INFECTION AND IMMUNITY, Oct. 1974, p. 810-815
Copyright © 1974 American Society for Microbiology
Vol. 10, No. 4
Printed in U.S.A.

Role of Interferon in Six Cell Lines Persistently Infected with Rubella Virus

TREVOR L. STANWICK AND J. V. HALLUM[1]

Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, Louisiana 70112

Received for publication 21 June 1974

Persistent infections with rubella virus were established in baby hamster kidney, BSC-1, HeLa, RK-13, rabbit embryo chondrocyte, and Vero cell lines. All of the cultures except Vero continually produced rubella virus and interferon to which the virus was sensitive. Concurrently, only the Vero cells did not display interference against superinfection with Newcastle disease and vesicular stomatitis viruses. The addition of 1,000 U of exogenous interferon to the cultures cured only the rabbit embryo and Vero cells of the persistent infection. That the interferon is not required for the initiation and maintenance of rubella viral persistence in vitro is implied by the following. (1) Vero cells were persistently infected in the absence of interferon; (2) actinomycin D or cortisone inhibited interferon synthesis but not the rubella viral infection; and (3) cells continuously cultured in the presence of cortisone maintained a viral persistence without interferon synthesis. On the other hand, interferon seems to be responsible for the viral interference; Vero cells infected with rubella virus and cultures inoculated with rubella virus in the presence of actinomycin D or cortisone did not display interference against Newcastle disease or vesicular stomatitis viruses.

Persistent infections with rubella virus have been reported previously for several cell lines (11, 12, 14, 23, 24, 29). Because of experimental variations among laboratories, many of the data are either inconclusive or contradictory regarding the function of interferon in rubella viral persistence and interference against superinfection with heterologous viruses. The purpose of this study was to establish persistent infections with rubella virus in cultures of baby hamster kidney, BSC-1, HeLa, RK-13, rabbit embryo chondrocyte, and Vero cells under identical environmental conditions, and to determine what underlying factor(s) contributes to this persistence. This report correlates the role of interferon in establishing and maintaining a persistent infection with rubella virus in these cell lines.

MATERIALS AND METHODS

Cell lines. Primary African green monkey kidney cells and cell lines of baby hamster kidney-21, clone 13 (BHK), BSC-1, HeLa, human amnion, RK-13, and Vero were obtained from Grand Island Biological Co., Grand Island, N.Y. The rabbit embryo chondrocyte culture was supplied by E. M. Early of Gulf South Research Laboratory, New Orleans, La. (23). All cell lines were cultured in Eagle minimal essential medium supplemented with 10% fetal calf serum for growth and 2% fetal calf serum for maintenance (Grand Island Biological Co.).

Viruses. The Herts strain of Newcastle disease virus (NDV) and the Indiana strain of vesicular stomatitis virus (VSV) were obtained from J. S. Youngner (30) and propagated in BHK cells. Rubella virus stock was grown in primary African green monkey kidney cells and titered by the interference assay against Coxsackie A9 virus (23). Rubella virus titers are expressed as mean tissue culture interfering dose ($TCInD_{50}$).

Cultures persistently infected with rubella virus ($cultures_{RV}$). Monolayers of BHK, BSC-1, HeLa, RK-13, and Vero cell lines in French square bottles were inoculated with 1,000 $TCInD_{50}$ of rubella virus. On day 5 after inoculation, the monolayers were dispersed with trypsin and seeded at a split ratio of 1:4. Upon completion of the monolayers, the growth medium was replaced with the maintenance medium. Subsequent cell transfers were performed on day 5 after formation of a confluent cell monolayer. Initiation of a persistent infection with rubella virus in rabbit embryo chondrocytes is described elsewhere (23).

HAd assay. A modification of the hemadsorption (HAd) and the HAd-inhibition techniques employed by Schmidt et al. (21) was used. Five days after the $cultures_{RV}$ formed confluent monolayers in Leighton tubes, the medium was removed and the cells were washed with phosphate-buffered saline (pH 7.4). A

[1] Present address: Department of Microbiology, University of Oregon Medical School, Portland, Ore. 97201.

one-ml amount of a 0.25% suspension of one-day-old chicken erythrocytes in dextrose-gelatin-Veronal buffer containing 10^{-3} M $CaCl_2$ (pH 7.4) was added to each Leighton tube. After 1 h of incubation at room temperature, the unattached erythrocytes were aspirated, and the cultures were carefully rinsed once with phosphate-buffered saline. From 100 to 300 cells were observed for determination of the percentage of HAd-positive cells. Specificity of the HAd reaction was verified by inhibition of HAd with rubella immune serum (but not with normal serum) and by the absence of HAd in the uninfected cultures. The percentage of cells displaying nonspecific HAd was insignificant.

