Proc. Nat. Acad. Sci. USA
Vol. 71, No. 8, pp. 2956–2960, August 1974

Persistent Noncytocidal Vesicular Stomatitis Virus Infections Mediated by Defective T Particles that Suppress Virion Transcriptase

(virus transcription/rabies)

JOHN J. HOLLAND AND LOUIS PEREZ VILLARREAL

Department of Biology, University of California, San Diego, La Jolla, Calif. 92037

Communicated by Frank J. Dixon, May 2, 1974

ABSTRACT **Infectious B virions of vesicular stomatitis virus were 100% lethal to BHK_{21} (baby hamster kidney) cells when infecting alone, and persistent noncytocidal infection could not be achieved with cloned B virions alone. However, a mixture of B virions and homologous, short, defective, interfering particles (T particles) of a temperature-sensitive mutant of the virus regularly established persistently infected, noncytocidal carrier cultures. A long T particle was generated during establishment of the carrier culture; we show that this long T particle can establish and maintain persistent noncytocidal infection even when it infects cells along with virulent wild-type B virions. This long T particle causes the production of wild-type B virions with greatly reduced virion transcriptase (EC 2.7.7.6; RNA nucleotidyltransferase) levels when co-infecting the same cells, so it appears to prevent cytopathology by regulating virus transcription. The implications of these findings for rabies and other slowly progressing noncytocidal infections are discussed.**

In 1966 Rustigian (1, 2) demonstrated that measles virus can readily establish persistent noncytocidal infections of cells in culture. Measles virus may be a latent causative agent in slow progressive neurologic diseases such as subacute sclerosing panencephalitis (3) and multiple sclerosis (4). The causes of viral latency and persistent infection in these diseases are unknown, but virus temperature sensitivity may be important (5, 6). Antibody may predispose to measles latency in carrier cultures (2), but almost nothing is known about molecular mechanisms of slow persistent infection by normally virulent viruses (either *in vitro* or *in vivo*).

Huang and Baltimore (7) were the first to speculate that defective interfering particles might play a role in the slow virus diseases *in vivo*, and we have recently reported that defective interfering particles (T particles) of vesicular stomatitis virus (VSV) can, in fact, alter viral pathogenesis in mice (8). Although rabies virus is a markedly slow Rhabdovirus that regularly produces noncytopathic carrier states in culture (9), VSV is very virulent and rapidly cytocidal under most conditions. In 1963 Wagner *et al.* (10) showed that a small-plaque mutant of VSV could establish a persistent slowly cytopathic carrier infection of L cells in culture. The carrier cells underwent recurrent crises of extensive cytopathology with alternating periods of recovery and growth. The mechanism of carrier establishment was not explored, but Wagner *et al.* suggested a possible role for interferon. Mudd *et al.* (11) recently observed that wild-type VSV Indiana regularly establishes persistent noncytocidal infection of Drosophila cells in culture, but again the mechanism remains to be elucidated.

Abbreviations: VSV, vesicular stomatitis virus; T particles, defective interfering particles of VSV; B virions, infectious, full-sized virus particles of VSV.

MATERIALS AND METHODS

Cells and Virus. BHK_{21} hamster kidney cells were used for virus growth and assay. Cells were grown in Eagle's minimum essential medium plus 7% (v/v) calf serum. Wherever wild-type VSV strain Indiana is referred to without further identification as to source, it refers to a highly virulent strain that produces 5 mm or greater plaques in 24 hr. It was originally obtained from Dr. John Mudd (12), and we cloned it six consecutive times before use in the present study. The New Jersey serotype VSV was obtained from Dr. F. Schaffer originally. Table 1 lists the sources of all other VSV strains used. All infectious, full-sized virus particles (B virions) were freed of T particles by at least two consecutive clonings. T particles were purified as described (8) to free them of B virions. The carrier, long T particle arose spontaneously in carrier cultures, as described in *Results*, and all other T particles were derived by serial undiluted passage of cloned homologous B virions.

