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Effect of Papaverine Treatment on Replication of Measles Virus in Human Neural and Nonneural Cells

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The replication of measles virus in human neural and nonneural cell lines in terms of growth and cytopathic effect was affected by treatment of the cells with papaverine, which increases endogenous cyclic AMP. Suppression of virus growth was most prominent in neuroblastoma cells, followed by that in epidermoid carcinoma and glioblastoma cells, whereas the suppressive effect was relatively weak in oligodendroglioma cells. The papaverine-induced suppression of virus growth in neuroblastoma cells was studied in detail. The suppression that occurred was dependent on the dose of papaverine and was reversible. By treatment with 10 μM papaverine, virus-cell interactions were modified as follows: (i) early replication steps such as adsorption, penetration, and uncoating of the virus were not affected; (ii) synthesis of viral RNAs, including genomic RNA and mRNA, was inhibited; (iii) translation of viral proteins from mRNA was not blocked; and (iv) glycosylation and transport of viral glycoproteins to the cell membrane were not inhibited, but phosphorylation was blocked. The significance of suppressed virus replication in neural cells is discussed in relation to the persistence mechanisms of measles virus in the central nervous system.

The mode of replication of measles virus (MV) has been studied extensively with the African green monkey kidney cell lines Vero and CV-1 because of their high susceptibility to MV (3). Other cell lines, such as those of neural origin, were recently used to investigate virus-cell interactions in a simulation of the growth of MV in the brain (2, 8). However, most of the neural cell lines were established from spontaneous tumors and show properties of cells at relatively undifferentiated or embryonic stages, in contrast to the situation in the brain, which consists mainly of differentiated cells (1). Several investigators reported that cyclic AMP (cAMP) and its analogs induce the differentiation of the established neural cell lines (1, 11, 12). Therefore, analysis of the replication of MV at different levels of cAMP in these cell lines may bring understanding of virus-cell interactions in the brain.

Robbins and Rapp (15) first reported that the treatment of human amnion AV_3 cells with cAMP caused the suppression of MV growth and the appearance of viral cytopathic effect (CPE) with selective suppression of the synthesis of viral P and M proteins, and they speculated about a restricted replication of MV in the nervous system because of the high level of cAMP in these tissues.

Miller and Carrigan (11) reported that elevation of the endogenous cAMP level by papaverine caused a marked, but reversible, suppression of MV growth in neural cells, with selective suppression of viral M protein synthesis. Thus, they postulated that viral persistence may be related to the state of neural differentiation.

In two experiments, we examined the effect of papaverine on the replication of MV in various neural and nonneural cells. Subsequently we examined the mechanisms for the inhibition of MV replication in the human neuroblastoma cell line in which the most marked suppression was observed and found that viral RNA synthesis and phosphorylation of the viral proteins were inhibited by treatment with papaverine but that virus adsorption, penetration, uncoating, translation of viral proteins from the mRNAs, and glycosylation of the viral proteins were not inhibited. These results suggest that the replication of MV in vivo may be easily modified by change in cellular conditions, such as cell differentiation, regeneration, hyperplasia, and hypertrophy, and that these kinds of modifications may participate in the establishment of a persistent MV infection in vivo.

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MATERIALS AND METHODS

Virus. The Edmonston strain of MV propagated in Vero cells was used. It had a titer of 10^6 PFU/ml when titrated in Vero cells.

Cells. Three human neural cell lines (12, 14, 19, 21), IMR-32 neuroblastoma (IMR), 118-MGC glioblastoma (MGC), and KG-1 oligodendroglioma (KG), and a human oral epidermoid carcinoma cell line, KB cells (KB), were maintained by DM 160 medium (Kyokuto Pharmaceutical Industrial Co. Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine or newborn calf serum and 60 μg of kanamycin per ml. Vero cells were used to titrate virus infectivity, since they were found to be insensitive to papaverine treatment in the preliminary study (data not shown).

Treatment of cells with papaverine. Papaverine (Sigma Chemical Co., St. Louis, Mo.) was dissolved in distilled water at a concentration of 1 mM and stored at −20°C before use. Before or after virus infection, monolayer cells were treated with papaverine, which was appropriately diluted with a maintenance medium consisting of DM 160 medium supplemented with 5% heat-inactivated fetal bovine serum and kanamycin.

Growth curve of virus. The assay methods were described elsewhere (22). Briefly, monolayer cells in multiwell plastic dishes (Linbro no. 76-033-05) were pretreated with 10 μM papaverine for 48 h, inoculated with 10^5 PFU of MV, and cultured in the maintenance medium containing 10 μM papaverine. Between 1 and 4 days post-inoculation (pi), the infected cells were disrupted by sonication, frozen once at −80°C, and clarified by centrifugation at 3,000 rpm for 15 min. The infectivity titer of virus in the supernatant was assayed in Vero cells.

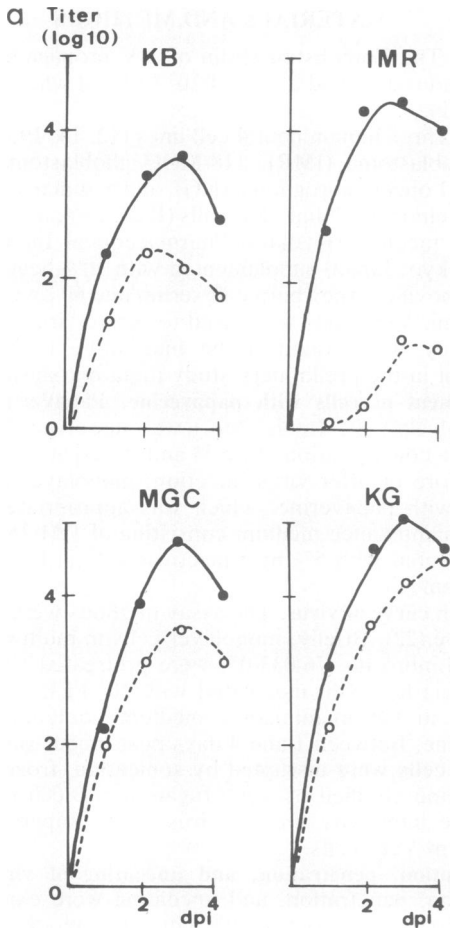
Adsorption, penetration, and uncoating of virus. Virus adsorption, penetration, and uncoating were examined by the method of Kohno et al. (9) with slight modifications for

