DIFFERENTIATION OF CLOSELY RELATED CELLS BY A VARIANT OF POLIOVIRUS, TYPE 2, MEF$_1$ STRAIN*

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The conceptual and technical problems associated with latent viral infections have been summarized in recent reviews (1–3) from quite different points of view. Investigation of persistent infection by viruses of mammalian cells in culture has failed to disclose the anticipated occurrence of a lysogenic-like relationship. Numerous studies (4–12) have revealed, instead, a much more complex dynamic equilibrium which permits cells and virus to multiply simultaneously. Since it may not be too fruitful to insist that a lysogenic relationship must constitute a phase of mammalian cell-virus interaction, future experimental design should be more profitable if it takes into accord the multiple phenomena known to be operative. Although substantial progress has been made in establishing some of the factors that determine the nature of cellular susceptibility or resistance to mammalian virus infection (13), its applicability to the "non-cytopathogenic" virus-cell relationship has not been evaluated. Thus, in the case of "moderate" or "temperate" mammalian viruses the basis for the nature of cellular resistance to viral infection and ensuing cytopathogenicity has not been characterized within the framework of classical concepts (14).

Experiments which provided an experimental system that appeared to be satisfactory for such studies (15) are the subject of this report which describes the interaction between a variant of poliovirus, Type 2, MEF$_1$ strain and a spectrum of primate cell lines. The reaction between this virus and HeLa cells appears to be particularly suitable because of the: (*a*) relative thermal stability of virus; (*b*) sharp differential cytopathogenicity of virus for closely related cells; (*c*) availability of a number of genetic markers for virus including animal and cell culture hosts; (*d*) simultaneous serial propagation of both virus and cells without resort to the protective effect of homotypic antiserum; (*e*) observed stability of the virus-cell relationship over a period of 4 years (16).

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Materials and Methods

Virus.—The variant of poliovirus, Type 2, MEF_1 strain (MEF virus) employed for studies was obtained from Dr. Peter Olitsky as a suspension of infected mouse brain tissue. The large pool of stock virus used for experiments was prepared from infected *cynomolgus* monkey renal cell cultures maintained in a fluid consisting of Hanks' solution (87 per cent), calf serum (1 per cent), 4.4 per cent bicarbonate solution (2 per cent), and 5 per cent lactalbumin hydrolysate (10 per cent). Penicillin and streptomycin were added to virus preparations to yield a final concentration of 500 units and 100 μg. per ml. respectively. Virus was stored at −20°C. in sealed ampoules until used.

Cell Strains.—The strains of cells employed for studies have been described (17). A line of HeLa cells (Hoye) adapted to growth in horse serum 20 per cent and yeast extract medium (YEM) 80 per cent was also used. Cell strains selected for special purposes are described under individual experiments. Culture tubes used in serial passage experiments to study cytopathogenicity of virus were prepared by routine procedures (18). Medium employed for maintenance of cells grown in human serum (HuS) consisted in all cases of YEM (95 per cent), calf serum (CaS) 5 per cent, penicillin (500 units/ml.), and streptomycin (100 μg./ml.). Maintenance medium used for monkey kidney cells was described above. Cells grown in horse serum (HoS) were maintained in a solution of YEM (95 per cent) and HoS (5 per cent). Cell cultures were incubated at 37°C. and maintained under observation usually for 7 days; in some cases incubation was continued for 3 weeks to provide adequate opportunity for cytopathogenicity of virus to be expressed.

Virus Assays.—Monkey kidney tube cultures were employed to titrate virus in fluids obtained from serial passages of virus in cell strains; five tube cultures were used to assay each virus dilution; virus titer (19) was expressed in terms of $TCID_{50}$ (50 per cent tissue culture infectious doses).

Monolayer cultures of cell strains were prepared for plaque titrations in efficiency-of-plating experiments from vigorously growing stock cultures by dispersion of cells in 0.05 per cent Difco trypsin suspended in a buffered glucose solution (GKN) (17). Bottles (20) used for plaque titrations received an inoculum of cells (10^6) sufficient to assure that monolayers of young cells formed in 24 to 48 hours. Cultures grown in HuS were washed three times with GKN prior to addition of virus. Virus inoculum, 1.0 ml. of each dilution made up in Scherer's maintenance solution (MS) (95 per cent) and CaS (5 per cent), was incubated with cells at 37°C. for 1 hour. After incubation the inoculum was decanted and 5 ml. of overlay medium (3 parts of a solution of MS-80 per cent, CaS-20 per cent, and one part 3 per cent Noble agar made up in Hanks' solution) was added to monolayers. Five bottles were used for each virus dilution tested; cultures were incubated for 3 to 5 days at 37°C. Cell sheets were stained to reveal plaques by addition of 2.0 ml. of 1.5 per cent agar containing neutral red (final concentration 1:10,000). Virus titer was expressed as plaque-forming units (PFU) per ml. of undiluted virus.