Transcriptase Assays. Assays of virion RNA polymerase (EC 2.7.7.6; RNA nucleotidyltransferase) (13) were done as described (14). In the present study, the labeled nucleoside triphosphate was [^{3}H]uridine triphosphate (Schwarz Bio-Research; 14 Ci/mmol) used in the presence of added unlabeled UTP.

RESULTS

Requirement for Defective Particles to Establish Persistently Infected Carrier Cultures. In order to study the factors involved in persistent VSV infection, we used various wild-type and mutant strains of VSV Indiana to infect BHK_{21} cells in culture. After infection for 24 hr at 37° with wild-type virus or for 48 hr at 33° with temperature-sensitive (*ts*) mutants, dead and dying nonattached cells were discarded, fresh medium was added, and remaining attached cells were incubated at 37° to allow survivors to grow. Table 1 shows that all wild-type VSV strains and all but one *ts* mutant strain completely destroyed all cells regardless of the presence or absence of defective T particles. In only one situation were surviving cells obtained after infection of 2×10^6 BHK_{21} cells at high multiplicity. As is shown in Table 1, double infection with a cloned stock of B virions of Pringle's mutant *ts*31 (16) and the

short T particles that it generates (17) regularly produced slow cytopathology with many surviving cells from which permanent carrier cultures were established. This experiment has been repeated four times, and a persistently infected carrier cell line has been produced each time. The presence of defective particles was essential since cloned *ts*31 B virions alone caused rapid cell destruction with no survivors. All of the studies reported below were done on the fourth persistently infected carrier culture cell line established in this laboratory. We designate this carrier cell line BHK_{21} VSV_{IND} CAR_4. It has been growing continuously in culture for 150 days. During the first months after establishment of this carrier cell line, the cells exhibited severe cytopathology and underwent frequent crises from which a few surviving cells reestablished the culture each time. This is similar to the observations of Wagner *et al.* (10) with an L cell carrier line. However, by the 90th day in culture the carrier cells had stabilized and no longer exhibited severe cytopathology or massive crises of cell death. Since that time the carrier cells have multiplied much like normal BHK_{21} cells, with a doubling time of 20–30 hr. Unlike the L cell carrier culture described by Wagner *et al.* (10), they can be dispersed by trypsin treatment without severe damage and they form confluent monolayers. However, nearly all carrier cells exhibit cytoplasmic inclusions, and multinucleated syncitial masses can be observed in all cultures.

Continuous Virus Production by Carrier Cultures. Table 2 shows that carrier cells have continuously shed virus into the culture medium at low levels during the entire period since infection. Even though cytopathology and cell death have been minimal since the 90th day in culture, an average of several plaque-forming units per cell are produced each day during incubation at 37°. However, shifting the temperature down to 33° causes severe cytopathology within 48 hr and induces the production of up to 100 plaque-forming units per cell. Likewise, cultivation of carrier cells at 37° with equal numbers of normal uninfected BHK_{21} cells leads to extensive cytopathology and increased virus production.

Production of a New Long T Particle in Persistently Infected Carrier Cultures. When we purified virus from the medium from 10^8 cells in carrier cultures maintained at 37°, no virus band could be seen on sucrose gradients. However, cultivation of 10^8 carrier cells with 10^8 normal BHK_{21} cells at 37° led to the production of large amounts of VSV virions. Surprisingly, most of the yield consisted of a new kind of long T particle. The original short T particle used to establish the carrier culture was absent or was present in barely detectable amounts. Fig. 1 shows a sucrose gradient containing a mixture of B virions and long T particles recovered from carrier cultures plus a smaller number of original short T particles added to show their much smaller size (and slower sedimentation rate). Whereas the original short T particle has a sedimentation rate $s_{20,w} = 150$ (17), the long T particle that arises during establishment of the carrier culture has a sedimentation rate of about $s_{20,w} = 470$.

