Effect of Persistent Fibroma Virus Infection on Susceptibility of Cells to Other Viruses

BILLIE L. PADGETT AND DUARD L. WALKER

Department of Medical Microbiology, University of Wisconsin, Madison, Wisconsin 53706

Received for publication 15 October 1969

Shope fibroma virus establishes a persistent cytoplasmic infection in primary (RK) and serially cultivated (DRK₃) rabbit kidney cells which is accompanied by a morphological alteration of the cells. The response of such cells to superinfection by other viruses was compared with that of control cells by determining plaque production and virus yield of superinfecting viruses. It was found that the growth of other poxviruses, myxoma and vaccinia, was greatly inhibited in the fibroma virus-infected cells, but that of pseudorabies and herpes simplex viruses, which are unrelated deoxyribonucleic acid viruses, was virtually unaffected. The ribonucleic acid (RNA) viruses, poliovirus 1 and coxsackievirus B1, did not produce plaques on either RK or fibroma virus-infected (F-RK) monolayers. However, the growth of several other RNA viruses, vesicular stomatitis virus, encephalomyocarditis virus, Sindbis virus, and Newcastle disease virus, was enhanced in F-RK cells. None of these latter RNA viruses produced any infectious progeny in DRK₃ cells, but they all plaqued on and produced good yields in DRK₃ cells persistently infected with fibroma virus. This phenomenon is termed facilitation. Facilitation results from the infection of DRK₃ cells by fibroma virus. Neither interference nor facilitation were due to changes in the adsorption or eclipse of the superinfecting virus.

Infection of rabbit kidney cells with Shope fibroma virus under conditions favoring cell multiplication results in a unique virus-cell relationship characterized by a persistent viral infection in the cell cytoplasm, an altered cell morphology, and continued cell multiplication with loss of contact inhibition (6). However, the initial stages of fibroma virus multiplication in these cells are quite similar to those of other poxviruses. Within 3 hr of viral entry, nuclear synthesis of deoxyribonucleic acid (DNA) ceases and cellular division is inhibited (Hinze and Walker, *unpublished data*). These cellular activities remain blocked while viral proteins and DNA are synthesized in the cytoplasm. Viral maturation ensues and the accumulation of infectious virus reaches a peak about 36 hr after infection.

At this point, fibroma virus infection diverges sharply from that of virulent poxviruses, such as vaccinia, and a persistent infection is established. The infected rabbit kidney cells do not die; instead, 36 to 48 hr after infection, nuclear synthesis of DNA commences and the cells later divide at a rate similar to that of uninfected cells. Concomitantly there is an absolute and rather abrupt decline in the amount of infectious fibroma virus in the culture until a level of about 1 plaque-forming unit (PFU)/cell is reached (Tompkins,

Walker, and Hinze, *in press*). This level of infectious virus is maintained as the cells continue to divide. Also, the amount of viral antigen detectable in the cells decreases, but all cells contain antigen.

What causes the rapid decrease in the level of infectious fibroma virus? Why, when the level drops to a low value, is not a new cycle of fibroma virus multiplication initiated? Is the limitation of viral multiplication directed by the viral genome or is it due to some repressive, cellular influence present in persistently infected cells? If it is due to a cellular influence, does this result in a specific or general repression of virus multiplication?

The question of repression of viral multiplication in cells persistently infected with fibroma virus was investigated by comparing the plaquing abilities and growth of various viruses in control and fibroma virus-infected cells. It was found that the multiplication of related DNA viruses is inhibited markedly in cells infected with fibroma virus, but that of unrelated DNA viruses is virtually unaffected. A surprising result was obtained with certain ribonucleic acid (RNA) viruses that normally are unable to multiply in a serially cultivated rabbit kidney cell line. Such viruses were found to yield large amounts of infectious virus in and produce plaques on mono-

layers of cells persistently infected with fibroma virus. This latter phenomenon we have termed facilitation. Preliminary investigations into the mechanisms of interference and facilitation are reported.

MATERIALS AND METHODS

Cells. A cloned line of serially cultivated domestic rabbit kidney cells (DRK₃) was obtained from H. Hinze, University of Wisconsin. Primary rabbit kidney cells (RK) were prepared as previously described (9) and used as secondary cultures. Other cells used were HeLa, Vero, and primary chick embryo cells (CEC). The basic medium for all cells was medium 199 with antibiotics and 10% inactivated calf serum. For the rabbit cells this was supplemented with 0.25% lactalbumin hydrolysate.