plastic dishes (Falcon no. 3001). Monolayer cells were chilled in a melting ice bath, inoculated with 10^5 PFU of MV, settled on ice, and harvested at intervals. The cells were subjected to sonication and light centrifugation, and the titer of adsorbed virus was determined on the supernatant.

To determine the penetration and uncoating rates, virus was adsorbed at 0°C for 60 min on the monolayer cells, and the cells were incubated for various intervals at 37°C. The cells were first chilled in a melting ice bath, to terminate virus penetration and confer resistance to low-pH treatment, and then treated with cold glycine-hydrochloride-buffered saline (0.14 M NaCl, 8 mM glycine, 0.001 N HCl, 1.2 mg of bovine serum albumin per ml, pH 2.5) for 5 min to inactivate unadsorbed virus, and then pH was neutralized by 1/15 M Na_2HPO_4. Thereafter the monolayer cells were further cultured in the maintenance medium, and the numbers of virus-induced plaques were counted 2 days later for penetrated virus. To determine the uncoating rate, the monolayer cells were subjected to sonication and light centrifugation immediately after inactivation of the unadsorbed virus, and the infectivity titer in the supernatant was assayed. The low-pH-resistant virus titer, representing penetrated virus, minus the supernatant infectivity titer, representing penetrated virus before uncoating, gave a remainder that was considered to represent a titer of the uncoated virus.

Preparation and agarose gel electrophoresis of the viral RNAs. Monolayer cells grown in plastic dishes (Falcon no. 3002) were infected with 2×10^5 PFU of MV. When CPE occupied about 30 to 50% of the cell sheet, the cells were treated with 20 μg of actinomycin D (AMD) (Sigma) per ml for 2 h at 37°C. Viral RNA was labeled by culturing the cells with P-free Eagle minimal essential medium supplemented with [^{32}P]orthophosphate (500 μCi/2 ml per dish; carrier-free, 64 mCi/ml) (Japan Radioisotope Association, Tokyo, Japan), 1.0 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), and 10 μg of AMD per ml for 5 h at 37°C.

The labeled cells were scraped, washed with cold phosphate-buffered saline, and lysed in TNE buffer (0.1 M NaCl, 10 mM Tris-hydrochloride, 1 mM EDTA-2Na, pH 7.2) containing 0.5% Nonidet P-40 at 0°C. The nuclear fraction was removed by light centrifugation, and the supernatant was mixed with an equal volume of TNE buffer containing 7.0 M urea (pH 7.4) (Ultra pure; Schwarz/Mann, Orangeburg, N.Y.). RNA was extracted by mixing the lysate with an equal volume of a phenol-chloroform-isoamyl alcohol mixture (50:50:1) and centrifuging the mixture at 10,000 rpm for 5 min. RNA was precipitated by addition of a 0.1 volume of 2.0 M sodium acetate buffer (pH 5.4) and incubation overnight with pure ethanol at −20°C. After centrifugation at



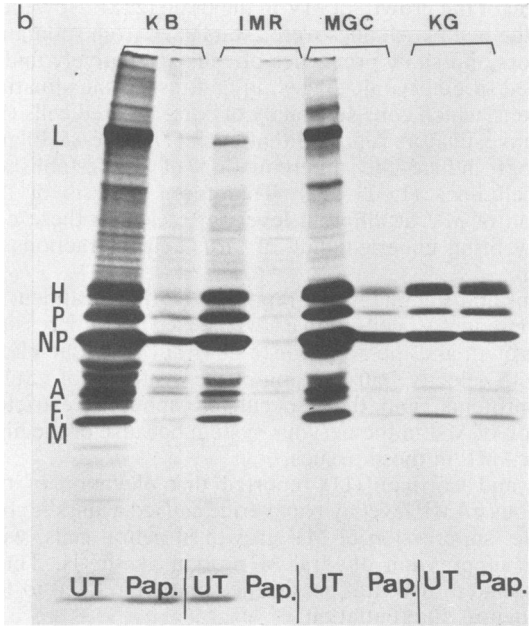


FIG. 1. Effect of papaverine on MV replication in various cell lines. (a) Virus growth curve. Monolayer cells were infected with MV and cultured in medium containing 10 μM papaverine. The cells were sonicated, freeze-thawed once, and centrifuged. Infectious virus was titrated in Vero cells. Ordinates indicate virus titers ($\log_{10}$ PFU/ml), and abscissa indicate days dpi. Symbols: ○, papaverine-treated cells; ●, control cells. (b) Synthesis of viral proteins. Virus-infected cells, either papaverine-treated or untreated ones, were labeled with [^{35}S]methionine for 2 h at the time when CPE occupied more than 50% of the cell sheet in untreated cells. Viral proteins were immunoprecipitated and analyzed by SDS-PAGE. Pap., Papaverine-treated cells; UT, untreated cells.