EXPERIMENTAL

Latent Infection of HeLa Cells by a Variant of Poliovirus, Type 2, MEF_1 Strain (MEF Virus).—The term latent infection as used herein designates a persistent infection of cell cultures by virus without attendant cytopathogenic changes and in which both agent and host cells undergo serial propagation. MEF virus was added to bottles of HeLa cells grown in human serum proven to be free of antibody to poliovirus, Type 2, by neutralization tests. Parallel

experiments were carried out in which HeLa cells were grown in *cynomolgus* monkey serum demonstrably free of poliovirus antibodies. During the period that tests were in progress MEF virus-infected cultures were fed a solution of HuS 50 per cent MS 50 per cent. Rate of cell growth determined when culture bottles became overcrowded and required subculture. Supernatant fluid containing virus was removed and cells in donor bottles dispersed with trypsin. New bottles were seeded with cells suspended in 10 ml. of fresh growth medium. Virus content of supernatnant fluid from donor bottles was titrated either in mice or in monkey kidney cell cultures. Table I summarizes data from representative findings which provided evidence for serial propagation of both host

TABLE I

Persistent Infection of Parent HeLa Cells by a Non-Cytopathogenic Variant of Poliovirus, Type 2, MEF_1 Strain

Serial passage level of parent HeLa cells	0	3	6	8
Virus dilution (accumulative)	2×10^{-1}	1.6×10^{-5}	3.2×10^{-7}	1.0×10^{-11}
Titer of virus in* supernatant cell culture fluid	$10^{-3.7}$	$10^{-3.2}$	$10^{-3.4}$	$10^{-4.1}$

* Assays for virus were made by intracerebral inoculation of serial dilutions of supernatant fluids from cell cultures into 21 day old Swiss albino mice; LD_{50} titers were calculated according to the method of Reed and Muench (19).

cells and virus. Such results were regularly repeatable in the years 1954–58 and thus afforded evidence for a stable host-virus system.

Characterization of MEF Virus and Host Cell Relationships.—The observations described above led to basic inquiries concerning the nature of the virus strain used. Foremost among these was the question of whether the virus employed was in fact a poliovirus. The following survey experiments were undertaken to characterize MEF virus and the test strain of HeLa cells:

(*a*) *Infectivity of MEF Virus for cynomolgus Monkeys and Mice.*—MEF virus as a 10 per cent suspension of infected mouse brain tissue in Hanks' solution was inoculated intrathalamically, 1.0 ml., bilaterally, into each of two monkeys. One monkey died with symptoms of poliomyelitis; examination of sections from the cord and brain revealed histopathologic changes characteristic of poliomyelitis. Mice challenged by the intracerebral route (0.03 ml. of decimal dilutions of virus suspension) showed symptoms of typical poliomyelitis; MEF virus was not infective for either mice or monkeys by peripheral routes of inoculation. The titer of MEF virus in freshly prepared mouse brain preparations in repeated tests did not exceed an LD_{50} (19) of $10^{-4.7}$.

(*b*) *Neutralization of Poliovirus, Type 2, YSK Strain, by MEF Virus Antiserum.*—Serum from the monkey which survived MEF virus infection in spite of partial paralysis, was tested for its capacity to neutralize poliovirus, Type 2, YSK strain (YSK virus). Heat inactivated (56°C. for 30 minutes) MEF virus antiserum neutralized challenge doses of from 10^2 to 10^5 $TCID_{50}$ of YSK virus added to HeLa cell cultures.