Ability of Long T Particles From Carrier Cultures to Establish New Persistent Carrier Infections in the Presence of Carrier B Virions or Wild-Type B Virions. Since the newly generated, long T particle has always been associated with carrier cells throughout the period from 20 to 150 days after establishment of persistent infection, we performed experiments to determine

TABLE 1. *Attempts to establish persistent infection of BHK_{21} cells with different strains and mutants of VSV Indiana serotype*

Virus strains	Results
Infection with cloned B virions alone	
Wild type[a]	Complete cytopathology; no surviving cells.
Wild type[b]	"
Wild type[c]	"
Wild type of Pringle[d]	"
Wild type[e]	"
*ts*11 Mutant of Pringle[d]	"
*ts*22 Mutant of Pringle[d]	"
*ts*31 Mutant of Pringle[d]	"
*ts*41 Mutant of Pringle[d]	"
HR mutant[c]	"
Infection with mixture of cloned B virions and homologous T particles	
Wild type[a]	Slow cytopathology; no surviving cells.
Wild type[b]	"
Wild type[c]	"
Wild type of Pringle[d]	"
Wild type[e]	"
*ts*11 Mutant of Pringle[d]	"
*ts*22 Mutant of Pringle[d]	"
*ts*31 Mutant of Pringle[d]	Surviving cells regularly established carrier state.
*ts*41 Mutant of Pringle[d]	Slow cytopathology; no surviving cells.
HR mutant[c]	"

In all cases 2×10^6 cells were infected at a multiplicity of 100. Purified T particles were used at a multiplicity of about 800–1500 (where indicated). All experiments were done in triplicate with identical results. *ts* Mutants were allowed to infect cells for 48 hr at 33° before we attempted to grow surviving cells at 37.5°.

[a] From F. Schaffer; [b] from A. Huang; [c] from A. Howatson; [d] from M. E. Reichmann; [e] from J. Mudd.

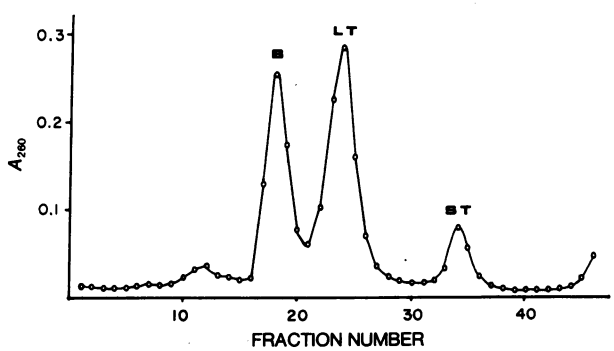

FIG. 1. Sucrose gradient comparison of a new long T particle from carrier cultures, with B virions, and with the original short T particle used to establish persistent infection. Purified short T particles (*ST*) were added to a mixture of B virions (*B*) and long T particles (*LT*) derived from VSV carrier cultures by cultivation with normal BHK_{21} cells, and layered on top of 5–40% sucrose gradients (8). The gradients were centrifuged for 60 min at 34,000 rpm in a Spinco SW41 rotor. Fractions were collected from the bottom of the tube, and the A_{260} was determined on each fraction.

TABLE 2. *Continuous production of infectious virus by VSV carrier culture of BHK_{21} cells*

Total no. of days in culture*	Degree of cytopathology in carrier cells†	PFU/ml of culture medium
13	3+	7×10^5
24	2+	4×10^4
40	1+	3×10^4
57	2+	2×10^3
73	2+	5×10^4
83	2+	2×10^5
95	—	1×10^6
105	—	9×10^5
124	—	1×10^6
139	—	8×10^5

PFU, plaque-forming units; —, no cytopathology.

* On the indicated day after establishment of the persistently infected carrier culture, the culture medium was sampled. Plaque assays were performed on monolayers of normal BHK_{21} cells at 33°.