Viruses. A cloned line of the Patuxent strain of Shope fibroma virus was used. Fibroma virus stocks were prepared by sonic treatment of infected RK cells in culture medium 3 days after infection. Other viruses used were the Moses strain of myxoma virus, IHD strain of vaccinia virus, HF strain of herpes simplex virus, pseudorabies virus, poliovirus type 1, coxsackievirus B1, encephalomyocarditis (EMC) virus, New Jersey serotype of vesicular stomatitis virus (VSV), Sindbis virus, and Victoria strain of Newcastle disease virus (NDV). Stocks of these viruses were prepared in an appropriate cell line, embryonated eggs (NDV), or mouse brain (coxsackie B1). All virus stocks were stored at -70 C.

Virus assay. All viruses were assayed by the plaque technique with monolayers of an appropriate cell in 1-oz (28 g) glass or 30-ml plastic bottles. Mixtures of myxoma and fibroma viruses were differentiated by assaying on RK or Vero cells. All cells except HeLa received a semisolid nutrient methylcellulose overlay (6). To reveal the plaques, the cell sheets were fixed (6) and stained with crystal violet. HeLa cells received a 0.9% agar overlay containing the same nutrients as other cells supplemented with diethylaminoethyl-dextran (200 µg/ml) and MgCl₂ (40 mM) but only 3% serum. HeLa cell sheets were stained with tetrazolium salt as described for RHF-1 cells (9). Myxoma and fibroma virus assays were incubated at 35 C; those of all other viruses were incubated at 37 C. Viruses were diluted in medium 199 and 3% inactivated calf serum or medium 199 and 3% inactivated horse serum (VSV, herpes simplex, and coxsackie B1).

Adsorption. Unless otherwise stated, all adsorptions were carried out on a rocking platform at 37 C. For plaque assays, EMC virus was adsorbed for 1 hr, myxoma virus for 3 hr, fibroma virus for 4 hr, and all other viruses for 2 hr. For all yield experiments in shell vials, the superinfecting virus was adsorbed for 1 hr.

Monolayers of cells persistently infected with fibroma virus. Preformed monolayers of RK or DRK₃ cells in shell vials (flat-bottomed vials, 21 by 70 mm, each containing a cover glass) or plaque bottles were inoculated with approximately 1 PFU of fibroma virus/cell. After adsorption for 2 hr at 37 C, growth medium was added. Plaque bottles were

incubated at 37 C in a CO₂ incubator set to maintain slightly alkaline conditions. Intact monolayers of morphologically altered cells were formed in 3 to 5 days, at which time over 95% of the cells were infected. If the bottles were not used on the 3rd day, the medium was changed. Shell vials were incubated at 37 C and the medium was changed daily. RK and DRK₃ cells infected with fibroma virus are termed F-RK and F-DRK₃ cells, respectively.

Mycoplasma cultivation. Cells and virus stocks were cultured for mycoplasma on the solid and diphasic media described by Allen et al. (1). Subcultures from diphasic to agar medium were made after 7 and 14 days of incubation. If all cultures were negative, the samples were considered free of mycoplasma.

RESULTS

Plaque production in monolayers of fibroma virus-infected cells. Comparative titrations of representative viruses were performed in normal and fibroma virus-infected primary (RK, F-RK) and serially cultivated (DRK₃, F-DRK₃) rabbit kidney cells. Additionally, reference assays of the RNA viruses were done in CEC or HeLa cells. These comparisons revealed four types of response (Table 1). Two DNA viruses, myxoma and vaccinia, produced plaques in the uninfected cells but were completely inhibited from doing so in cells infected with fibroma virus. Both are poxviruses; myxoma virus, in particular, is closely related to fibroma virus. Two other DNA viruses, herpes simplex and pseudorabies, produced plaques in both normal and fibroma virus-infected rabbit cells. In fact, the presence of fibroma virus in the cells had no noticeable effect on either the number or appearance of plaques produced by these superinfecting viruses. Two RNA viruses, poliovirus 1 and coxsackievirus B1, that have no capacity for adsorbing to normal rabbit cells, formed no plaques in either the normal or fibroma virus-infected cells. Four other RNA viruses, EMC, VSV, Sindbis, and NDV, formed no plaques in normal DRK₃ cells at any concentration tested, and only two, EMC and VSV, produced plaques in normal RK cells. However, all of these RNA viruses, representing four different viral groups, produced plaques at high dilution in the cells infected with fibroma virus. Indeed, their titers in the fibroma virus-infected cells approached those attained in their reference assay cell.