(*c*) *Cytopathogenicity of Prototypic Polioviruses for HeLa Cells.*—To determine whether the test line of HeLa cells was inherently resistant to polioviruses, 10 $TCID_{50}$ of poliovirus, Type

TABLE II

Differential Susceptibility of Primate Cells to MEF virus Cytopathogenicity

Cell strain	Effect of virus*	Log $TCID_{50}$ titer of virus‡ in supernatant fluid
HeLa, parent	0	4.5
" , clone 4	0	4.5
Hoye, parent§	4^+	3.6
Conjunctiva, parent	0	4.5
" , clone B_2A_2	2^+	5.5
" , clone B_2A_4	2^+	3.83
Esophageal, parent	2^+	5.6
" , clone A_2b	4^+	5.5
Liver, parent	4^+	5.5
" , clone A_1A_1	2^+	3.83
" , clone A_2b	4^+	5.63
" , clone D_1B_1	0	4.4
Maben, parent	2^+	5.17
Cynomolgus monkey kidney cells	4^+	7.2
rhesus monkey kidney cells	4^+	7.5
Human amnion‖	4^+	Not done

* Cytopathogenicity was scored: (4^+) complete cell destruction; (2^+) incomplete cellular destruction; (0) no evident cytopathogenic changes.

‡ Titers from two replicate tests varied 0.5 log units when virus was assayed in tube monkey kidney cell cultures.

§ All stable human cell lines were grown in human serum except line Hoye grown in horse serum; the cells listed in this table were stock Minnesota cell strains.

‖ Cytopathogenic changes ensued only after 3 weeks incubation at 37°C.

1, Mahoney strain, Type 2, YSK strain, and Type 3, Saukett strain, were separately added to HeLa cell cultures, 10 tubes per test. Typical cell destruction occurred with all test viruses.

(*d*) *Possible Viral Contaminant of HeLa Cells.*—The possibility that the test strain of cells was contaminated by a virus which interfered with MEF virus multiplication and attendant cytopathogenicity was considered, although experiments with the same parent line of HeLa cells (17) indicated that this was a very remote possibility. To explore the question further undiluted MEF virus was added to HeLa cells (0.1 ml. to each of 5 culture tubes per test) and allowed to propagate for 3 days. Cultures were then challenged with 10^3 to 10^5 $TCID_{50}$ of cytopathogenic polioviruses (Type 1, Mahoney, and Type 2, YSK). Cells observed daily for a period of 7 days failed to show evidence of the cytopathogenic effect of challenge viruses. These findings were confirmed in two replicate experiments and provided evidence that

MEF virus, and not a contaminant virus, served as an interfering agent for fully cytopathogenic polioviruses (11).

Differential Cytologic Response of Cells to MEF Virus Infection.—A survey was undertaken to determine the spectrum of cytopathogenicity of MEF virus for primate cells and to provide a cell culture system which could be used for virus assay. Routine procedures were applied to assess the cytopathogenic effect of MEF virus for cells.

Monolayers of test cells grown on coverslips in Leighton tubes were stained with hematoxylin and eosin to provide a record of viral induced morphologic changes. Undiluted stock virus suspension (titer $10^{6.88}$ $TCID_{50}$/ml. in monkey kidney cells), 0.1 ml., was added to each of 10 culture tubes per test cell strain. Cells were maintained under optimal conditions for demonstration or normal cell morphology by addition of minimal amounts of feeding solution (equivalent volumes of 1.4 per cent bicarbonate solution and CaS 50 per cent or HoS 50 per cent). Cells were observed daily for as long as 3 weeks for evidence of viral cytopathogenicity: complete destruction of cells was scored 4+, intermediate 2+, and absence of cytopathogenicity 0. Three replicate experiments for each cell strain established the reliability of results.

The spectrum of response of cells to MEF virus is evident from Table II and Figs. 1 to 8. Morphologic reactions to viral infection ranged from complete cellular destruction to absence of cytopathogenicity. The intermediate type of cellular response was manifested in two qualitatively different categories of reaction which were readily differentiated. For example, in the case of clonal conjunctival cells B_2A_2 destruction was incomplete (Fig. 8) although all cells of the culture were similarly affected. In contrast, incomplete destruction of parent esophageal cells was characterized by the occurrence of colonies of cells entirely normal in morphology (Fig. 7). Titration experiments to be discussed in detail below, showed that MEF virus multiplied in all test cell strains.

Susceptibility of Cells of Diverse Origin to MEF Virus.—The possibility was considered that the cell strains employed for the preceding experiments may have been biologically unique compared with similar strains used in other laboratories. Lines of Chang's strains of conjunctival and liver cells adapted to growth in horse serum medium were obtained from the Department of Virus Diseases, Walter Reed Army Medical Center. Representative cell strains also were received from two commercial sources. The method for testing the susceptibility of cells to the cytopathogenic effect of MEF virus was described above.