† During the first 3 months after establishment of the carrier culture, the persistently infected cells underwent frequent crises of severe cytopathology, followed by periods of recovery and growth. After 90 days, the carrier cells commenced continuous, uninterrupted growth without periodic crises.

whether it is the carrier B virion or the carrier long T particle that is responsible for maintenance of the persistent slow carrier state of infection. After 105 days of persistent infection, B virions were isolated from the carrier culture and cloned twice to free them from T particles. Likewise, carrier long T particles were produced in large quantity by cocultivation of carrier cells and normal BHK_{21} cells, and the long T particles were purified by repeated sucrose gradient centrifugation (18, 8) to free them of B virions. These carrier B virions and long T particles were then tested separately and in combinations to determine the requirements for reestablishment of persistent carrier infection after inoculation onto monolayers of normal BHK_{21} cells. Table 3 shows that carrier B virions freed of T particles cause total cell destruction and produce large yields of infectious virus; furthermore, they are unable to establish persistent carrier infection of BHK_{21} cells when they infect alone. However, in the presence of purified long T particles, the same B virions produce much lower yields of virus, cause greatly reduced cytopathology, and readily establish persistent carrier infections in BHK_{21} cells. Obviously the carrier B virion by itself is not responsible for the persistent slow noncytopathic infections in the carrier state, but it can readily reestablish persistent noncytocidal infection in the presence of the carrier long T particle. In fact, the long T particle seems to be the major prerequisite for establishment of the carrier state since it facilitates persistent infection even when it infects with virulent wild-type B virions (Table 3). Purified long T particles alone cannot establish persistent infection (Table 3) because they are unable to replicate without B virions.

TABLE 3. *Infection of BHK_{21} cells with B virions and T particles isolated from carrier cultures after 105 days of persistent infection*

Inoculum	Yield (PFU/cell)	New carrier state established?
Yield from induced carrier culture (mixture of B virions and T particles)	65	Yes
Cloned B virions from induced carrier culture (no T particles)	8,700	No
Cloned B virions plus purified long T particles (both from carrier culture)	90	Yes
Purified carrier culture long T particles alone (no B virions)	0	No
Cloned wild-type VSV B virions (no T particles)	18,000	No
Cloned wild-type VSV B virions plus purified long T particles from carrier culture	240	Yes*

B virions were used at multiplicity of infection of 200 in all cases; T particles were used at multiplicity of infection of about 1000.

* Between 20 and 140 days after establishment of this carrier culture with long T particles plus wild-type VSV B virions, the persistently infected cells were producing B virions that are not temperature-sensitive. Therefore, temperature sensitivity is not a requirement for persistent noncytocidal infection to be established and maintained. However, these persistently infected cells grow more slowly than the original carrier culture, and occasionally undergo crises of cytopathology.

Homologous Superinfection Immunity of Carrier Cultures and Lack of Interferon Production. Table 4 shows that at 130 days of persistent infection the carrier cells show strong homologous autointerference. The yield of superinfecting Indiana strain B virions is reduced over 99%, as compared to normal BHK_{21} cells. This finding indicates that all cells (or greater than 99%) are carrying interfering T particles, since a very high challenge virus input was used (multiplicity of infection of 1000 plaque-forming units per cell) to assure synchronous infection of all cells. Note that New Jersey challenge virus was suppressed only about 90%. This is typical of T particle interference with a heterologous VSV serotype (19). Note also that no detectable interferon was present in the medium from any carrier cultures, and that carrier cultures were completely susceptible to superinfection with unrelated RNA viruses such as influenza virus and mengovirus. We conclude that the persistent carrier state involves rather specific homologous autointerference mediated by long T particles and not by interferon or other nonspecific mechanisms.

Evidence that All or Nearly All Persistently Infected Carrier Cells are Infectious Centers Containing both B Virions and Long T Particles. The homologous (Indiana) interference data of Table 4 strongly suggest that over 99% of carrier cells contain T particles. Therefore, we dispersed cells from carrier cultures with trypsin, counted them, and plated various dilutions on monolayers of normal BHK_{21} cells to determine the percentage of carrier cells that are infectious centers (i.e., that produce or contain infectious B virions). In three separate experiments it was found that all or nearly all thoroughly washed cells from carrier cultures register as infectious centers (Table 5). Furthermore, when plaques were picked at terminal dilution of infectious center assays and virus from each of five different infectious center plaques was analyzed on sucrose gradients (after one passage in 10^7 BHK_{21} cells), a mixture of B virions and long T particles was observed in each case. This is evidence that each infectious center was a cell producing both B