That the plaques appearing on F-DRK₃ monolayers were caused by the superinfecting RNA virus was suggested by the observation that these plaques appeared at the same time as those on the reference assay cell monolayers and were of similar sizes. Virus was recovered from 10 plaques that appeared in F-DRK₃ monolayers 2 days after inoculation of VSV. In all cases, the recovered

TABLE 1. *Comparative titers of representative viruses in rabbit cells persistently infected with fibroma virus and in uninfected cell monolayers*

Virus	Plaque titer (PFU/ml) in cell monolayers					
	Test cells				Reference cells	
	RK	F-RK ^a	DRK ₃	F-DRK ₃ ^b	CEC	HeLa
Myxoma.....	1.2×10^8	$<5 \times 10^1$	1.2×10^8	$<5 \times 10^1$		
Vaccinia.....	2.7×10^8	$<5 \times 10^2$	4.9×10^8	$<5 \times 10^2$		
Herpes simplex.....	3.7×10^6	6.7×10^6	1.1×10^6	2.1×10^6		
Pseudorabies.....	1.5×10^8	4.4×10^7	2.9×10^8	2.2×10^8		
Polio type 1.....	$<5 \times 10^1$	$<5 \times 10^1$				4.2×10^7
Coxsackie B1.....	$<5 \times 10^1$	$<5 \times 10^1$				$>10^8$
Encephalomyocarditis...	1.9×10^4	1.4×10^6	$<5 \times 10^1$	2.3×10^6		2.2×10^6
Vesicular stomatitis.....	7.1×10^7	1.9×10^8	$<5 \times 10^2$	4.2×10^7	5.0×10^8	
Sindbis.....	$<5 \times 10^2$	1.3×10^7	$<5 \times 10^2$	1.9×10^7	1.1×10^8	
Newcastle disease.....	$<5 \times 10^2$	1.9×10^8	$<5 \times 10^2$	6.8×10^6	1.8×10^8	

^a RK cells persistently infected with fibroma virus.^b DRK₃ cells persistently infected with fibroma virus.

virus produced plaques on CEC monolayers, was neutralized by anti-VSV serum, and was not neutralized by anti-fibroma serum.

Viral yields in control and fibroma virus-infected cells. Replicate shell vials containing monolayers of approximately equal numbers of normal or fibroma virus-infected cells were inoculated with various viruses in 0.1 ml of diluent. After adsorption the inoculum was removed, the cells were washed three times, and growth medium was added. One group of vials was frozen at this time, which was set as zero time. Additional vials were incubated at 37 C for various periods of time and then frozen. Later, the vials were frozen and thawed three times, the contents of duplicate vials were pooled, and infectious virus was assayed in an appropriate cell line.

The results (Table 2) generally parallel those of the comparative plaque titrations. The yields of myxoma and vaccinia viruses were much lower in the fibroma virus-infected than in control cells, even though input multiplicities of 10 PFU/cell of these viruses were used. In contrast, the yields of herpes simplex and pseudorabies viruses were unaffected by the concurrent presence of fibroma virus in the cells. VSV, NDV, and Sindbis viruses all multiplied to some extent in RK cells, but greater yields were obtained in F-RK cells. These same viruses apparently were unable to multiply at all in normal DRK₃ cells, as the titers recorded were no greater than those of the zero-time samples; however, they all produced excellent yields in F-DRK₃ cells.

Therefore, after infection of RK cells by

fibroma virus, the ability of other poxviruses to grow in the cell was inhibited, that of unrelated DNA viruses was virtually unaffected, and that of several RNA viruses was enhanced. Furthermore, infection of DRK₃ cells by fibroma virus resulted in some change that permitted the growth of certain RNA viruses which were unable to produce any infectious virus in the normal cell. This latter phenomenon will be termed facilitation.

