a decline in numbers, mimicking the decline expected to follow the release of sterile flies. Apparently two of the control populations (C_1, C_3) and all four of the treated populations (T_1, T_2, T_{3a}, T_{3b}) were stable before the introduction of sterile males. (The apparent fluctuations in numbers reflect unavoidable changes in size of samples and hence in sampling error.) Only C_2 appeared to be unstable, declining slowly throughout the period of observation in a way quite unlike the rapid falls seen in T_1, T_{3a}, and T_{3b} only *after* the releases.

In three of the four treated populations there was a sharp fall in numbers less than 48 hours after entry of the sterile flies. In T_2 the wild population did not decline despite the sterile flies, probably because the latter had been irradiated too early in development; the normal yellow spots on the thorax were bleached to white, a phenocopy induced by radiation in the middle stages of adult morphogenesis within the pupa. Laboratory observations had shown that such flies were very short lived and sluggish; in trial T_2 they tended to be easily caught and to remain in the lower foliage; abnormal behavior and appearance probably prevented them from interacting effectively with wild flies. In T_1, T_{3a}, and T_{3b} the released flies seemed normal in color and behavior. Accordingly, T_2 was rejected when the results were considered. Table 1 compares the most reliable estimates of numbers before and after release in the other six trials,