TABLE 4. *Virus susceptibility of VSV_{IND} carrier BHK_{21} cultures and control BHK_{21} cells*

Challenge virus*	Cell type	Interferon titer†	Yield (PFU/cell)	% Interference
VSV Indiana	Control BHK_{21}	0	20,000	—
VSV Indiana	Carrier BHK_{21}	0	30	99
VSV New Jersey	Control BHK_{21}	0	3,000	—
VSV New Jersey	Carrier BHK_{21}	0	200	90
NWS influenza virus	Control BHK_{21}	0	200	—
NWS influenza virus	Carrier BHK_{21}	0	150	None
Mengovirus	Control BHK_{21}	0	500	—
Mengovirus	Carrier BHK_{21}	0	1000	None

* Monolayers of 2×10^6 control BHK_{21} cells or VSV Indiana carrier cells (at 130 days of persistent infection) were infected at multiplicity of infection of 1000 with VSV Indiana or VSV New Jersey and at multiplicity of infection of 20 with influenza virus or mengovirus.

† Interferon assays were done on supernatant medium from each culture after treatment at pH 2.5 to destroy virus infectivity.

virions and T particles. We conclude that all or nearly all cells in the carrier culture are doubly infected with B virions and T particles.

Depressed Virion Transcriptase Levels in Purified B Virions from Cells Coinfected with Long T particles from Carrier Cells. It will be shown elsewhere (Villarreal and Holland, *J. Virol.*, in press) that carrier cells exhibit extremely low levels of virus transcriptase activity in comparison with cells productively infected with B virions. Surprisingly, we have also observed that B virions purified from carrier cells show greatly reduced levels of virion transcriptase. Since the data above indicate that the reduced virulence of B virions in persistently infected carrier cells is mediated by long T particles, we examined the possibility that carrier long T particles are able to suppress the virion transcriptase activity of any type of VSV_{IND} B virions produced in cells coinfected by these long T particles. Fig. 2 shows that they do, in fact, strongly suppress the specific activity of the virion transcriptase of purified B virions produced even by wild-type VSV Indiana coinfecting the same cells. We conclude that these carrier T particles are in some manner exerting regulatory control over virus transcription and thereby promoting persistent, noncytocidal carrier infection by B virions that are otherwise rapidly lethal.

Rabies Virus B Virions Lack any Detectable Transcriptase Activity In Vitro. Sokol *et al.* (20) recently reported that infectious B virions of rabies virus lack the virion-associated transcriptase activity that is associated with other members of the Rhabdovirus group, and that should, in theory, be associated with any negative-strand RNA virus (13). The bottom curve of Fig. 2 shows a confirmation of this. We have repeated this observation several times. Nevertheless, our rabies B virions carry out primary transcription in cells treated with cyclohexamide and actinomycin D, and they induce a sedimentable cytoplasmic RNA polymerase activity that can be assayed *in vitro* after isolation from cells late in infection (Villarreal and Holland, *J. Virol.*, in press).

TABLE 5. *Infectious center assays on BHK_{21} carrier cells, and association of long T particles with each infectious center*

No. of carrier cells assayed	No. of infectious centers (plaques on normal BHK_{21} monolayers)	Proportion of infectious center plaques yielding long T particles (along with B virions)*
8×10^6	1×10^7	3/3
5×10^7	4×10^7	5/5
1.7×10^7	1.6×10^7	4/4

Persistently infected carrier cells were treated 40 min at 37° with heat-inactivated rabbit antiserum against VSV to inactivate cell surface virus. The cells were then washed thoroughly, dispersed with trypsin, suspended in minimal essential medium, counted in a hemacytometer, and diluted in minimal essential medium. Appropriate dilutions of these carrier cells were carefully layered on top of triplicate monolayers of normal BHK_{21} cells under soft agarose, incubated at 33° for 48 hr for plaque development.

* In each of the three experiments, well-isolated plaques generated by each infectious center were picked. The virus from each was amplified by 1 passage on bottles of BHK_{21} cells, purified, and analyzed on 5–40% sucrose gradients for the presence of long T particles.