Relationship of facilitation to prior infection of DRK₃ cells with fibroma virus. Fibroma virus has been passed repeatedly in cell cultures in this laboratory, and stocks have been prepared by sonic treatment of the infected cells in their culture medium. It was conceivable that something from the cultured cells rather than fibroma virus could be responsible for the observed alterations in cell susceptibility. To test this possibility, a suitable dilution of stock fibroma virus was added to an equal volume of diluent and of rabbit anti-fibroma serum. The fibroma virus preparation used to stimulate this antiserum was a homogenate of infected rabbit testes; this line of fibroma virus had been passed exclusively in rabbits. After incubating at 37 C for 30 min, the mixtures were inoculated into DRK₃ cells in vials. The cells were superinfected 10 days later with VSV or Sindbis virus at a calculated input multiplicity of 10 PFU/cell. The yields were harvested 24 hr later and assayed on CEC monolayers.

The yields of VSV in five vials of DRK₃ cells previously inoculated with fibroma virus and

TABLE 2. *Comparative yields of representative viruses in fibroma virus-infected and control cells*

Virus	Input multiplicity of infection	Length of incubation	Virus yield (total PFU) in cell cultures			
			RK	F-RK	DRK ₃	F-DRK ₃
	PFU/Cell	hr				
Myxoma	10	24	7.4×10^6	3.3×10^8	8.3×10^6	6.2×10^4
Vaccinia	10	24	1.8×10^7	2.0×10^6	4.6×10^7	3.4×10^5
Herpes simplex	0.01	72	2.9×10^5	5.2×10^5	1.8×10^6	8.0×10^5
Pseudorabies	0.01	48	4.7×10^7	2.2×10^7	7.1×10^7	2.7×10^7
Vesicular stomatitis	0.1	24	7.6×10^7	2.9×10^8	7.0×10^4	1.6×10^8
Sindbis	0.1	48	2.4×10^6	8.2×10^7	4.8×10^2	2.7×10^7
Newcastle disease	0.1	24	8.8×10^4	3.4×10^6	1.1×10^2	7.3×10^6

diluent ranged from 4.8×10^7 to 8.2×10^7 , and the yields of Sindbis virus in three vials ranged from 4.2×10^6 to 11×10^6 . In contrast, the yields of VSV in three vials of DRK₃ cells previously inoculated with fibroma virus and anti-fibroma serum ranged from 8×10^3 to 20×10^3 , and yields of Sindbis virus in two vials were 4×10^8 and 86×10^8 . These results suggest that the change in susceptibility of the DRK₃ cell is a result of its infection by fibroma virus.

Additionally, all stocks of fibroma virus and lines of DRK₃ cells were tested for mycoplasma. Some virus stocks and cell sublines did contain mycoplasma; however, contaminated lines of DRK₃ cells were unable to support the growth of RNA viruses. The results shown in Table 1 were obtained with cells and viruses free of mycoplasma.

Further studies on interference and facilitation. The possibility that the observed changes in cellular susceptibilities were due to alterations in viral adsorption or eclipse was studied by comparing DRK₃ and F-DRK₃ cells with respect to these activities. Myxoma virus, NDV, and VSV were used, the former representing inhibited poxviruses and the latter two facilitated RNA viruses.

Replicate shell vials containing approximately equal numbers of DRK₃ or F-DRK₃ cells were inoculated with 0.1 ml of myxoma virus (10^6 PFU), NDV (2×10^4 PFU), or VSV (2×10^4 PFU). After adsorption at 4 C for a period of 2 hr for NDV and VSV and 4 hr for myxoma virus, the inocula were removed. Cell sheets were washed three times, and 2 ml of growth medium was added to each vial. Two vials from each group were harvested at this time. They were frozen and thawed three times, and the contents were pooled and later assayed to establish the amount of virus adsorbed to the cells. The rest of the vials were incubated at 37 C, and at intervals two vials from each group were harvested. The fluids were removed, pooled, and assayed to measure eluted

virus. The cells, in 2 ml of fresh medium, were frozen and thawed three times; they were then pooled and assayed to measure uneclipsed virus.

It is evident (Table 3) that myxoma virus did adsorb to F-DRK₃ cells and did so to the same extent as to DRK₃ cells. Also, myxoma virus apparently eclipses in the F-DRK₃ cells. After 1 hr at 37 C, the amounts of virus detectable on DRK₃ and F-DRK₃ cells had fallen to 25 and 31 %, respectively, of the zero-time levels. After 2 hr these values were 10 and 12 %. The virus levels in the supernatant fluids indicated that viral elution did not account for the difference between PFU on the cells at 0 and 120 min.