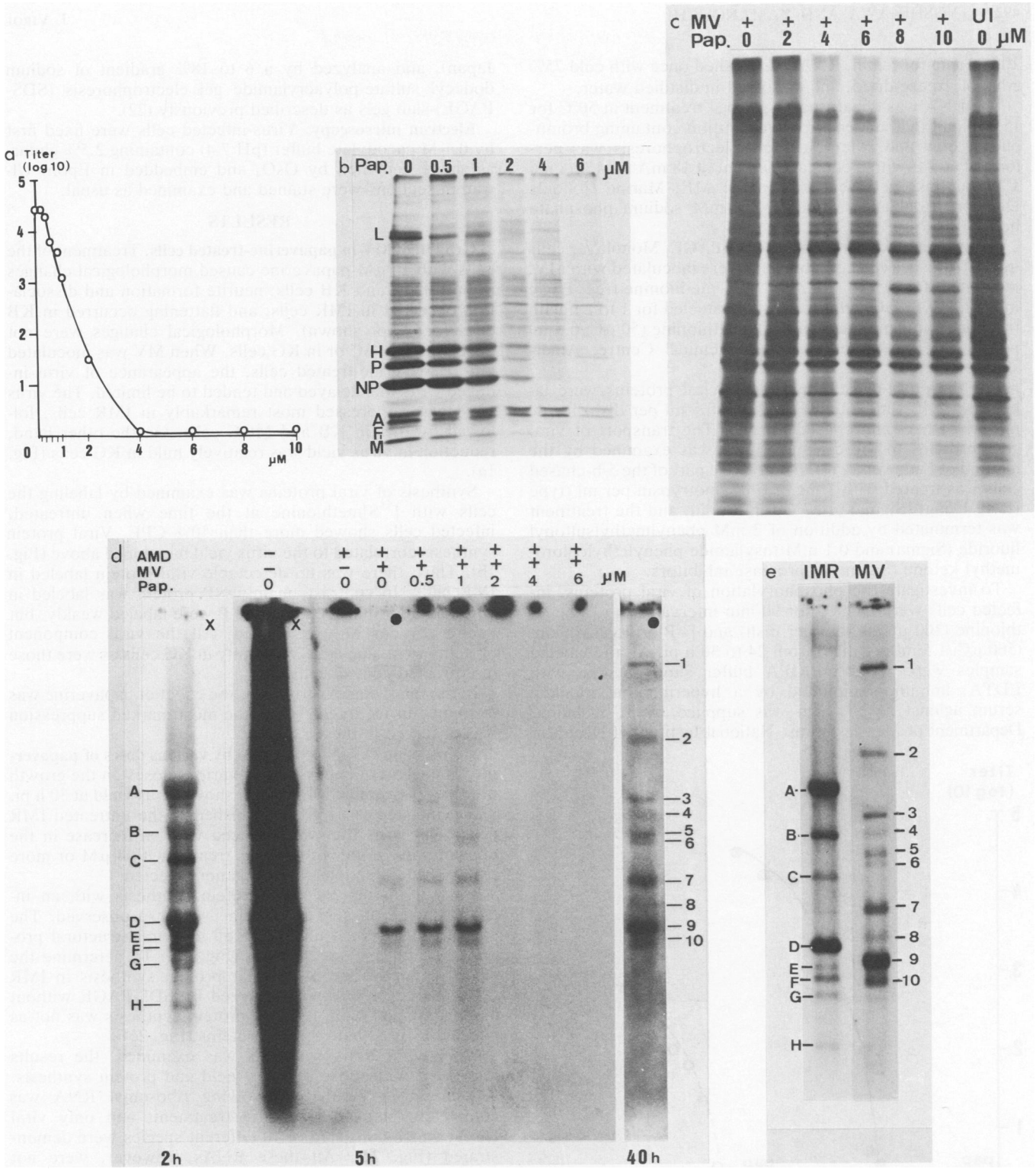


FIG. 2. Dose-dependent suppression by papaverine treatment of MV replication in IMR cells. (a) Virus yield. Monolayer cells were treated with 0 to 10 μM papaverine 24 h before virus inoculation. After virus adsorption, the cells were cultured in maintenance medium containing 0 to 10 μM papaverine. Virus yield at 30 h pi was titrated in Vero cells. Abscissa indicates the concentration of papaverine. (b and c) Synthesis of cellular and viral proteins (L, H, P, NP, A, F_1, and M). Monolayer cells were treated with papaverine (Pap.) as above, infected with virus, cultured in medium containing papaverine and labeled by [^{35}S]methionine from 28 to 30 h pi. The cells were lysed by RIPA buffer and analyzed by SDS-PAGE with (b) or without (c) immunoprecipitation, representing viral and cellular protein synthesis, respectively. UI, Uninfected cells; Pap., papaverine-treated cells. (d) Synthesis of viral RNAs. Monolayer cells were treated as above, and after inhibition of cellular RNA synthesis by AMD treatment, viral RNAs were labeled with [^{32}P]orthophosphate and analyzed by agarose gel electrophoresis. Pattern of autoradiography performed at −80°C for 5 h is shown. To demonstrate cellular and viral RNAs more clearly, autoradiography of the same gel was also performed for 2 h (lanes ×) or 40 h (lanes ●). AMD, AMD treatment. (e) Comparative patterns of cellular and viral RNAs. RNAs of IMR cells and those of MV were labeled with [^{32}P]orthophosphate. Major bands are completely different from each other. Among cellular RNAs, A and D are 28S and 18S ribosomal RNAs, respectively.

10,000 rpm for 5 min, RNA was washed once with cold 75% ethanol, freeze-dried, and dissolved in distilled water.

The RNA was denatured by glyoxal treatment at 50°C for 15 min and then mixed with dye solution containing bromophenol blue and xylene cyanol. Electrophoresis was performed at 8°C with a constant current of 18 mA for 17 h on a 1.1% agarose gel (Seakem agarose ME; Marine Colloids Division, Rockland, Maine) in 10 mM sodium phosphate buffer (pH 7.2).