Evidence for the existence of rabies T particles has emerged from several laboratories (9, 21–23), but they have only recently begun to be isolated and characterized as for VSV (24). Rabies virus generates various T particles as New Jersey serotype VSV does (25, 26), and even the broad B virion band of rabies (9) may include long T particles, as the original work of Sokol *et al.* (9) suggests. All of the above suggests the possibility that rabies T particles may be partly responsible for the persistent noncytocidal character of rabies virus infection *in vitro* and for the slow progression of rabies pathology *in vivo*.

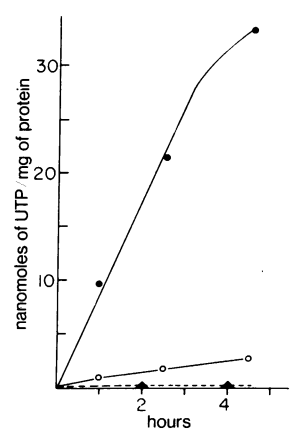

FIG. 2. Comparison of rates of transcription *in vitro* by purified B virions derived from cells infected with: wild-type VSV B virions alone (●); with wild-type VSV B virions plus 500 carrier long T particles per cell (○); with HEP rabies virions (▲). Cells were infected with a multiplicity of about 100 plaque-forming units/ml of B virions. The B virion yields from each infected cell group were purified 3-fold on sucrose gradients (8) and used in transcription assays *in vitro* (37) as described in *Materials and Methods*, using a final concentration of 100 mg/ml of B virion protein.

DISCUSSION

These results show a total dependence upon T particles for persistent injection of the highly susceptible BHK_{21} cell line. In less sensitive cell types T particles may or may not prove to be necessary for persistence (see ref. 11).

The striking similarities observed between noncytocidal VSV carrier infections of BHK_{21} cells and rabies virus noncytocidal infections in BHK_{21} cells suggests that a common mechanism may be involved. Rabies virus readily establishes slow, persistent, noncytocidal infections *in vitro*, and can be very slowly progressing *in vivo*. LCM virus also establishes slow persistent noncytocidal infections *in vivo* and *in vitro* (31–33). Although *in vivo* the host immune response plays a critical role in determining the outcome of noncytocidal infection, there is strong biological evidence that defective interfering virus is involved in LCM virus persistence (33–34). Neither the infectious LCM virus nor its defective particles has been purified, however.

It has recently been observed (35) that preparations of measles virions may contain small RNA molecules that could be defective particle RNA (in addition to larger 50 S virion RNA). Also, subviral-sized fragments of nucleocapsid have been described in infected cells after high multiplicity passage of cloned measles virions (36). These findings, implicating the presence of defective particles in measles virus, and our results, showing the involvement of defective T particles in persistent noncytocidal infection, suggest that experiments testing the role of defective particles in subacute sclerosing panencephalitis and other persistent virus infections should be attempted.

The precise molecular basis for defective particle autointerference and its relationship to persistence is unknown. We have no explanation for the reduced (or nonexistent) virus transcriptase levels in cells and virions from the VSV carrier culture and rabies-infected cells. The best available evidence suggests that T particles of VSV do not express their genetic information and that they interfere solely at the replicative level (17, 26–30) so it is not obvious how they might depress B virion transcriptase, although competition for limited numbers of polymerase molecules is one possibility.

The observations regarding normally slow, persistent virus like LCM, taken together with the present report and with findings *in vivo* (8) concerning the normally highly virulent VSV system, provide convincing preliminary support for the suggestion of Huang and Baltimore (7) that defective particles may play a major part in slow, persistent virus disease.

We thank Estelle Bussey for excellent technical assistance. This research was supported by Research Grant CA 10802 from the National Cancer Institute.

