

1981

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Recommended Citation

Menna, Jay H. and May, John D. (1981) "Non-Measles Hemadsorption in a Cell Line Persistently Infected with Measles Virus (BGM/MV)," *Journal of the Arkansas Academy of Science*: Vol. 35 , Article 14.

Available at: <http://scholarworks.uark.edu/jaas/vol35/iss1/14>

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NON-MEASLES HEMADSORPTION IN A CELL LINE PERSISTENTLY INFECTED WITH MEASLES VIRUS (BGM/MV)

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ABSTRACT

Adsorption of Rhesus monkey erythrocytes to the plasma membranes of measles virus-infected cells is frequently carried out to detect the presence of plasma membrane-associated measles virus hemagglutinin. The hemagglutinin is a viral genome-coded structural glycoprotein of the measles virus that is associated with the plasma membrane of the host cell during measles virus replication. BGM/MV, a non-virogenic line of African green monkey kidney cells persistently-infected with measles virus, adsorbed Rhesus monkey erythrocytes in an inverse fashion relative to the number of cells present in the culture and the time post-seeding. Serological studies employing the hemadsorption-inhibition and membrane immunofluorescence assay procedures, suggested that this phenomenon was not mediated by the viral hemagglutinin. Assays for Simian virus-5 and mycoplasma, contaminating agents that induce erythrocyte adsorption, were negative. Incubation of BGM/MV cells at 33°C or with graded concentrations of fetal calf serum, to stimulate the metabolism of resting (G_0) cells, suggested that adsorption was related to a phase(s) of the cell growth cycle other than G_0 , for adsorption was prolonged and stimulated in a dose-response fashion, respectively. Comparative adsorption studies employing the parent cell line (BGM), not infected with measles virus, were performed using various species of erythrocytes. While both cell lines adsorbed Rhesus monkey erythrocytes in an inverse fashion relative to cell density, differences were noted in the adsorption of some of the other species of erythrocytes. These data suggest that Rhesus monkey erythrocyte adsorption to BGM/MV cells was mediated by a receptor(s) of cellular origin.

INTRODUCTION

The adsorption of erythrocytes to the plasma membranes of cultured cells, hemadsorption (HAD), is frequently used to detect cell surface-associated viral antigens. Care must be taken in the interpretation of HAD results, as a number of apparently uninfected cell lines have been found to spontaneously adsorb various species of erythrocytes (Franks et al., 1963; Kano and Milgrom, 1965; Neuman and Tytell, 1965).

This report describes studies involving the BGM/MV cell line, which consists of the BGM cells (African green monkey kidney cells) (Barron et al., 1970) which are persistently infected with a mouse-adapted neurotropic strain of measles virus. The cell line was established by Menna et al. (1975a) and has been well characterized (Menna et al., 1975a,b; Flanagan and Menna, 1976). As determined by indirect immunofluorescence assay, > 99% of the cells contained cytoplasmic measles virus antigens, whereas, less than 1% of the cells possessed detectable levels of cell surface measles antigens (Menna et al., 1975a). Infectious measles virus was not recovered from the cells, nor could it be induced by treating the cells with various metabolic inhibitors or by enucleating the cells with cytochalasin B (Menna et al., 1975a). Although treatment of confluent cell monolayers with metabolic inhibitors failed to induce the synthesis of infectious measles virus, several of the metabolic inhibitors when added to BGM/MV cells induced the expression of measles cell surface antigens (Menna et al., 1975a; Flanagan and Menna, 1976).

Studies by May and Menna (1979) revealed changes in the stable virus host cell relationship characteristic of the BGM/MV line of cells. An apparent cyclic expression of cytopathic effect occurred in concert with changes in the percent of cells with intracellular and cell surface measles antigens. In the course of these studies it was noted that the BGM/MV cells exhibited spontaneous (non-induced) HAD activity of Rhesus monkey erythrocytes inversely to the total number of cells present in the culture. The results of experiments carried out to characterize this phenomenon are discussed in this report.

METHODS AND MATERIALS

Cell Lines.

BGM, a stable line of African green monkey kidney cells (Barron et al., 1970) were passaged weekly by trypsinization. The cells were seeded in Eagles' Minimum Essential Medium (EMEM) supplemented with 10% fetal calf serum (FCS) and 50 ug per ml of gentamicin. BGM cell cultures were incubated at 37°C in an atmosphere of 5% CO_2 .