The results of this experiment (Table III) revealed that MEF virus exerted a differential cytopathogenicity on very closely related cell lines. The data showed also that the resistance to cytopathogenic infection displayed by the Minnesota strains of HeLa and Chang's lines of epithelial cells was stable over a period of years.

Effect of Serial Passage of MEF Virus on Reaction of Cells to Infection.—

TABLE III

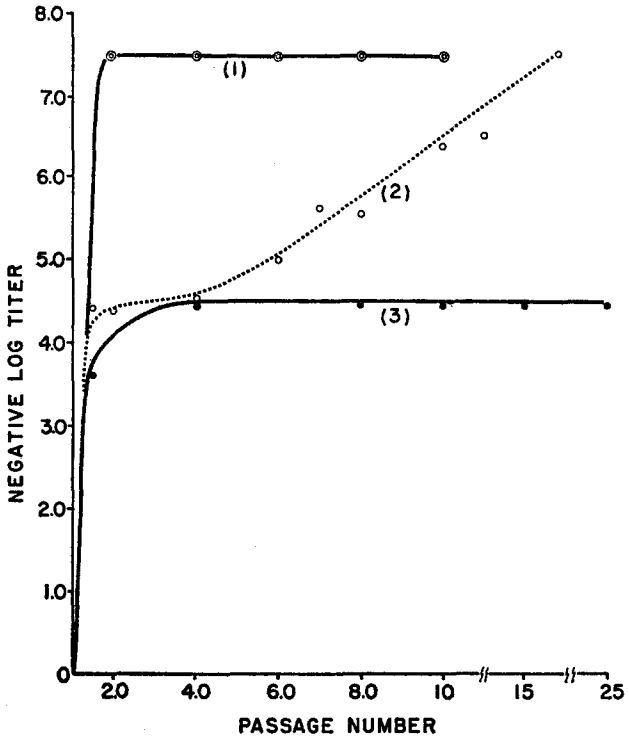
Susceptibility of Cells of Diverse Origin to MEF Virus

Cell strain	Date tested	Effect of virus
HeLa, clone S3 (C)* grown in human serum	1957	Multiplication, no cytopathogenicity
	1958	" " "
	1959	" " "
HeLa, parent (Gey) (C) grown in human serum	1957	Multiplication, complete cell destruction
	1958	" " " "
	1959	" " " "
HeLa, parent (Gey) (C) grown in horse serum	1957	Multiplication, complete cell destruction
	1958	" " " "
	1959	" " " "
HeLa, parent, grown in human serum	1954	Multiplication, no cytopathogenicity
	1955	" " "
	1956	" " "
	1957	" " "
	1958	" " "
	1959	" " "
Liver, Chang, grown in human serum	1957	Multiplication, complete cell destruction
	1958	" " " "
	1959	" " " "
Liver, Chang, (C), grown in horse serum	1957	Multiplication, complete cell destruction
Liver, Chang (WR)* grown in horse serum	1957	Multiplication, complete cell destruction
Conjunctiva, Chang, grown in human serum	1954	Multiplication, no cytopathogenicity
	1955	" " "
	1956	" " "
	1957	" " "
Conjunctiva, Chang, (WR) grown in horse serum	1957	Multiplication, no cytopathogenicity
Conjunctiva, Chang (C) grown in horse serum	1957	Multiplication, no cytopathogenicity

* The letters (C) or (WR) indicate that cells originated from a commercial source or Walter Reed Army Medical Center; the remaining cells were stock Minnesota strains.

Serial passage of MEF virus in primate cell strains was undertaken to characterize further their biological interaction. Ten tube cultures of each cell type were employed for each passage level of virus.