Labeling of viral proteins and SDS-PAGE. Monolayer cells in plastic dishes (Falcon no. 3001) were inoculated with MV, incubated for 15 to 30 min with methionine-free Eagle minimal essential medium, and then labeled for 1 to 2 h with the same supplemented with [^{35}S]methionine (50 μCi/0.5 ml per dish; 1,465 Ci/mmol) (Radiochemical Centre, Amersham, England).

For the pulse-chase experiment, viral proteins were labeled with [^{35}S]methionine (100 μCi/0.5 ml per dish) for 60 min at 24 h pi and chased for 5 h. The transport of viral glycoproteins to the cell membrane was examined by the method of Knipe et al. (7). Briefly, one part of the 5-h-chased cells was treated with 1 mg of α-chymotrypsin per ml (type II; 49 U/mg) (Sigma) at 37°C for 10 min, and the treatment was terminated by addition of 2 mM phenylmethylsulfonyl fluoride (Sigma) and 0.1 mM tosylamide phenylethylchloromethyl ketone (Sigma) as protease inhibitors.

To investigate the phosphorylation of viral proteins, infected cells were labeled for 90-min intervals with [^{35}S]methionine (100 μCi/0.5 ml per dish) and [^{32}P]orthophosphate (500 μCi/1.5 ml per dish) from 24 to 30 h pi. All the labeled samples were lysed in RIPA buffer supplemented with EDTA, immunoprecipitated by a hyperimmune monkey serum against MV (which was supplied by T. Kohama, Department of Measles Virus, National Institute of Health of Japan), and analyzed by a 6 to 18% gradient of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slab gels as described previously (22).

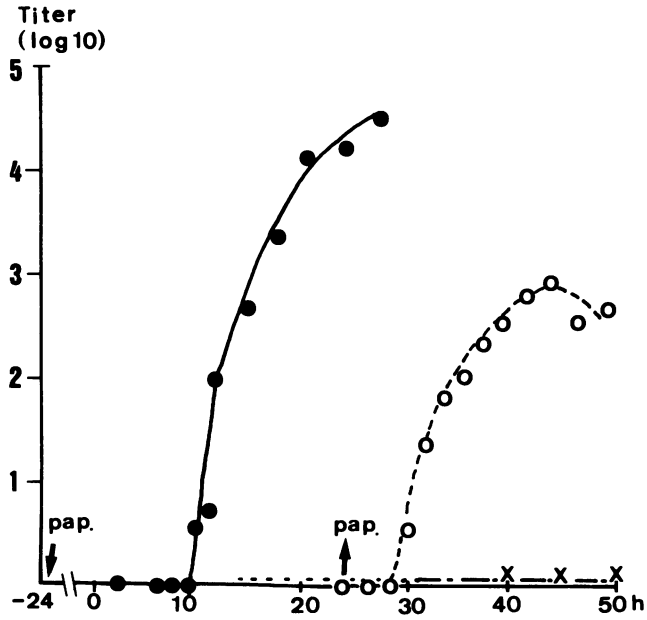


FIG. 3. Reversibility of the suppressive effect of papaverine on virus replication in IMR cells. Two cultures of papaverine-treated IMR cells were inoculated with MV and cultured in medium containing papaverine. The medium of one group was replaced 24 h pi by ordinary medium without papaverine (○), and that of the other group was not changed (x). As a control, untreated cells were inoculated with MV and cultured in medium without papaverine (●).

Electron microscopy. Virus-infected cells were fixed first by 0.1 M cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde and then by OsO_4 and embedded in Epon. Ultrathin sections were stained and examined as usual.

RESULTS

Growth of MV in papaverine-treated cells. Treatment of the cells with 10 μM papaverine caused morphological changes in both IMR and KB cells; neurite formation and dissociation occurred in IMR cells; and flattening occurred in KB cells (data not shown). Morphological changes were not observed in MGC or in KG cells. When MV was inoculated onto papaverine-treated cells, the appearance of virus-induced CPE was delayed and tended to be limited. The virus yield was suppressed most remarkably in IMR cells, followed by that in KB and MGC cells. On the other hand, reduction in virus yield was relatively mild in KG cells (Fig. 1a).

Synthesis of viral proteins was examined by labeling the cells with [^{35}S]methionine at the time when untreated, infected cells showed more than 50% CPE. Viral protein synthesis correlated to the virus yield mentioned above (Fig. 1b). Thus, there was no detectable viral protein labeled in IMR cells. In contrast, mainly NP protein was labeled in both KB and MGC cells, H and P were labeled weakly, but neither F_1 nor M was labeled. All the viral component proteins were labeled as efficiently in KG cells as were those in untreated control cells.

In the following experiments, the effect of papaverine was examined in IMR cells, since the most marked suppression was observed in these cells.

Suppression of MV replication by various doses of papaverine. The effect of papaverine at various doses on the growth of MV was examined. Figure 2a shows virus yield at 30 h pi, when one-step virus growth finished in the untreated IMR cells. The virus titer was reduced with an increase in the dose of papaverine. In the cells treated with 4 μM or more papaverine, infectious virus was not detected.

The suppression of viral protein synthesis with an increase in the dose of papaverine was also observed. The suppression seemed to involve all the viral structural proteins but not any special ones (Fig. 2b). To determine the effect of papaverine on cellular protein synthesis in IMR cells, the cell lysate was analyzed by SDS-PAGE without immunoprecipitation. Cellular protein synthesis was not as affected as was that of viral proteins (Fig. 2c).