The BGM/MV cell line was derived by the co-cultivation of BGM cells with mouse brain cells prepared from C3H mice infected *in vivo* with a neurotropic strain of measles virus (Menna et al., 1975a). BGM/MV cells are persistently infected with measles virus and are morphologically and antigenically BGM-like cells (Menna et al., 1975b).

BGM/MV cells were passaged every four days by trypsinization and were seeded in EMEM supplemented with 10% FCS and gentamicin at a concentration of 50 ug per ml. Unless otherwise noted, BGM/MV cells were incubated at 37°C in an atmosphere of 5% CO_2 .

Viruses.

Measles virus, Edmonston strain (MV-500E), was obtained from Dr. T. D. Flanagan, Buffalo, N.Y. Stock virus consisted of clarified infectious BGM cell lysates.

Stock Simian virus-5 (SV-5), originally obtained from the American Type Culture Collection, was prepared as an infectious clarified BGM cell lysate.

Antisera.

African green monkey serum directed against measles virus anti-

gens (Gallagher and Flanagan, 1976) was obtained from Dr. Flanagan. The antiserum was exhaustively absorbed with BGM cells before use.

Rabbit antisera to SV-5 was obtained from Microbiological Associates, Bethesda, MD. Prior to use, the antisera was exhaustively absorbed with BGM cells.

Fluorescein isothiocyanate (FITC)-labeled goat antiserum to human IgG and FITC-labeled goat antiserum to rabbit IgG (Hyland Co., Costa Mesa, CA.) were used in the indirect immunofluorescence assays for measles virus antigens and SV-5 antigens, respectively.

Erythrocytes.

Rhesus monkey erythrocytes were provided by Drs. D. E. Hill and A. A. Krum, University of Arkansas for Medical Sciences. Guinea pig, chicken, rabbit, and human group-O erythrocytes were provided by the Department of Microbiology and Immunology, University of Arkansas for Medical Sciences.

Cell Enumeration.

Monolayer cultures of BGM/MV cells were trypsinized, suspended in EMEM, and cells were counted using a Spencer hemacytometer.

Hemadsorption Assay.

Hemadsorption assays were performed using the procedure of Menna et al. (1975a). Briefly, cell cultures were washed three times in EMEM, and then were incubated at room temperature for 60 min with an appropriate volume of a 0.5% Rhesus monkey erythrocyte suspension prepared in EMEM. The cell cultures were then washed three times with EMEM and were observed microscopically (100 \times) for adherent erythrocytes. Cells with three or more adherent erythrocytes were considered HAD-positive. The degree of hemadsorption was quantified in replicates of three cultures, and the mean percent HAD-positive cells was determined.

Hemadsorption-inhibition (HADI) assay.

Hemadsorption-inhibition assays were performed using the procedure described by May and Menna (1979) to determine the specificity of the adsorption of Rhesus monkey erythrocytes to the plasma membranes of BGM/MV cells. Cultures of BGM/MV, BGM, and BGM cells lytically-infected with the MV-500E strain of measles virus, were washed three times with EMEM. Then 1 ml volumes of varying dilutions of heat-inactivated (56 $^{\circ}$ C for 30 min) African green monkey antiserum to measles virus antigens was added to replicate cultures of each series. As controls, replicate cultures of each series were treated with pre-immune African green monkey serum or were mock-treated with EMEM. The cell cultures were then incubated for 1 hr at 37 $^{\circ}$ C, washed three times with 1 ml volumes of EMEM, and an HAD assay employing Rhesus monkey erythrocytes was carried out as described above.

Membrane Immunofluorescence Assay.

BGM/MV cells were assayed for membrane-associated measles virus antigens using the procedure of Menna et al. (1975a). Briefly, unfixed BGM/MV cells, grown on 9 \times 22 mm coverslips (Bellco Biological Glassware, Vineland, N.J.) were rinsed three times with phosphate buffered saline (PBS, pH 7.2) and were then treated for 30 min at 4 $^{\circ}$ C with a 1:10 PBS dilution of African green monkey antiserum to measles virus antigens (heat-inactivated, 56 $^{\circ}$ C for 30 min). As controls, BGM/MV cells treated with pre-immune African green monkey antiserum, or with PBS alone, were assayed in parallel. In

each assay, to assess the activity of the anti-measles virus antiserum, a positive control was assayed in parallel, which consisted of BGM cells lytically-infected with the MV-500E strain of measles virus and uninfected BGM cells. Following incubation, all of the cultures were washed for 20 min in cold PBS to remove unreacted antiserum. The cultures then were treated with FITC-labeled goat antiserum to human IgG and incubated at 4 $^{\circ}$ C for 20 min. The cell cultures were washed in cold PBS for 10 min, the coverslips were mounted in buffered glycerol (1 part glycerol to 9 parts PBS), and the cells were observed for membrane immunofluorescence using a Zeiss microscope equipped with a fluorescence epi-illuminator.