At the first passage level stock MEF virus, 0.1 ml., was added to each tube in the passage series and 0.1 ml. amounts of pooled virus transferred for higher passage levels. The yield of



TEXT-FIG. 1. Curve 1 is representative of cells (*cynomolgus* and *rhesus* monkey renal cells, clone A_2B of esophageal cells, and Chang's line of liver cells, clone A_2B) fully susceptible to the cytopathogenic effect of an unadapted variant of poliovirus, Type 2, MEF_1 strain. Curve 2 is typical of cells (Chang's parent line of conjunctival cells, parent Maben cells, and clone D_1B_1 of Chang's line of liver cells) which propagated virus up to the seventh to tenth passage level without evidence of viral induced morphologic changes; higher passage levels of virus caused complete cellular destruction. Curve 3 is representative of five replicate experiments in which virus was passed serially in parent HeLa cells; no cytopathogenic mutants of virus evolved to become predominant.

virus at representative and critical passage levels was titrated in monkey kidney tube cultures, 5 tubes per decimal dilution of virus, and $TCID_{50}$ endpoints calculated as before. Serial passage experiments and titrations employing monkey kidney cells and parent conjunctival cells were conducted in duplicate; experiments with HeLa cells were carried out in quintuplicate: a single passage experiment in 1954, 1955, and 1956, respectively, and two experiments in 1957. MEF virus as infected mouse brain suspension served as inoculum for serial passages

of virus in monkey kidney cells to obviate selective pressure that may have operated in preparation of stock MEF virus pools used throughout other portions of the investigation.

The following observations were made (Text-fig. 1): (*a*) repeated passages of MEF virus in parent HeLa cells failed to result in the selection of cytopathogenic mutants by cells; (*b*) many cell lines were immediately susceptible to virus without "adaptation", *viz.*, monkey kidney cells, clonal esophageal cells, clone A_2B of Chang's liver cells, *etc.*; (*c*) with respect to parent conjunctival cells and D_1B_1 clone of liver cells, cytopathogenic "mutants" were observed at

TABLE IV

Relative Plating Efficiency of MEF Virus on Representative Cell Strains

Cell strain*	Log PFU/ml MEF-virus‡	Log PFU/ml YSK-virus§
HeLa, parent	No plaques	5.81 ± 0.1
Hoye, parent	5.64 ± 0.2	
Esophageal, parent	No plaques	
" clone A_2b	5.96 ± 0.1	
Liver, clone A_1A_1	No plaques	
" clone D_1B_1‖	No plaques	
Maben, parent‖	No plaques	
Monkey kidney	5.89 ± 0.1	

* Stock Minnesota strains of cells were used for all experiments; parent HeLa cells adapted to growth in horse serum were designated as the "Hoye" strain.

‡ Five plaque bottles were used for each dilution of virus tested and the counts for a single virus dilution averaged; variation in plaque counts were determined from not less than 5 replicate experiments per cell strain.

§ A "positive" control was established with each experiment to assure that the absence of plaques in resistant cell strains was not a result of technical error or some peculiarity of the cell growth or maintenance medium. Data were accumulated for HeLa parent-cytopathogenic poliovirus, Type 2, YSK strain, titrations to survey the degree of variation in plaque titrations with a prototypic poliovirus.

‖ High concentrations of MEF virus, *i.e.*, 10^0–10^2 dilutions of stock virus, were toxic to the D_1B_1 clone of liver cells and parent Maben cells.

the seventh to tenth passages but did not complete their competitive advantage until the fifteenth passage.

Efficiency-of-Plating Studies.—To provide confirmatory evidence for the differential susceptibility of cells to infection by MEF virus efficiency-of-plating experiments were undertaken.

The concentration of virus in the stock MEF virus pool was titrated in representative cell lines in terms of plaque-forming units (PFU). Five bottles were used for each virus dilution tested; tests were repeated five times for each cell line to assay precision of results. Cytopathogenic poliovirus, Type 2, was added to representative bottles of cells to assure that the absence of plaques in resistant cell strains did not result from technical errors or from media used for growth or maintenance of cultures.

Experimental findings (Table IV) were definitive: (*a*) cells fully susceptible

to the cytopathogenic effect of MEF virus as evidenced by tube tests reacted to viral infection by typical plaque formation; (*b*) cells resistant to viral cytopathogenicity as observed in tubes did not lyse to form plaques regardless of the dose of virus inoculum; (*c*) cells of intermediate susceptibility in culture tube experiments failed to develop plaques irrespective of challenge virus concentration. The data also showed that the susceptible stable epithelial cell lines were as sensitive to infection by MEF virus as monkey kidney cells. The interesting observation was made also that two cell lines, *i.e.*, clonal D_1B_1 liver cells and parent Maben cells, underwent rapid toxic degeneration without indication of plaque formation when exposed to high concentration of MEF virus.