1. Rustigian, R. (1966) *J. Bacteriol.* **92**, 1792–1804.
2. Rustigian, R. (1966) *J. Bacteriol.* **92**, 1805–1811.
3. Horta-Barbosa, L., Fuccillo, D. A., Sever, J. L. & Zeman, W. (1969) *Nature* **221**, 974–976.
4. Henson, T. E., Brody, J. A., Sever, J. L., Dyken, M. S. & Cannon, J. (1970) *J. Amer. Med. Ass.* **211**, 1985–1988.
5. Preble, O. T. & Youngner, J. S. (1973) *J. Virol.* **12**, 481–491.
6. Haspel, M. V., Knight, P. R., Duff, R. G. & Rapp, F. (1973) *J. Virol.* **12**, 690–695.
7. Huang, A. & Baltimore, D. (1970) *Nature* **226**, 325–327.
8. Doyle, M. & Holland, J. J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2105–2108.
9. Sokol, F., Kuwert, E., Wiktor, T. J., Hummeler, K. & Koprowski, H. (1968) *J. Virol.* **2**, 836–849.
10. Wagner, R. R., Levy, A., Snyder, R., Ratcliff, G. & Hyatt, D. (1963) *J. Immunol.* **91**, 112–122.
11. Mudd, J. A., Leavitt, R. W., Kingsbury, D. T. & Holland, J. J. (1973) *J. Gen. Virol.* **20**, 341–351.
12. Mudd, J. A. & Summers, D. F. (1970) *Virology* **42**, 328–340.
13. Baltimore, D., Huang, A. S. & Stampfer, M. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 572–576.
14. Reichmann, M. E., Villarreal, L. P., Kohne, D., Lesnaw, J. & Holland, J. J. (1974) *Virology* **58**, 240–249.
15. Staynov, D. Z., Pinder, I. C. & Gratzer, W. B. (1972) *Nature New Biol.* **235**, 197–199.
16. Pringle, C. R. (1970) *J. Virol.* **5**, 559–567.
17. Reichmann, M. E., Pringle, C. R. & Follett, E. A. C. (1971) *J. Virol.* **8**, 154–160.
18. Huang, A. S., Greenawalt, J. W. & Wagner, R. R. (1966) *Virology* **30**, 161–172.
19. Prevec, L. & Kang, C. Y. (1970) *Nature* **228**, 25–27.
20. Sokol, F. & Clark, H. F. (1973) *Virology* **52**, 246–263.
21. Hummeler, K., Koprowski, H. & Wiktor, T. J. (1967) *J. Virol.* **1**, 152–170.
22. Wiktor, T. J., Fernandes, M. V. & Koprowski, H. (1964) *J. Immunol.* **93**, 353–366.
23. Yoshino, K., Taniguchi, S. & Arai, K. (1966) *Proc. Soc. Exp. Biol. Med.* **123**, 387–392.
24. Crick, J. & Brown, F. (1974) *J. Gen. Virol.* **22**, 147–151.
25. Perrault, J. & Holland, J. J. (1972) *Virology* **50**, 148–158.
26. Perrault, J. & Holland, J. J. (1972) *Virology* **50**, 159–170.
27. Huang, A. S. & Manders, E. (1972) *J. Virol.* **9**, 909–916.
28. Emerson, S. & Wagner, R. R. (1972) *J. Virol.* **10**, 297–309.
29. Roy, P., Repik, P., Hefti, E. & Bishop, D. H. L. (1973) *J. Virol.* **11**, 915–925.
30. Mori, H. & Howatson, A. F. (1973) *Intervirology* **1**, 168–175.
31. Hotchin, J. (1962) *Cold Spring Harbor Symp. Quant. Biol.* **27**, 479–499.
32. Oldstone, M. B. A. & Dixon, F. J. (1970) *J. Exp. Med.* **131**, 1–19.
33. Lehmann-Grubbe, F. (1971) *Virol. Monogr.* **10**, 1–173.
34. Welsh, R. M., O'Connell, C. M. & Pfau, C. J. (1972) *J. Gen. Virol.* **11**, 926–932.
35. Winston, S. H., Rustigian, R. & Bratt, M. A. (1973) *J. Virol.* **13**, 721–728.
36. Kiley, M. P., Gray, R. H. & Payne, F. E. (1974) *J. Virol.* **13**, 721–728.
37. Villarreal, L. P. & Holland, J. J. (1973) *Nature New Biol.* **246**, 17–19.