Immunofluorescence Assay for Intracellular Antigens.

The indirect method of immunofluorescence was used for detecting SV-5 antigens in BGM/MV cells. BGM/MV cells grown on 9 \times 22 mm coverslips were fixed in acetone at 4 $^{\circ}$ C for 10 min. Following fixation, the coverslips were air dried at room temperature and washed for 5 min in cold PBS. Appropriately diluted rabbit antiserum to SV-5 antigens was then added to the cell monolayers, and the cultures were incubated for 30 min at 37 $^{\circ}$ C. Additional acetone-fixed monolayers of BGM/MV cells were assayed in parallel, using pre-immune rabbit serum and PBS alone. Also, in each assay, a virus control consisting of SV-5-infected BGM cells and a negative-control consisting of uninfected BGM cells were assayed in parallel. Following incubation, the cell monolayers were washed for 20 min in cold PBS and treated with appropriately diluted FITC-labeled goat antisera to rabbit IgG for 30 min at 37 $^{\circ}$ C. Then, they were washed for 20 min in cold PBS, mounted in buffered glycerol, and observed using a Zeiss microscope equipped with a fluorescence epi-illuminator.

Assay of Cell Viability.

Viability of BGM/MV cells was assessed using the trypan blue (Sigma Chemical Co., St. Louis, MO.) exclusion staining procedure of Merchant et al. (1964).

RESULTS

Although spontaneous HAD activity occurred during both the cytopathic and non-cytopathic phases of the cyclic expression of the measles virus infection of the BGM/MV cells, only the non-cytopathic phases were investigated. This was necessitated by the difficulty in quantifying cells during periods of syncytial cell expression. The results of a representative experiment demonstrating the inverse relationship between cell number and the spontaneous adsorption of Rhesus monkey erythrocytes are shown in Figure 1.

BGM/MV cells were seeded at a concentration of 2.5×10^4 cells per ml, and the cultures were incubated at 37 $^{\circ}$ C in an atmosphere of 5% CO $_2$. At daily intervals for four days, replicates of three cultures were harvested for total cell determination, and three cultures were harvested for determination of the percent hemadsorbing cells. Greater than 99% of the BGM/MV cells adsorbed Rhesus monkey erythrocytes during the initial 48 hrs post-seeding (PS). Further incubation of the cells at 37 $^{\circ}$ C resulted in an increased number of cells and a pronounced decrease in HAD activity. At 72 and 96 hrs PS, successive doublings occurred essentially in the number of cells while the HAD activity had decreased to 20% and <1%, respectively. Cell viability at all times was >95%.

The apparent association of cell growth density with the expression of cell surface receptors for Rhesus monkey erythrocytes was further investigated by comparing the growth rate and HAD activity of BGM/MV cells grown at 33 $^{\circ}$ C and 37 $^{\circ}$ C. BGM/MV cells were seeded at 8×10^4 cells per ml. One-half of the cultures were incubated at 37 $^{\circ}$ C in an atmosphere of 5% CO $_2$; the remaining cultures, in an identical atmosphere at 33 $^{\circ}$ C. At daily intervals thereafter for

five days, three cultures from each series were harvested for enumeration of cells and three for HAD assay. The results of this experiment are shown in Figure 2. Cells grown at 33° C replicated at a slower rate and had an elevated HAD activity for an extended period of time when compared with cells grown at 37° C. At 96 hrs PS, <1% of the cells grown at 37° C were HAD-positive, whereas >99% of the cells grown at 33° C were positive. At 120 hrs PS, the HAD activity of the cultures grown at 33° C had decreased by approximately 75% from the level observed during the initial 96 hrs PS.

To determine if the spontaneous HAD activity was measles virus-specific, an HADI assay was performed using heat-inactivated (56° C

for 30 min) African green monkey serum directed against measles virus antigens. Treatment of replicate monolayers of BGM/MV cells at 24 hrs PS for 1 hr at 37° C with the monkey anti-measles serum failed to block or reduce the adsorption of Rhesus monkey erythrocytes to the cells. But the antiserum blocked the adsorption of Rhesus monkey erythrocytes to the parent BGM cells which were lytically-infected with the MV-500E strain of measles virus. To further document that the HAD activity was not mediated by measles virus hemagglutinin, BGM/MV cells were examined daily PS for four days for the presence of plasma membrane-associated measles virus antigens by the indirect immunofluorescence assay procedure employing unfixed cells. No correlation was observed between the cell density-associated HAD activity of the cells and membrane immunofluorescence, for the percent of cells spontaneously expressing antigen failed to fluctuate with time PS.