TABLE V

Effect of PPLO Contamination on Reaction of Cells to MEF Virus Infection

Cell strain*	Occurrence of PPLO	Reaction of cells to viral infection
HeLa, parent (Gey), commercial 1 and 2	+	Virus multiplication, cell destruction
HeLa, clone S3, commercial 1 and 2	+	" " , no cytopathogenicity
HeLa, parent (Gey), commercial 1 and 2	−	Virus multiplication, cell destruction
HeLa, clone S3, commercial 1 and 2	−	" " , no cytopathogenicity

* The parent and S3 lines of HeLa cells obtained from two commercial sources were freed from PPLO by serial cultivation for at least 4 weeks in standard growth medium containing added tetracycline at a level of 25 μg./ml.

Effect of PPLO Contamination on Morphologic Response of Cells to MEF Virus Infection.—Since it is common knowledge that many cell lines have been contaminated by L forms or/and pleuropneumonia-like organisms (PPLO) (21) it was essential to provide evidence that the differential response of cells to MEF virus was not attributable to the presence or absence of PPLO.

Parent and the S3 strains of HeLa cells obtained from commercial sources were freed of PPLO contaminants by serial cultivation for a period of not less than 4 weeks in standard growth medium containing added (25 μg./ml.) tetracycline (tetracyn, Charles Pfizer and Company, Inc., Brooklyn). Before addition of tetracycline cultures contained PPLO in concentrations as high as 10^6 per culture. After treatment cultures were free of PPLO on prolonged subculture in the absence of tetracycline.[1] Tube cultures of test cells were exposed to stock virus as indicated above.

Data tabulated in Table V reveal that the differential morphologic response of the parent and S3 lines of HeLa cells to MEF virus infection was independent

[1] The authors are indebted to Dr. M. F. Barile, National Institutes of Health, who conducted the bioassays for L forms and PPLO. Tetracycline has not been found to be universally effective in freeing cells from PPLO and is very toxic for the L strain of mouse fibroblasts.

of the presence or absence of PPLO. Virus multiplication occurred in all test cell strains. No attempt was made to assay the effect of PPLO on yield of virus per cell.

DISCUSSION

Long term studies of the interaction between a variant of poliovirus, Type 2, MEF_1 strain (MEF virus) and HeLa cells have shown that MEF virus was consistent in its capacity to multiply in such cells without induction of observable cytopathogenic changes. Moreover, sumultaneous serial propagation of both cells and virus occurred without the need to invoke the protective effect of homotypic antiserum (4, 7, 10). Evidence was provided to assure that MEF virus was in fact a strain of Type 2 poliovirus and that the qualitative relationship between cells and virus was stable over a period of years.

A comparison of the differential susceptibility of cells of diverse origin was undertaken to determine whether the Minnesota strains of cells employed were unique in their susceptibility to MEF virus. Experiments with cell strains obtained from two commercial sources and from Walter Reed Army Medical Center confirmed the fact that the selective cytopathogenicity of virus distinguished closely related cells. For example, commercial strains of the Gey line of HeLa cells grown in either human or horse serum were fully susceptible to the necrotic effect of MEF virus, whereas the S3 clone of HeLa cells from the same sources supported virus multiplication without evidence of morphologic change. The destructive effect of MEF virus on cells grown in horse serum evidently did not result from its effect on virus cell relationships *per se*, since similar results did not occur with the Chang strain of conjunctival cells grown in horse serum, nor did use of horse serum in 5 per cent concentration in maintenance medium for resistant cell lines result in viral induced cytopathogenicity. These observations are of significance for two reasons: (*a*) they afford additional definitive evidence that qualitative changes in cell lines can result from failure to use *rigidly* standardized media for continuous growth of stock cell cultures, and (*b*) that cells propagated in different laboratories need not necessarily yield similar findings in comparable experiments in spite of the fact that one might assume that they should.

Visual evidence of the distinctive response of closely related cells to MEF virus infection was confirmed by serial passage experiments and by efficiency-of-plating studies. Direct microscopic examination disclosed that unadapted virus completely destroyed lines of certain cell strains, *e.g.*, clonal esophageal cells and *rhesus* and *cynomolus* monkey kidney cells. A second characteristic reaction of cells to infection was "partial" resistance manifested by incomplete cellular destruction in which all cells of the culture were similarly affected, *e.g.*, clonal conjunctival cells, B_2A_2, or by outgrowth of resistant cells as observed with the parent line of esophageal cells. The third category of reaction

to infection involved viral multiplication without induction of observable cytopathogenic changes which was observed with parent HeLa, or the Chang parent strain of conjunctival cells grown in either human or horse serum.