When viral RNA synthesis was examined, the results correlated with those of virus yield and protein synthesis. Cellular RNA synthesis involving ribosomal RNA was completely blocked by AMD treatment, and only viral RNAs which consisted of 10 different species were demonstrated (Fig. 2d). All these RNAs, however, were not detected when the cells were treated with 2 μM or more papaverine. The patterns of major cellular RNAs were markedly different from those of viral ones (Fig. 2e).

Reversible effect of papaverine on replication of MV. To examine whether the suppressive effect of papaverine on the growth of MV was reversible or not, papaverine was removed from the culture medium 24 h pi, and virus yield was measured at intervals. In untreated IMR cells, the first progeny virus was detected 10 h pi, and one-step virus growth finished within 30 h (Fig. 3). In the cells treated with 10 μM papaverine, no infectious virus was detected up to 24

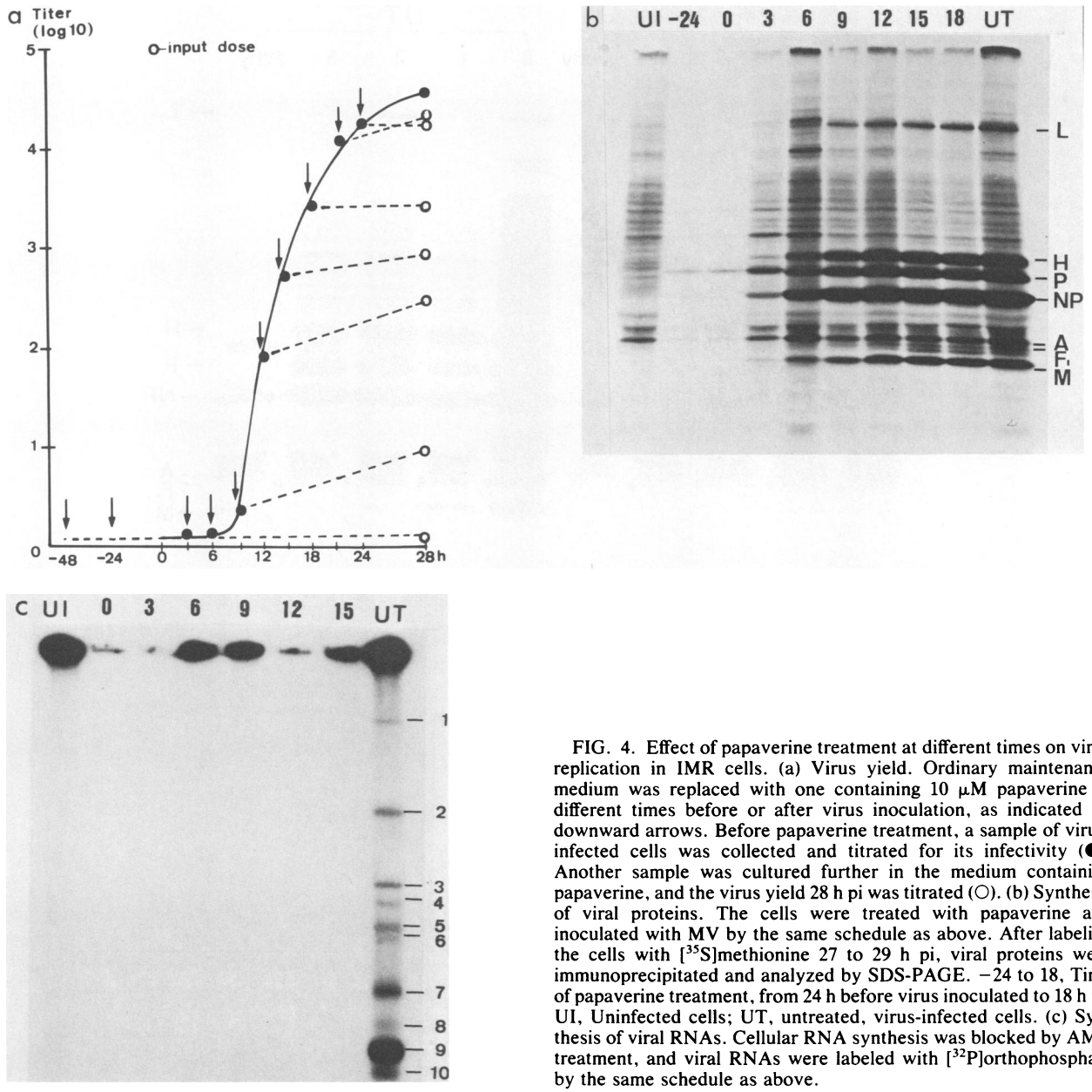


FIG. 4. Effect of papaverine treatment at different times on virus replication in IMR cells. (a) Virus yield. Ordinary maintenance medium was replaced with one containing 10 μM papaverine at different times before or after virus inoculation, as indicated by downward arrows. Before papaverine treatment, a sample of virus-infected cells was collected and titrated for its infectivity (●). Another sample was cultured further in the medium containing papaverine, and the virus yield 28 h pi was titrated (○). (b) Synthesis of viral proteins. The cells were treated with papaverine and inoculated with MV by the same schedule as above. After labeling the cells with [^{35}S]methionine 27 to 29 h pi, viral proteins were immunoprecipitated and analyzed by SDS-PAGE. −24 to 18, Time of papaverine treatment, from 24 h before virus inoculated to 18 h pi. UI, Uninfected cells; UT, untreated, virus-infected cells. (c) Synthesis of viral RNAs. Cellular RNA synthesis was blocked by AMD treatment, and viral RNAs were labeled with [^{32}P]orthophosphate by the same schedule as above.

h pi. When the maintenance medium containing papaverine was replaced with the medium free of papaverine, infectious virus was detected 6 h after the change in medium, i.e., 30 h pi, and its titer increased thereafter. When the papaverine-treated, virus-infected cells were maintained by the culture medium containing papaverine without a change in medium, no infectious virus was detected until 50 h pi, the termination of this experiment. Thus, it is clear that this suppressive effect was reversible.