Interferon production and assay. Interferon endogenous to the $cultures_{RV}$ was harvested with the culture fluid, dialyzed for 72 h against glycine buffer (pH 2) and then for 24 h against phosphate-buffered saline (pH 7.4) (30). The solution was sterilized by filtration, stored at −70 C, and assayed by the reduction of VSV plaques. Twofold dilutions (each 3 ml) of interferon were added to homologous cell monolayers in plastic petri dishes (Falcon Plastic, Oxnard, Calif.; 60 by 15 mm). After incubation for 24 h at 37 C, the interferon was aspirated and an amount of 50 plaque-forming units of VSV (0.5 ml) was absorbed to the cell monolayers for 1 h at 37 C. The inoculated cultures were overlaid with 1% carboxymethyl cellulose (4,000 centipoise, Fisher Scientific Co., Pittsburgh, Pa.) in culture medium supplemented with 5% fetal calf serum (BHK cells received 2% serum), and incubated at 37 C for 3 days in 5% CO_2. The monolayers were gently washed with phosphate-buffered saline to remove the semisolid overlay, stained for 5 min with 1% crystal violet in 20% methanol, and examined for the number of plaques.

Double-stranded heteropolymers of inosine and cytosine (20 μg/ml) were used to stimulate exogenous interferon in uninfected cultures. After incubation of the inoculated cultures at 37 C for 24 h, the culture fluid containing the interferon was harvested, stored at −70 C, and assayed by the plaque reduction method.

The sensitivity of rubella virus to the exogenous and endogenous interferon was determined by the reduction in HAd specific for rubella virus antigen. Uninfected cell monolayers in Leighton tubes were inoculated with twofold dilutions of interferon prepared in the respective cell lines. Twenty-four hours later the cultures were inoculated with 1,000 $TCInD_{50}$ of rubella virus without removing the interferon. Five days after viral inoculation the reduction in HAd due to interferon was determined.

Interferon from BSC-1 cells was also assayed in Vero cells. Human amnion cells were also used for the assay of HeLa cell interferon.

Mycoplasma. Cell cultures and virus stocks were tested for *Mycoplasma* (1). Those found to be contaminated were discarded.

RESULTS

Characterization of $cultures_{RV}$. Persistent infections were initiated in BHK, BSC-1, HeLa, RK-13, rabbit embryo chondrocyte, and Vero cells. Five days after initial viral inoculation and on the fifth day after formation of confluent monolayers (upon subsequent cell transfers) the cultures were monitored for the extracellular virus titer, the percentage of cells HAd positive for rubella virus antigen, the extracellular interferon titer, and the interference to superinfection with NDV and VSV.

Rubella virus was continually produced for 85 transfers of the rabbit $cells_{RV}$ and 20 transfers of the other $cultures_{RV}$ (Table 1). Approximately 70% of the BHK_{RV} and rabbit $embryo_{RV}$ cells, and 90 to 100% of the cells in the remaining $cultures_{RV}$ were HAd positive for the presence of rubella virus. All of the $cultures_{RV}$ except the

TABLE 1. *Characterization of cultures persistently infected with rubella virus (cultures$_{RV}$)*

$Cultures_{RV}$	Cell passage no.[a]	Rubella virus titer[b]	Hemadsorption-positive cells (%)	Interferon titer (U/ml)	Viral interference[c]	
					NDV	VSV
BHK_{RV}	10	5.0	76	12	3.5	4.5
	20	6.2	73	16	3.5	4.5
$BSC\text{-}1_{RV}$	10	3.0	92	24	4.5	3.5
	20	2.4	95	27	4.5	3.5
$HeLa_{RV}$	10	3.6	97	22	5.5	3.0
	20	2.4	94	28	5.5	3.0
$RK\text{-}13_{RV}$	10	3.3	98	20	2.5	3.5
	20	3.8	100	26	2.5	3.5
Rabbit $embryo_{RV}$	85	4.7	64	16	3.0	4.0
$Vero_{RV}$	10	3.5	100	<1	0	0
	20	2.8	98	<1	0	0

[a] Cell passage numbers after initial infection of the cultures.

[b] Titers are $\log_{10}$ $TCInD_{50}$/0.2 ml.