To gain insight to the mechanisms by which virus adapted to cells as evidenced by full cytopathogenicity, a large initial inoculum, 0.1 ml., of undiluted stock MEF virus was employed for serial passage experiments. Undiluted virus was used purposefully to learn whether the stock preparation contained virus particles destined to reveal their cytopathogenicity through their competitive advantage on serial passage. The yield of virus from the different cell types was titrated at selected passage levels until opportunity for full "adaptation" occurred. The following picture emerged: (*a*) although 25 passages of virus were made in HeLa cells in each of three replicate experiments there was no evidence for the selection of cytopathogenic mutants; (*b*) during the first three passages most cell lines exerted a marked selection for cytopathogenic variants of virus; (*c*) a mechanism of mutation without significant host-cell selection appeared to operate in two cell lines, *i.e.*, cytopathogenic mutants became evident at the seventh to tenth passage levels but did not complete their competitive advantage until the fifteenth passage.

Results of efficiency-of-plating experiments yielded additional data for conclusive demonstration of the differential effect of MEF virus on cell strains. Cells resistant to viral cytopathogenicity failed to lyse whereas cells susceptible to virus gave essentially the same plaque titers when exposed to stock virus. Partially resistant cells failed to give rise to plaques despite addition to bottles of $10^{3.5}$ to $10^{5.5}$ PFU of virus. Higher concentrations of virus were cytotoxic to Maben cells and clonal D_1B_1 liver cells; similar cytotoxicity resulting from addition of high multiplicities of virus to resistant cells was reported by Deinhardt and Henle (22). During studies with partially resistant cells "abortive" plaques (transient appearance of pin-point necrotic areas) were sometimes seen instead of anticipated cloudy plaques. Addition of lactalbumin hydrolysate to the overlay medium infrequently permitted abortive plaques to enlarge to ordinary size. These observations suggested that cell nutrition as well as pH (23, 24) of the environment were determinant factors which collectively governed the physiologic capacity of cells to support virus replication.

The findings reported here are of interest in relation to comparisons of the differential susceptibility of cells to viruses described by many authors (8, 11, 22, 25). The observations of Ho and Enders (11) and Dutcher and colleagues (26) are of particular interest because the virus used in the studies summarized by this report appeared to have been derived from the same common source but had not been adapted to growth in the embryonated egg (27). Even more extraordinary are the similarity in results in regard to the susceptibility to virus of cells adapted to growth in horse serum (26).

Finally, it may be pertinent in this era of enthusiastic *in vitro* virus-cell cul-

ture research to paraphrase a comment from a recent review which directs our attention to the *in vivo* aspects of latent virus infection (3): *The ability of tissue cells to survive and continue to function during the infectious process is conditioned by the physiologic state of the infected individual. Unfortunately, so few facts are available concerning this important problem that it cannot be profitably discussed at the present time.*

SUMMARY

By application of a variant of poliovirus, Type 2, MEF_1 strain, as a selective agent it was possible to distinguish among stable parent strains of epithelial cells and their clonal derivatives by their differential morphologic response to infection. The variant of poliovirus grew in a number of cell strains without induction of observable cytopathogenic changes. Other strains of cells reacted to viral infection by manifesting partial or complete degeneration. Parent HeLa cells and virus underwent simultaneous serial propagation in the absence of homotypic antiserum to virus. The stability of the virus-cell relationship was established by results from replicate experiments conducted over a period of years. Some cell strains of common origin maintained in different laboratories did not react similarly to the cytopathogenic effect of virus. Representative experiments revealed that the morphologic response of HeLa cells to MEF virus infection was not influenced by the presence or absence of pleuropneumonia-like organisms. The differential morphologic response of cells to infection was confirmed by efficiency-of-plating experiments which revealed differences in the capacity of MEF virus to form plaques in the test cell strains. Serial passages of MEF virus in cell strains demonstrated differences in their selection for cytopathogenic "mutants" of virus.

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EXPLANATION OF PLATES

Cells stained with hematoxylin and eosin were photographed at magnification of 160.