To further characterize the non-measles virus-mediated density-dependent HAD activity of BGM/MV cells, comparative adsorption studies were performed using the parent uninfected cell line. BGM/MV and BGM cells were seeded at a concentration of 2.5×10^4 cells per ml and the cell cultures were incubated at 37° C in an atmosphere of 5% CO₂. At 24 and 96 hrs PS, standard HAD assays were performed using replicates of three cultures of each cell line per species of erythrocyte tested. These erythrocytes were guinea pig, chicken, human group-O, rabbit, and Rhesus monkey. The results of this experiment are shown in Table 1. BGM/MV cells adsorbed only two species of erythrocytes, Rhesus monkey and human group-O, and only at 24 hrs PS. In contrast, the parent BGM cell line adsorbed all species of erythrocytes except guinea pig. Of particular interest is that the parent BGM cell line also adsorbed Rhesus monkey erythrocytes inversely to the time PS, and presumably inversely to cell number.

Guinea pig and chicken erythrocytes failed to adsorb to the BGM/MV cells at 24 hrs PS, a time at which >99% of the cells were capable of adsorbing Rhesus monkey erythrocytes. This failure suggested that the cell density-dependent HAD of Rhesus monkey erythrocytes by BGM/MV cells was not due to contamination with SV-5, a virus that will induce the HAD of both guinea pig and chicken erythrocytes (White, 1962). This contention was further supported by indirect immunofluorescence assays for SV-5 antigens in BGM/MV cells. No SV-5 antigens were detected when the cells were assayed at various times PS.

Since cells contaminated with mycoplasma have been shown capable of inducing HAD (Berg and Frothingham, 1961), living BGM/MV cells and spent-culture media from two passage levels were submitted to Flow Laboratories (Rockville, MD) for mycoplasma analysis. No mycoplasma were detected using the agar plate and Hoechst staining method.

The age of the indicator erythrocytes used in HAD assays is important for specificity. Dowdle and Robinson (1966) reported non-viral mediated HAD of guinea pig erythrocytes to confluent monolayers of primary Rhesus monkey kidney cells when using erythrocytes stored for more than 72 hrs at 4° C in PBS. In our studies the

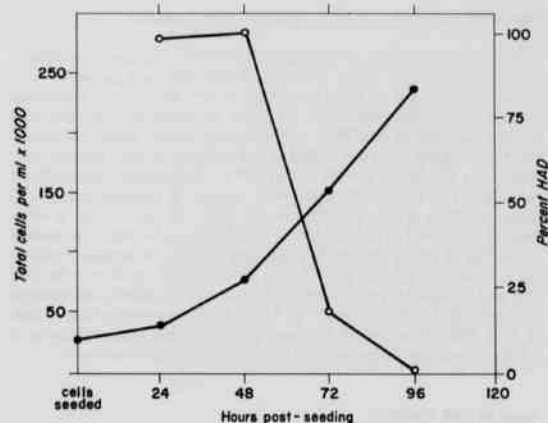


Figure 1. The number of cells per ml and the percent of cells adsorbing Rhesus monkey erythrocytes during the growth of BGM/MV cells. BGM/MV cells were seeded at 2.5×10^4 cells per ml in growth medium and were incubated at 37° C in an atmosphere of 5% CO₂. All points represent the mean of three replicates; ●—●, total number of cells per ml; ○—○, percent HAD-positive cells.

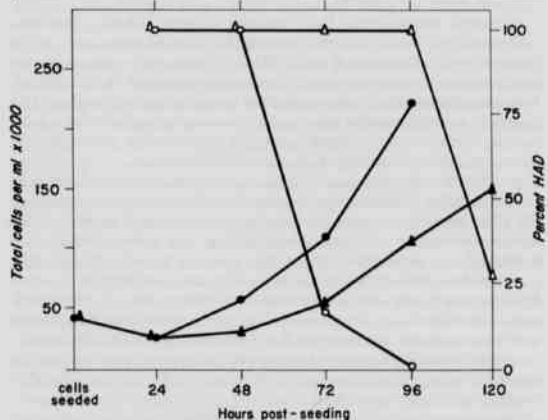


Figure 2. Comparison of the rate of growth and the capacity to adsorb Rhesus monkey erythrocytes of BGM/MV cells grown at 33° C and 37° C. All points represent the mean of three replicates; ●—●, total cells per ml at 37° C; ○—○, percent HAD-positive cells at 37° C; ▲—▲ total cells per ml at 33° C; △—△ percent HAD-positive cells at 33° C.