[c] $\log_{10}$ reduction in $TCID_{50}$ of NDV and VSV in $cultures_{RV}$ compared to the titers in homologous uninfected cultures.

$Vero_{RV}$ cells produced a low titer of extracellular interferon and displayed interference to superinfection with NDV and VSV.

Effect of exogenous interferon on $cultures_{RV}$. With the exception of $Vero_{RV}$ cells, the exogenous interferon used to treat the $cultures_{RV}$ was stimulated with double-stranded heteropolymers of inosine and cytosine in homologous cell lines; $Vero_{RV}$ cells received interferon from BSC-1 cells. The $cultures_{RV}$ were incubated for three routine cell passages in the presence of 1,000 U of exogenous interferon.

After the third passage of the interferon-treated cells, the extracellular virus titer and the percentage of cells that were HAd-positive for rubella virus antigen were determined (Table 2). Only in the $Vero_{RV}$ and rabbit $embryo_{RV}$ cells did the virus titer and percentage of cells displaying HAd decrease to an undetectable level. The addition of interferon did not noticably affect the other $cultures_{RV}$. Furthermore, even after removal of the exogenous interferon from the $Vero_{RV}$ and rabbit $embryo_{RV}$ cells, the cultures remained free of detectable rubella virus and antigen.

Sensitivity of rubella virus to exogenous and endogenous interferon. The HAd reduction method for rubella virus antigen was used so that the sensitivity of rubella virus to endogenous and exogenous interferon could be determined. Uninfected cultures were treated with twofold dilutions of interferon, challenged 24 h later with 1,000 $TCInD_{50}$ of rubella virus, and observed 5 days later for the percentage of cells displaying HAd.

The reduction in the percentage of HAd-positive cells in the interferon-treated cells compared to untreated cells is recorded in Table 3.

TABLE 2. *Effect of exogenous interferon on $cultures_{RV}$*

$Cultures_{RV}$[a]	Rubella virus titer[b]	Hemadsorption-positive cells[c] (%)
BHK_{RV}	4.3	97
$BSC\text{-}1_{RV}$	2.5	83
$HeLa_{RV}$	2.6	98
$RK\text{-}13_{RV}$	3.5	94
Rabbit $embryo_{RV}$	<1	0
$Vero_{RV}$	<1	0

[a] $Cultures_{RV}$ were routinely passed in the presence of 1,000 U of exogenous interferon produced with double-stranded heteropolymers of inocine and cytosine in homologous cells. $Vero_{RV}$ cells received BSC-1 cell interferon.

[b] Virus titer (log_{10} $TCInD_{50}$/0.2 ml) of the third passage of the $cultures_{RV}$.

[c] Specific hemadsorption for rubella virus antigen after the third passage of the $cultures_{RV}$.

TABLE 3. *Sensitivity of rubella virus to endogenous and exogenous interferon*

Cell source of interferon	Assay cell	Interferon titer[a]	
		Endogenous[b]	Exogenous[c]
BHK	BHK	12 (18)	146
BSC-1	BSC-1	21 (26)	256
	Vero	ND	183
HeLa	Human amnion	18 (28)	220
RK-13	RK-13	19 (24)	232
Rabbit embryo	Rabbit embryo	16 (21)	195

[a] Interferon titers represent the dilution of interferon which produced a 50% reduction in the number of cells displaying hemadsorption specific for rubella virus antigen, compared to control cultures not treated with interferon. ND, Not done.

[b] Endogenous interferon was harvested from the indicated cell lines persistently infected with rubella virus. The numbers in the parenthesis represent the interferon titers assayed by the reduction of VSV plaques.

[c] The amount of exogenous interferon stimulated with double-stranded heteropolymers of inosine and cytosine in the indicated cell lines was 1,000 U when calculated by the reduction of VSV plaques.

The sensitivity of the rubella virus varied with the cell culture. In all instances, rubella virus was less sensitive than VSV. There was no difference in interferon sensitivity when rubella virus from the respective $cultures_{RV}$ was substituted for the stock rubella virus from primary African green monkey kidney cells.

Initiation of rubella virus infection in the presence of actinomycin D. In order that the relationship of interferon production to the initiation of a persistent infection could be determined, the various cell lines were simultaneously inoculated with 1,000 $TCInD_{50}$ of rubella virus and actinomycin D (AD). BHK, RK-13, HeLa, and rabbit embryo cells received 20 ng of AD/ml; Vero and BSC-1 cells received 50 ng of AD/ml. The control cultures were inoculated only with rubella virus. Five days after inoculation rubella virus and interferon titers in the culture fluids were assayed, and the cultures that received AD were trypsinized and transferred once in the absence of AD. On day 5 after the transferred cultures formed a monolayer, the culture fluids were again examined for rubella virus and interferon synthesis.