Effect of papaverine on early steps of virus replication. The effect of papaverine on early steps of virus replication such as adsorption, penetration, and uncoating in IMR cells was examined. There was no difference in the kinetics of these early steps of MV replication between papaverine-treated and untreated cells (data not shown).

Time-sequential effect of papaverine on growth of MV. IMR cells were treated with 10 μM papaverine before or after virus inoculation and then examined for virus yield and the synthesis of viral proteins and RNAs. Infectious virus was not detected when the cells were treated before the production of progeny virus, i.e., 6 h pi (Fig. 4a). If the cells were treated later than 12 h pi, when virus-induced CPE had appeared, the virus titers did not increase as did those of untreated controls, although CPE tended to spread gradually.

The kinetics of viral protein synthesis correlated to the time papaverine treatment started (Fig. 4b). If treatment was started before virus inoculation or immediately after virus adsorption, only P protein was detected. When treatment was started 3 h pi, P and NP proteins were demonstrated

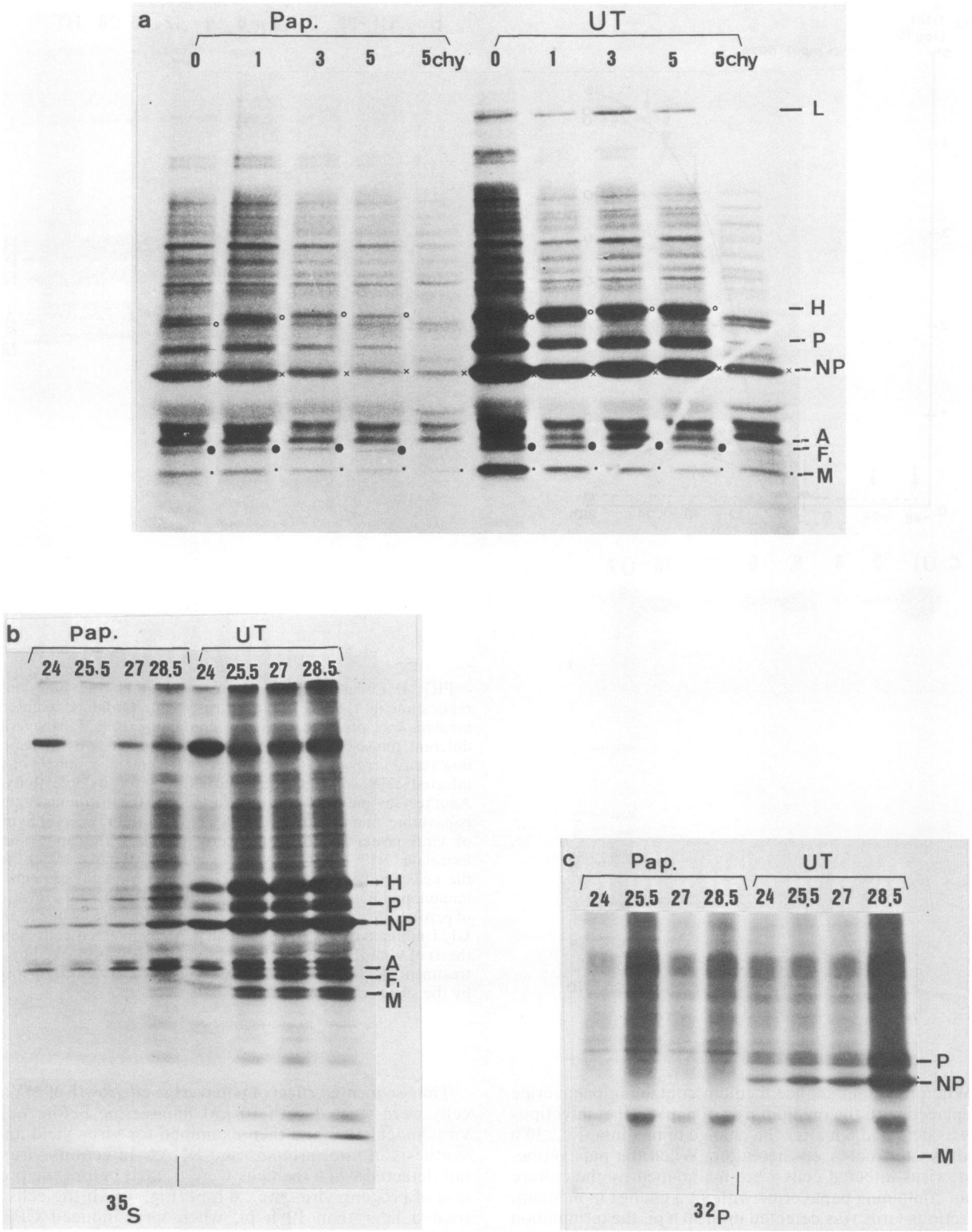


FIG. 5. Effect of papaverine on processing of viral proteins in IMR cells. (a) Pulse-label and -chase of viral proteins. Virus-infected cells were treated with papaverine (Pap.) at 12 h pi or were untreated (UT), labeled with [^{35}S]methionine for 1 h at 24 h pi, and then chased for 1, 3, or 5 h. To remove outer membrane proteins, 5-h-chased monolayer cells were treated with chymotrypsin (5chy) before harvesting the cells. In both papaverine-treated and untreated cells, increase in molecular weight of H protein (○) and appearance of F_1 protein (●) are noted. With chymotrypsin treatment, H and F_1 proteins disappear, whereas inner proteins such as NP (x) and M (■) do not disappear. (b) Kinetics of viral protein synthesis. Monolayer cells were treated with papaverine 12 h pi, or untreated, and labeled with [^{35}S]methionine from 24 to 30 h pi at 90-min intervals. Numbers indicate the starting time (h pi) of the labeling. (c) Kinetics of viral protein phosphorylation. Cells were treated and labeled with [^{32}P]orthophosphate by the same schedule as above.

dominantly, but H, F_1, and M proteins were not detected. If treatment was started later than 6 h pi, all the viral component proteins were produced; labeling of viral proteins was increased parallel with the delayed starting of papaverine treatment.