Table 1. Adsorption of erythrocytes to BGM/MV and BGM cells

ERYTHROCYTES	PERCENT HAD			
	BGM/MV		BGM	
	24 HRS	96 HRS	24 HRS	96 HRS
Rhesus Monkey	>99	<1	60	15
Human Grp. O	45	<1	<1	15
Guinea Pig	<1	<1	<1	<1
Chicken	<1	<1	30	90
Rabbit	<1	<1	40	90

Table 2. Induction of HAD activity in BGM/MV cells by fetal calf serum^a

PERCENT FETAL CALF SERUM (VOL/VOL)	PERCENT HAD
0	<1
5	10
10	30
15	60
20	90

^aReplicate confluent monolayers of BGM/MV cells were treated with various concentrations of fetal calf serum (vol/vol) in growth medium for 24 hours at 37° C and an HAD assay employing Rhesus monkey erythrocytes was performed on replicates of three cell monolayers per fetal calf serum concentration. Each value represents the mean percent HAD activity. HAD values at each fetal calf serum concentration varied <5%.

adsorption of Rhesus monkey erythrocytes occurred using freshly acquired blood and decreased as a function of increasing BGM/MV cell number using the same lots of erythrocytes.

Experiments were performed to further substantiate that the non-measles virus mediated adsorption of Rhesus monkey erythrocytes to BGM/MV cells was related to a phase of cellular metabolism not associated with the resting cell. To this end, confluent BGM/MV cell monolayers were treated with varying amounts of FCS, a complex biological material known to stimulate cell division in resting confluent monolayers of contact-inhibited cells (Temin, et al., 1972). Treatment of confluent monolayers of HAD-negative BGM/MV cells with EMEM supplemented with varying amounts of FCS for 24 hrs at 37° C resulted in increased HAD of Rhesus monkey erythrocytes relative to the amount of FCS present (Table 2). The FCS-induced HAD activity was similar to the cell density-dependent HAD activity in that it could not be blocked by monkey anti-measles virus serum and the percent of cells with plasma membrane-associated measles virus antigens did not increase when the cells were analyzed by membrane immunofluorescence assay.

DISCUSSION

While several other reports of non-viral mediated HAD activity have been published (Franks et al., 1963; Kano and Milgrom, 1965; Neuman and Tytell, 1965) the present report is the first documenting a cell density-dependent, HAD.

Characterization of the cell density-dependent HAD manifested by BGM/MV cells indicated that it was not due to measles virus hemagglutinin or to contamination of the cells with SV-5 or mycoplasma. These observations suggested that the receptor(s) mediating the attachment of the Rhesus monkey erythrocytes is coded for by a cellular gene(s) and are further supported by the observation that the parent uninfected cell line, BGM, also adsorbed Rhesus monkey erythrocytes in an apparent cell density-dependent fashion. Also, BGM/MV cells adsorb human group-O erythrocytes in a cell density-dependent fashion, cells not capable of interacting with measles virus hemagglutinin (Waterson, 1965).

A dose-response relationship was shown between FCS concentration and the percent induction of HAD in confluent monolayers of HAD-negative BGM/MV cells. This relationship and the sustained HAD activity in BGM/MV cells maintained at 33° C, relative to cells maintained at 37° C, suggest that the spontaneous HAD of Rhesus monkey erythrocytes is related to some phase(s) of active cell replication and not solely to cell density.

The expression of specific cell surface antigens and receptors as a function of certain phases of the cell growth cycle have been reported by others. Thomas (1971) found that the expression of B and H blood group antigens on the surface of cultured mouse P815Y cells was cyclic in nature. Resting cells were negative for the B antigen and positive for the H antigen, while actively replicating cells were B antigen positive and negative for the H antigen. It was also found that phytohemagglutinin stimulation of mouse (BALB/C) lymphocytes resulted in the expression of the B antigen.

The agglutination of normal 3T3 mouse fibroblasts by concanavalin A was found to be cell cycle-dependent by Collard et al. (1975) and to occur only in cell mitosis. The agglutination of synchronized cultures of transformed 3T3 cells, however, was maximum in G1 and in mitosis.