Disappointingly small amounts of NDV and VSV adsorbed to the cells at 4 C, but the results suggest that these viruses adsorbed to DRK₃ cells about as well as to F-DRK₃ cells. Eclipse of these viruses, however, was more rapid than with myxoma virus, and it appeared to proceed at similar rates in DRK₃ and F-DRK₃ cells. Growth of NDV and VSV in F-DRK₃ cells was to be expected. The increase in PFU of VSV at 180 min represents the first progeny of the VSV growth cycle. As with myxoma virus, viral elution did not account for the difference between the PFU on the cells at 0 and 30 min.

Because of the limited adsorption of RNA viruses at 4 C in the previous experiment and the importance of establishing the role of adsorption in the facilitation phenomenon, adsorption of RNA viruses to CEC, DRK₃, and F-DRK₃ cells was compared in a different way. Adsorption was determined indirectly by measuring the virus that remained unadsorbed after a period of contact with the test cells.

Three plaque bottles each of CEC, DRK₃, and F-DRK₃ cells were inoculated with a suitable quantity of NDV or Sindbis virus in 0.2 ml of diluent. Three empty, wetted plaque bottles were inoculated also to allow measurement of heat inactivation and nonspecific adsorption during

the experiment. After adsorption for 2 hr at 37 C, the inoculum from each bottle was transferred to a plaque bottle of CEC to measure virus unadsorbed by the initial cell monolayer. The first set of cell monolayers was covered with methylcel overlay to determine the number of plaques that would develop.

The results are recorded in Table 4. The differences between the numbers of PFU recovered from the empty bottles and those recovered from the cell monolayers are presumably due to specific adsorption. These differences were large with NDV. Almost all of the available virus adsorbed to the cell monolayers during the time allowed. It is evident that NDV did adsorb to the insusceptible (nonpermissive) DRK₃ cells, and it did so to almost the same extent as to the susceptible F-DRK₃ cells. With Sindbis virus the differences were only twofold, but Sindbis virus adsorbed equally well to DRK₃ and F-DRK₃ cells. As would be expected, no plaques appeared on the DRK₃ monolayers. However, these results indicate that facilitation is not due to a change in the adsorptive abilities of the DRK₃ cells.

DISCUSSION

The present studies were undertaken to determine whether there is a general repression of viral

multiplication in cells persistently infected with fibroma virus. The results presented in Tables 1 and 2 lead to the conclusion that there is not. There is a definite interference with the growth of myxoma and vaccinia viruses, viruses related to fibroma, but this interference does not extend to any other virus tested. Since the observed interference is quite selective, it cannot be attributed to any general factor, such as interferon. This example of interference therefore adds to the list of noninterferon-mediated or intrinsic interference systems (3, 7, 8).

In these studies, the interference was manifested by persistently infected cells; that is, cells in which little active multiplication of fibroma virus is occurring, and it was such as to withstand a high input multiplicity of the superinfecting virus. The interference seems not to be due to alterations in adsorption or eclipse of the poxviruses. This conclusion is based presently on equating loss of infectivity with eclipse, but we recognize that in fact these may not be the same thing. Even in persistently infected cells, the interference could be due to some product of or a competition with fibroma virus multiplication. However, another possibility is that the cell somehow represses fibroma virus multiplication during the persistent infection and that this repression affects related

TABLE 3. Adsorption and eclipse of viruses in DRK₃ and F-DRK₃ monolayers

Time, at 37 C ^a	Total PFU of virus on cell monolayer					
	Myxoma virus		NDV		VSV	
	DRK ₃	F-DRK ₃	DRK ₃	F-DRK ₃	DRK ₃	F-DRK ₃
<i>min</i>						
0	1.1×10^5	2.3×10^5	324	772	232	476
30			16	52	64	92
60	2.7×10^4	7.2×10^4				
90			8	28	12	28
120	1.1×10^4	2.7×10^4				
180	1.5×10^4	4.3×10^3	7	12	1	220

^a Myxoma virus adsorbed for 4 hr at 4 C; NDV and VSV adsorbed for 2 hr at 4 C.

TABLE 4. Adsorption of NDV and Sindbis viruses to CEC, DRK₃, and F-DRK₃ cell monolayers

Cell monolayer	Newcastle disease virus		Sindbis virus	
	Plaques on monolayer	PFU in supernatant fraction ^a	Plaques on monolayer	PFU in supernatant fraction ^a
CEC	295, ^b 305, 309	68, ^b 39, 41	85, 58, 43	25, 40, 31
DRK ₃	0, 0, 0	25, 20, 23	0, 0, 0	40, 25
F-DRK ₃	8, 13, 5	6, 12, 12	15, 12, 8	43, 39, 23
Empty bottle		187, 182, 153		49, 62, 68

^a PFU in supernatant fractions were determined by assay on CEC monolayers.