Interferon production was not detected in the cultures inoculated with AD; however, the rubella virus titer was unaffected when compared to the controls (Table 4). After removal of the AD, the level of interferon reached that of the controls, and the virus titer remained relatively

TABLE 4. *Initiation of rubella virus infection in the presence of actinomycin D*

Cell lines	AD not added[a]		AD present[b]		AD removed[c]	
	Virus titer[d]	Interferon titer[e]	Virus titer	Interferon titer	Virus titer	Interferon titer
BHK	4.3	16	4.0	<1	5.0	16
BSC-1	3.5	13	3.4	<1	2.7	14
HeLa	2.2	18	2.8	<1	3.5	22
RK-13	3.4	14	4.0	<1	3.7	12
Rabbit embryo	2.5	12	3.5	<1	2.4	16
Vero	3.7	<1	3.0	<1	2.8	<1

[a] Control group of cells without AD were inoculated with 1,000 $TCInD_{50}$ of rubella virus. Virus and interferon yields were determined five days after inoculation.

[b] BSC-1 and Vero received 50 μg of AD/ml; the other cell lines received 20 μg of AD/ml. All cultures were inoculated with 1,000 $TCInD_{50}$ of rubella virus and were assayed 5 days after inoculation for the virus and interferon titers.

[c] Five days after inoculation the cultures receiving AD were trypsinized and transferred. On day 5 after the transferred cultures formed a monolayer, the virus and interferon titers were assayed.

[d] Virus titers are $\log_{10}$ $TCInD_{50}$/0.2 ml.

[e] Recorded are the units of interferon per milliliter.

constant. When $cultures_{RV}$ treated with AD were challenged with NDV or VSV, there was no viral interference when compared to uninfected cells treated with equivalent amounts of AD.

Effect of cortisone on initiation and maintenance of a persistent infection with rubella virus. Further evaluation of the role of interferon in the initiation and maintenance of persistent infection with rubella virus was done with cortisone. Cortisone (50 μg/ml of medium) was added to the uninfected cells 24 h prior to inoculation with 1,000 $TCInD_{50}$ of rubella virus. Control cultures were not pretreated with cortisone before inoculation. After three transfers of the inoculated cultures, the culture fluids were assayed for the production of extracellular virus and interferon. The cortisone-treated cultures were transferred in the presence of cortisone.

In the cortisone-treated cultures, the production of interferon was undetected; however, the virus titer was similar to the control cultures (Table 5). $Cultures_{RV}$ initiated and maintained in the presence of cortisone did not display any more interference against NDV and VSV.

DISCUSSION

Persistent infections with rubella virus were established in BHK, BSC-1, HeLa, RK-13, rabbit embryo chondrocyte, and Vero cells. Only the $Vero_{RV}$ cells failed to produce interferon and to display interference to challenge with NDV or VSV. Persistent infections of BHK, rabbit embryo, RK-13, and Vero cells by rubella virus have been published previously (11, 14, 23, 24). To our knowledge, persistent infections of HeLa and BSC-1 cells by rubella virus and the demonstration of interferon in BHK_{RV}, $RK\text{-}13_{RV}$, and $HeLa_{RV}$ cells have not been reported to date. In contrast to other reports, neither BHK_{RV} nor $RK\text{-}13_{RV}$ developed any cytopathic effect due to rubella virus (10, 13, 17, 22, 24, 25, 27). Cytologic alterations were only observed in the rabbit embryo chondrocytes, which changed from epithelial cells to cells similar to fibroblasts after infection with rubella virus (23).

Although it has been suggested that interferon may play a role in maintaining the persistent infection of cells by rubella virus in vitro (6, 12, 29), there are several reports of rubella viral persistence in the absence of detectable interferon (8, 14, 19, 20). Our data indicated that neither the initiation nor maintenance of rubella viral persistence was due to the presence of interferon. Inoculation of the cell lines with rubella virus in the presence of AD or cortisone inhibited the interferon synthesis, but not the establishment of viral persistence. Furthermore, the viral persistence was maintained for three cell passages although interferon production was suppressed by the continued presence of cortisone in the culture media.

Interference to NDV and VSV coincided with the presence of interferon in the $cultures_{RV}$.