Viral RNA synthesis was completely inhibited by treatment with papaverine at any time after virus inoculation (Fig. 4c). These results suggest that papaverine inhibits the transcription of viral RNAs but not the translation of the proteins from viral mRNAs.

Effect of papaverine on processing of viral proteins. To investigate the processing of viral proteins in the presence of papaverine, IMR cells were treated with 10 μM papaverine after 12 h pi. In this condition, the virus yield was markedly suppressed, as mentioned above, whereas translation of viral proteins proceeded.

Electron microscopic examination revealed that the number of budding virions was remarkably smaller in the papaverine-treated sample than was that of untreated controls (data not shown). However, the virions budding from the papaverine-treated cells were not morphologically distinguishable from those of untreated cells.

Glycosylation and the transport of viral glycoproteins to the cell membrane were examined by combination of a pulse-chase experiment and protease treatment of the infected cells. The molecular weight of H protein increased during the chase periods, suggesting that glycosylation of H protein occurred in papaverine-treated cells, as it did in untreated controls (Fig. 5a). The cleavage of fusion protein from F_0 to F_1 was also observed in both cases. Moreover, H and F_1 proteins, which were detected in 5-h-chased samples, disappeared after treatment of the cells with chymotrypsin, although the inner proteins such as NP and M remained. These results indicate that viral glycoproteins are normally processed and transported to the membrane even in papaverine-treated cells.

Phosphorylation of viral proteins was examined by temporal pulse-labeling of the cells with [^{35}S]methionine and [^{32}P]orthophosphate. All the viral component proteins were demonstrated in papaverine-treated cells by labeling with [^{35}S]methionine, although the labeling efficiency was not as good as that of untreated controls (Fig. 5b). When the infected cells were pulse-labeled with [^{32}P]orthophosphate by the same time schedule, the isotope was incorporated into P and NP proteins and weakly into M protein in untreated controls. However, the phosphorylation did not occur in any of these proteins in papaverine-treated cells (Fig. 5c).

DISCUSSION

MV is known to persist in the central nervous system and to induce a slow virus disease, subacute sclerosing panencephalitis. The mechanisms of the persistent infection and virus-cell interactions in the brain, however, have not been well understood. Recently. Wechsler and Meissner (20) reviewed MV-cell interactions in terms of the replication mode of both MV and subacute sclerosing panencephalitis viruses in various cell lines and concluded that host cell and viral factors play important roles in persistent infections.

In this study, cellular conditions were experimentally modified by increasing endogenous cAMP by papaverine treatment. The treatment generally caused suppression of MV-induced CPE and virus replication. As reported by Miller and Carrigan (11), neuroblastoma cells were highly susceptible to the treatment, although oligodendroglioma cells were relatively resistant. Moreover, nonneural KB cells were also susceptible, as were AV_3 cells (15). Thus, cAMP-associated suppression of MV growth occurred in both neural and nonneural cells. Papaverine is known to block the activity of cAMP phosphodiesterase, resulting in an increase of the endogenous cAMP. The low efficiency of papaverine-induced suppression in the resistant cells may be responsible for the low permeability of papaverine, the low-level production of cAMP, or the abundant production of phosphodiesterase. It may be necessary to measure the in situ cAMP level in brain cells to investigate MV-cell interactions, including persistent infections in the central nervous system.

In neuroblastoma cells, in which the most marked suppressive effect was noticed, early steps of virus growth, such as adsorption, penetration, uncoating, translation of the viral proteins from the mRNAs, and processing of the viral glycoproteins, occurred normally, whereas synthesis of viral RNAs and phosphorylation were inhibited. Thus, suppression of viral RNA synthesis is suggested as a mechanism of papaverine-induced inhibition of MV replication. In a similar study, Miller and Carrigan (11) speculated that selective suppression of M protein production caused inhibition of MV replication in neuroblastoma cells. The discrepancy of these results might be ascribed to the difference in susceptibility to papaverine of the neuroblastoma cell lines. In fact, the suppressive effect of papaverine treatment differed from cell to cell; synthesis of F_1 and M proteins seemed to be selectively blocked by papaverine treatment in KB and MGC cells, in contrast to the suppression of all viral proteins in IMR cells.

Reversible suppression of MV growth by papaverine was reported already by Miller and Carrigan (11). A state of silent infection, in which MV growth is inhibited, was reported for small lymphocytes, and active production of the progeny virus is induced in lymphoblasts which were transformed by phytohemagglutinin (10, 18). Although mechanisms for virus persistence in small lymphocytes are unknown, this situation seems to superficially resemble that of MV infection in papaverine-treated IMR cells. The inhibition of viral RNA synthesis and the reversibility of the papaverine-induced inhibition may suggest that MV replication is modified to a nonproductive genomic form by change in the microenvironment of host cells, such as differentiated neural cells. This type of persistent infection in a genomic form has been reported for herpes simplex virus infection, in which the virus undergoes latency at the regional ganglion and is reactivated when the peripheral region is injured and neuronal regeneration occurs (17).