^b The number of plaques developing on the monolayer and the PFU in the corresponding supernatant fraction are listed in order.

viruses. Pseudorabies and herpes simplex viruses may escape this inhibition because replication of their DNA and viral assembly occur in the cell nucleus.

A surprising finding was the facilitation of several RNA viruses in fibroma virus-infected cells. Production of infectious progeny by these viruses in DRK₃ cells was shown to be dependent on the concurrent infection of the cells by fibroma virus. The mechanism of facilitation was not established. As with the interference, it apparently affects a stage in the multiplication of the viruses subsequent to eclipse. All of the facilitated viruses were RNA viruses, but it has not been determined if facilitation is limited to this group. The DNA viruses used, other than poxviruses, were unaffected but were ones that multiply extensively in normal rabbit cells. The RNA viruses not facilitated were ones that do not adsorb to rabbit cells. It would be interesting to try other DNA viruses and an RNA virus, such as influenza A, whose multiplication involves the cell nucleus.

Although the results obtained with RNA viruses were unexpected, a search of the literature revealed that a similar phenomenon has been observed in rabbits infected with Shope fibroma virus. Ginder and Friedewald (5) reported that Semliki Forest virus would multiply in and cause necrosis of fibromas on domestic rabbits and the virus would grow in cultures of minced fibromas but not in normal adult rabbit tissues.

Many examples of dual infection resulting in an increased yield of one virus are known (reviewed in 2, 4), but this particular example is unusual in that growth of RNA viruses is facilitated by a DNA virus. Other examples described have generally involved pairs of DNA viruses or pairs of RNA viruses. There is as yet no standard terminology for this type of phenomenon. *Complementation* has been used as a general term (4), but it has a more restricted meaning to geneticists. *Helper virus systems* is somewhat awkward and has been associated particularly with systems in which the rescued virus has the

protein coat of the helper virus. *Enhancement* implies that some infectious virus is produced in the control system which is increased in the experimental system. Although enhancement, as so limited, describes the effect of concurrent fibroma virus infection on the yield of RNA viruses in RK cells, it does not reflect accurately the results in DRK₃ cells where the RNA viruses produce no progeny alone. Therefore, we offer the term *facilitation* as a general term to cover all situations in which dual infection results in increased yields of one or both viruses. As the mechanism(s) of facilitation are established, it may be possible to assign more specific terms to the various systems.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grants CA-05682 from the National Cancer Institute and AI-06334 from the National Institute of Allergy and Infectious Diseases.

We thank Eva Hann for her skilled technical assistance.

LITERATURE CITED

1. Allen, V., S. Sueltmann, and C. Lawson. 1967. Laboratory diagnosis of *Mycoplasma pneumoniae* in a public health laboratory. *Health Lab. Sci.* 4:90-95.
2. Cantell, K. 1968. Enhancement of animal virus growth by another virus, p. 31-44. *In* M. Sanders and E. H. Lennette (ed.), *Medical and applied virology*. Warren H. Green, Inc., St. Louis.
3. Cords, C. E., and J. J. Holland. 1964. Interference between enteroviruses and conditions effecting its reversal. *Virology* 22:226-234.
4. Fenner, F. 1968. *The biology of animal viruses*, vol. 1. Molecular and cellular biology. Academic Press Inc., New York.
5. Ginder, D., and W. Friedewald. 1951. Effect of Semliki Forest virus on rabbit fibroma. *Proc. Soc. Exp. Biol. Med.* 77:272-276.
6. Hinze, H. C., and D. L. Walker. 1964. Response of cultured rabbit cells to infection with the Shope fibroma virus. I. Proliferation and morphological alteration of the infected cells. *J. Bacteriol.* 88:1185-1194.
7. Ledinko, N. 1963. An analysis of interference between active polioviruses types 1 and 2 in HeLa cells. *Virology* 20:29-44.
8. Marcus, P. I., and D. H. Carver. 1967. Intrinsic interference: a new type of viral interference. *J. Virol.* 1:334-343.
9. Padgett, B. L., M. S. Moore, and D. L. Walker. 1962. Plaque assays for myxoma and fibroma viruses and differentiation of the viruses by plaque form. *Virology* 17:462-469.