TABLE 5. *Effect of cortisone on initiation and maintenance of a persistent infection with rubella virus*

Cell lines[a]	Cortisone absent		Cortisone present	
	Virus titer[b]	Interferon titer[c]	Virus titer	Interferon titer
BHK	2.5	20	2.5	<1
BSC-1	2.0	16	1.7	<1
HeLa	2.3	19	2.5	<1
RK-13	3.8	13	4.5	<1
Rabbit embryo	3.3	24	3.5	<1
Vero	2.5	<1	2.3	<1

[a] All cells were inoculated with 1,000 $TCInD_{50}$ of rubella virus and routinely transferred three times. Cultures receiving 50 μg of cortisone per ml were pretreated 24 h prior to viral inoculation and were continuously incubated with cortisone for the three cell passages.

[b] Virus titers are $\log_{10}$ $TCInD_{50}$/0.2 ml.

[c] Units of interferon per milliliter.

Concurrently, when interferon synthesis was inhibited by AD or cortisone, no viral interference was observed. It has been reported that interferon may function in rubella viral interference against various viruses for several cell cultures (2, 3, 12, 15, 27, 28, 29). On the other hand, rubella virus has also been shown to interfere with challenge viruses in the absence of detectable interferon (5, 8, 16, 19, 20, 28). Inhibition of rubella viral interference by AD has been observed by Desmyter et al. (2) and Wong et al. (29). In contrast to other reports, the addition of AD or cortisone had no effect on interferon synthesis once interferon production was initiated in our cultures (24, 29).

Rubella virus and the endogenous interferon to which it was sensitive were produced simultaneously in all the cultures except $Vero_{RV}$ cells. The addition of large quantities of exogenous interferon to the $cultures_{RV}$ cured only the $Vero_{RV}$ and rabbit $embryo_{RV}$ cells of the viral infection; rubella virus yields in the other $cultures_{RV}$ were unaffected. Mifune et al. (14) also cured $Vero_{RV}$ cells with exogenous interferon from BSC-1 cells. However, $cultures_{RV}$ that produced interferon were not altered by the addition of low concentrations of exogenous interferon (4, 14).

The cure of rabbit $embryo_{RV}$ cells is similar to that reported for L cells persistently infected with NDV (9). Both persistent infections exist in the presence of interferon to which the virus is sensitive. It has been suggested that the NDV persistence might be due to the presence of the viral genome in a protected state, or to cells which are refractory to the action of interferon (9). Subsequently, persistent NDV has been shown to possess the enzyme, ribonucleic acid-dependent deoxyribonucleic acid polymerase, which could account for the viral persistence in the presence of interferon (7). It remains to be shown whether a similar mechanism exists to explain the persistance of rubella virus irrespective of the presence of interferon.

ACKNOWLEDGMENTS

This investigation was supported by the John A. Hartford Foundation, Inc., New York, N.Y.