Several possibilities have been proposed for the persistence mechanism of MV in subacute sclerosing panencephalitis: split tolerance of cell-mediated immunity to MV, blocking factors, silent infection in lymphocytes, a defect in M protein, and antigenic modulation by the antibody (10, 13, 20). Persistence of virus in genomic form needs to be considered as another possibility. By use of an in situ hybridization technique, Haase et al. (5) demonstrated the existence of viral RNA in a part of the brain of subacute sclerosing panencephalitis patients, in which immunofluorescence testing failed to detect viral antigens. The result is compatible with the hypothesis of virus persistence in genomic form.

Electrophoretic patterns of the MV mRNAs were reported by Hall et al. (6), Gorecki and Rozenblatt (4), and Rozenblatt et al. (16). In this study, 10 different species of RNA were detected under conditions in which cellular RNA synthesis was completely inhibited by AMD. The largest one was considered to be equivalent to the negative-stranded viral

genome, since major parts could not bind to oligodeoxyribosylthymine-cellulose (data not shown). The characterization of another nine mRNAs is now in progress.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Ten different species of MV RNAs, as indicated in the present study, were recently described by S. A. Udem and K. A. Cook (J. Virol. **49**:57–65, 1984).

LITERATURE CITED

1. **Bigner, D. D., S. H. Bigner, J. Ponten, B. Westermark, M. S. Mahaley, Jr., E. Ruoslahti, H. Herschman, L. F. Eng, and C. J. Wikstrand.** 1981. Heterogeneity of genotypic and phenotypic characteristics of fifteen permanent cell lines derived from human gliomas. J. Neuropathol. Exp. Neurol. **40**:201–229.
2. **Fleury, H., and P. D. Pasquier.** 1977. Replication of measles virus in a cell culture from a glioblastoma of human origin. J. Neuropathol. Exp. Neurol. **36**:842–845.
3. **Fraser, K. B., and S. J. Martin.** 1978. Measles virus and its biology. Academic Press, Inc., London.
4. **Gorecki, M., and S. Rozenblatt.** 1980. Cloning of DNA complementary to the measles virus mRNA encoding nucleocapsid protein. Proc. Natl. Acad. Sci. U.S.A. **77**:3686–3690.
5. **Haase, A. T., P. Ventura, C. J. Gibbs, Jr., and W. W. Tourtellotte.** 1981. Measles virus nucleotide sequences: detection by hybridization in situ. Science **212**:672–675.
6. **Hall, W. W., W. Kiessling, and V. ter Meulen.** 1978. Membrane proteins of subacute sclerosing panencephalitis and measles viruses. Nature (London) **272**:460–462.
7. **Knipe, D. M., H. F. Lodish, and D. Baltimore.** 1977. Localization of two cellular forms of the vesicular stomatitis viral glycoprotein. J. Virol. **21**:1121–1127.
8. **Kobune, K., K. Yamanouchi, Y. Yoshikawa, M. Hayami, and A. Shishido.** 1979. Growth of measles virus in continuous cell lines derived from the nervous tissues of human and rat. Arch. Virol. **61**:115–125.
9. **Kohno, S., M. Kohase, and M. Suganuma.** 1968. Growth of measles virus in a mouse derived established cell line L cells. Jpn. J. Med. Sci. Biol. **21**:301–311.
10. **Lucas, C. J., J. C. Ubels-Postma, A. Rezee, and J. M. D. Galama.** 1978. Activation of measles virus from silently infected human lymphocytes. J. Exp. Med. **148**:940–952.
11. **Miller, C. A., and D. R. Carrigan.** 1982. Reversible repression and activation of measles virus infection in neural cells. Proc. Natl. Acad. Sci. U.S.A. **79**:1629–1633.
12. **Miyake, E.** 1979. Establishment of a human oligodendroglial cell line. Acta Neuropathol. (Berlin) **46**:51–55.
13. **Morgan, E. M., and F. Rapp.** 1977. Measles virus and its associated diseases. Bacteriol. Rev. **41**:636–666.
14. **Ponten, J., and E. H. Macintyre.** 1968. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. **74**:465–486.
15. **Robbins, S. J., and F. Rapp.** 1980. Inhibition of measles virus replication by cyclic AMP. Virology **106**:317–326.
16. **Rozenblatt, S., C. Gesang, V. Lavie, and F. S. Neumann.** 1982. Cloning and characterization of DNA complementary to the measles virus mRNA encoding hemagglutinin and matrix protein. J. Virol. **42**:790–797.
17. **Stroop, W. G., and J. R. Baringer.** 1982. Persistent, slow and latent viral infections. Prog. Med. Virol. **28**:1–43.
18. **Sullivan, J. L., D. W. Barry, S. J. Lucas, and P. Albrecht.** 1975. Measles infection of human mononuclear cells. I. Acute infection of peripheral blood lymphocytes and monocytes. J. Exp. Med. **142**:773–784.
19. **Tumilowicz, J. J., W. W. Nichols, J. J. Cholon, and A. E. Greene.** 1970. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res. **30**:2110–2118.
20. **Wechsler, S. L., and H. C. Meissner.** 1982. Measles and SSPE viruses: similarities and differences. Prog. Med. Virol. **28**:65–95.
21. **West, G. J., J. Uki, H. R. Herschman, and R. C. Seeger.** 1977. Adrenergic, cholinergic, and inactive human neuroblastoma cell lines with the action-potential Na^+ ionophore. Cancer Res. **37**:1372–1376.
22. **Yoshikawa, Y., K. Yamanouchi, Y. Morikawa, and M. Sakaguchi.** 1983. Characterization of canine distemper viruses adapted to neural cells and their neurovirulence in mice. Microbiol. Immunol. **27**:503–518.