Our experience with the BGM/MV cell line suggests that HAD, especially if present in cell lines persistently-infected with virus, should be confirmed as viral specific by serological assay. Two types of HAD of Rhesus monkey erythrocytes have been observed in BGM/MV cells: 1) HAD activity which can be serologically shown to be measles virus-specific (Menna et al., 1975a; Flanagan and Menna, 1976; May and Menna, 1979); and 2) an inherent HAD activity which is non-measles in nature and is apparently associated with the cell-growth cycle.

ACKNOWLEDGMENTS

This work was supported by the Graduate Student Research Allocation Fund of the University of Arkansas for Medical Sciences and by Public Health Service Grant 5RESO 171502 EVR from the National Institute of Environmental Health Sciences.

LITERATURE CITED

- BARRON, A. L., C. OLSHEVSKY and M. M. COHEN. 1970. Characteristics of the BGM line of cells from African Green monkey kidney. *Archiv Gesamte Virusforsch.* 32:289-392.
- BERG, R. B. and T. E. FROTHINGHAM. 1961. Hemadsorption in monkey kidney cell cultures of mycoplasma (PPO) recovered from rats. *Proc. Soc. Exp. Biol. & Med.* 108:616-618.
- COLLARD, J. G., J. H. M. TEMMINK and L. A. SMETS. 1975. Cell cycle-dependent agglutinability, distribution of concanavalin-A binding sites and surface morphology of normal and transformed fibroblasts. *Adv. Exp. Med. Biol.* 55:221-244.
- DOWDLE, W. R. and R. Q. ROBINSON. 1966. Non-specific hemadsorption by Rhesus monkey kidney cells. *Proc. Soc. Exp. Biol. & Med.* 121:193-198.
- FLANAGAN, T. D. and J. H. MENNA. 1976. Induction of measles virus hemagglutinin in a persistently infected, nonvirogenic line of cells (BGM/MV). *J. of Virol.* 17:1052-1055.
- FRANKS, D. R., R. A. COOMBS, T. S. L. BESWICK and M. M. WINTER. 1963. Recognition of the species origin of cells in culture by mixed agglutination. III. Identification of the cells of different primates. *Immunol.* 6:64-72.
- GALLAGHER, M. R. and T. D. FLANAGAN. 1976. Replication of measles virus in continuous lymphoid cell lines. *J. Immunol.* 116:1084-1088.
- KANO, K. and F. MILGROM. 1965. Serological adhesion in mammalian cell cultures. *Inter. Arch. Allergy.* 28:231-239.

- MAY, J. D. and J. H. MENNA. 1979. Changes in the virus-host cell relationship in a stable nonvirogenic cell line persistently infected with measles virus. (BGM/MV). *J. Gen. Virol.* 45:185-194.
- MENNA, J. H., A. R. COLLINS and T. D. FLANAGAN. 1975a. Characterization of an *in vitro* persistent-state measles virus infection: Establishment and virological characterization of the BGM/MV cell line. *Infect. & Immun.* 11:152-158.
- MENNA, J. H., A. R. COLLINS and T. D. FLANAGAN. 1975b. Characterization of an *in vitro* persistent-state measles virus infection: Species characterization and interference in the BGM/MV cell line. *Infect. & Immun.* 11:159-163.
- MERCHANT, D. J., R. H. KAHN and W. H. MURPHY. 1964. Handbook of cell and organ culture. Chapter 5, p. 157. Burgess Pub. Co., Minneapolis.
- NEUMAN, R. E. and A. A. TYTELL. 1965. Anomalous hemadsorption by cell cultures. *Proc. Soc. Exp. Biol. & Med.* 119:180-186.
- TEMIN, H. M., R. W. PIERSON and N. C. DULAK. 1972. The role of serum in the control of multiplication of avian and mammalian cells in culture. Vol. 1, p. 59. *In Growth Nutrition and Metabolism of Cells in Culture*, (G. H. Rothblat and V. J. Cristofolo, Eds.), Academic Press, New York.
- THOMAS, D. B. 1971. Cyclic expression of blood group determinants in marine cells and their relationship to growth control. *Nature* 233:317-321.
- WATERSON, A. P. 1965. Measles virus. *Arch. Gesamte Virusforsch.* 16:57-60.
- WHITE, D. O. 1962. Hemadsorption by uninoculated cultures of monkey kidney epithelium. *Aust. J. Exp. Biol. & Med. Sci.* 40:523-526.