LITERATURE CITED

1. Chanock, R. M., L. Hayflick, and M. F. Braile. 1962. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. Proc. Nat. Acad. Sci. U.S.A. **48**:41-49.
2. Desmyter, J., P. De Somer, W. E. Rawls, and J. L. Melnick. 1968. The mechanism of rubella virus interference. International symposium on rubella vaccines (London). Symp. Ser. Immunobiol. Stand. **11**:139-148.
3. Desmyter, J., J. L. Melnick, and W. E. Rawls. 1968. Defectiveness of interferon production and of rubella virus interference in a line of African green monkey kidney cells (Vero). J. Virol. **2**:955-961.
4. Desmyter, J., W. E. Rawls, J. L. Melnick, M. D. Yow, and F. F. Barrett. 1967. Interferon in congenital rubella. Response to live attenuated measles vaccine. J. Immunol. **99**:771-777.
5. De Somer, P., A. Billiau, E. De Clercq, and E. Schoone. 1967. Rubella virus interference and interferon production. Antonie van Leeuwenhoek J. Microbiol. Serol. **33**:237-245.
6. Downie, J. C., and J. S. Oxford. 1969. Persistent rubella virus infection in hamster lung cells. J. Gen. Virol. **5**:11-17.
7. Furman, P. A., and J. V. Hallum. 1973. RNA-dependent DNA polymerase activity in preparations of a mutant of Newcastle disease virus arising from persistently infected L cells. J. Virol. **12**:548-555.
8. Grayzel, A. I., and C. Beck. 1969. Rubella infection of synovial cells and the resistance of cells derived from patients with rheumatoid arthritis. J. Exp. Med. **131**:367-373.
9. Hallum, J. V., H. R. Thacore, and J. S. Youngner. 1972. Effect of exogenous interferon on L cells persistently infected with Newcastle disease virus. Infect. Immunity **5**:145-146.
10. Hekker, A. C., P. Huisman, and W. Jongling. 1968. A plaque method for the titration of rubella virus and the determination of antibodies. International symposium on rubella vaccines (London). Symp. Ser. Immunobiol. Stand. **11**:187-192.
11. Kilburn, D. G., and A. L. Wezel. 1970. The effect of growth rate in continuous-flow cultures on the replication of rubella virus on BHK cells. J. Gen. Virol. **9**:1-7.
12. Maassab, H. F., and J. A. Veronelli. 1966. Characteristics of serially propagated monkey kidney cell cultures with persistent rubella infection. J. Bacteriol. **91**:436-441.
13. McCarthy, K., C. H. Taylor-Robinson, and S. E. Pillinger. 1963. Isolation of rubella virus from cases in Britian. Lancet **2**:593-598.
14. Mifune, K., J. Desmyter, and W. E. Rawls. 1970. Effect of exogenous interferon on rubella virus production in carrier cultures of cells defective in interferon production. Infect. Immunity **2**:132-138.
15. Neva, F. A., and T. H. Weller. 1964. Rubella interferon and factors influencing the indirect neutralization test for rubella antibody. J. Immunol. **93**:466-473.
16. Parkman, P. D., E. L. Buescher, M. S. Artenstein, J. M. McCown, F. K. Mundon, and A. D. Druzd. 1964. Studies of rubella. I. Properties of the virus. J. Immunol. **93**:595-607.
17. Plotkin, S. A. 1965. Plaquing of rubella virus in RK-13 cells. Arch. Gesamte Virusforsch. **16**:423-425.
18. Plotkin, S. A., A. Boue, and J. G. Boue. 1965. The *in vitro* growth of rubella virus in human embryo cells. Amer. J. Epidemiol. **81**:71-85.
19. Rawls, W. E., J. Desmyter, and J. L. Melnick. 1968. Virus carrier cells and virus-free cells in fetal rubella. Proc. Soc. Exp. Biol. Med. **129**:477-483.
20. Rawls, W. E., and J. L. Melnick. 1966. Rubella virus carrier cultures derived from congenitally infected infants. J. Exp. Med. **123**:795-816.
21. Schmidt, N. J., J. Dennis, and E. H. Lennette. 1968. Hemadsorption and hemadsorption inhibition tests for rubella virus. Arch. Gesamte Virusforsch. **25**:308-320.
22. Sedwick, W. D., and T. J. Wiktor. 1967. Reproducible plaquing system for rabies, lymphocytic choriomeningitis, and other ribonucleic acid viruses in BHK-21/13S agarose suspensions. J. Virol. **1**:1224-1226.

23. Smith, J. L., E. M. Early, W. T. London, D. A. Fuccillo, and J. L. Sever. 1973. Persistent rubella virus production in embryonic rabbit chondrocyte cell cultures. Proc. Soc. Exp. Biol. Med. **143**:1037-1041.
24. Svedmyr, A. 1965. Persistent infection with rubella virus in RK-13 cells. Arch. Gesamte Virusforsch. **16**:446-465.
25. Taylor-Robinson, C. H., K. McCarthy, S. G. Grylls, and C. M. O'Ryan. 1964. Plaque formation by rubella virus. Lancet **1**:1364-1365.
26. Thomssen, R., E. Suhrkamp, and S. Bonk. 1972. Inability of rubella virus interference to reverse the inhibition of cellular protein synthesis caused by poliovirus. Arch. Gesamte Virusforsch. **37**:62-70.
27. Vaheri, A., W. D. Sedwick, S. A. Plotkin, and R. Maes. 1965. Cytopathic effect of rubella virus in BHK-21 cells and growth to high titers in suspension cultures. Virology **27**:239-241.
28. Weller, T. H., and F. A. Neva. 1965. Biological characterization of rubella virus as assayed in a human amnion culture system. Arch. Gesamte Virusforch. **16**:393-400.
29. Wong, K. T., S. Baron, and T. G. Ward. 1967. Rubella virus: role of interferon during infection of African green monkey kidney tissue cultures. J. Immunol. **99**:1140-1149.
30. Youngner, J. S., A. W. Scott, J. V. Hallum, and W. R. Stinebring. 1966. Interferon production by inactivated Newcastle disease virus in cell cultures and in mice. J. Bactriol. **92**:862-868.