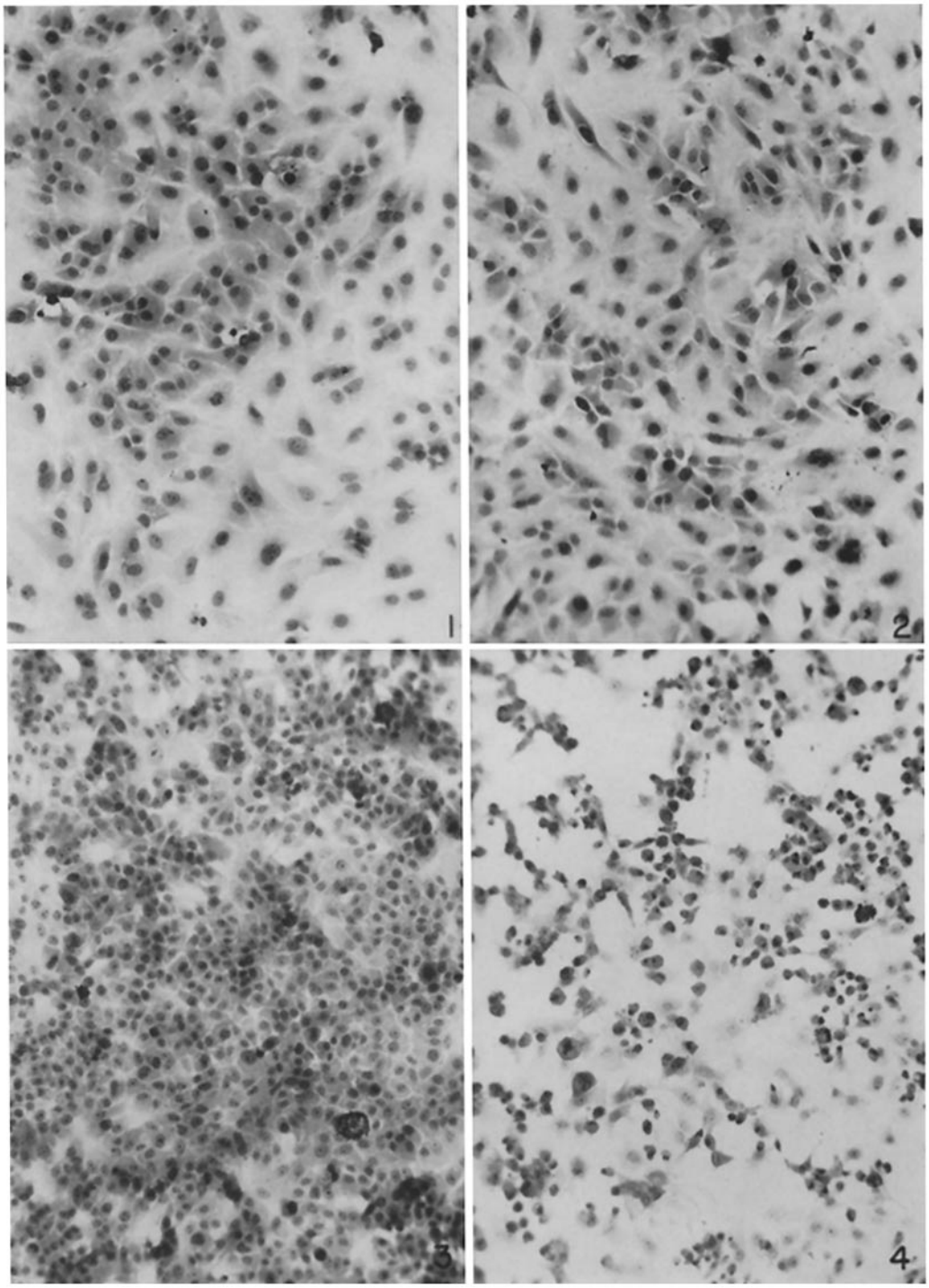
PLATE 41

FIG. 1. Normal HeLa cells.

FIG. 2. MEF virus infected HeLa cells maintained normal morphologic characteristics until cell growth resulted in crowding and consequent non-specific morphologic changes.

FIG. 3. Normal HeLa cells adapted to growth in horse serum (line Hoye). Hoye cells were noticeably smaller than parent HeLa cells grown in human serum. The population of Hoye cells in monolayer culture vessels often exceeded that of HeLa cells by 2- to 3-fold.

FIG. 4. Hoye cells 2 days after infection by MEF virus; the reaction observed was graded as incomplete destruction; at the end of 3 to 5 days' incubation at 37°C. all cells were destroyed by virus.



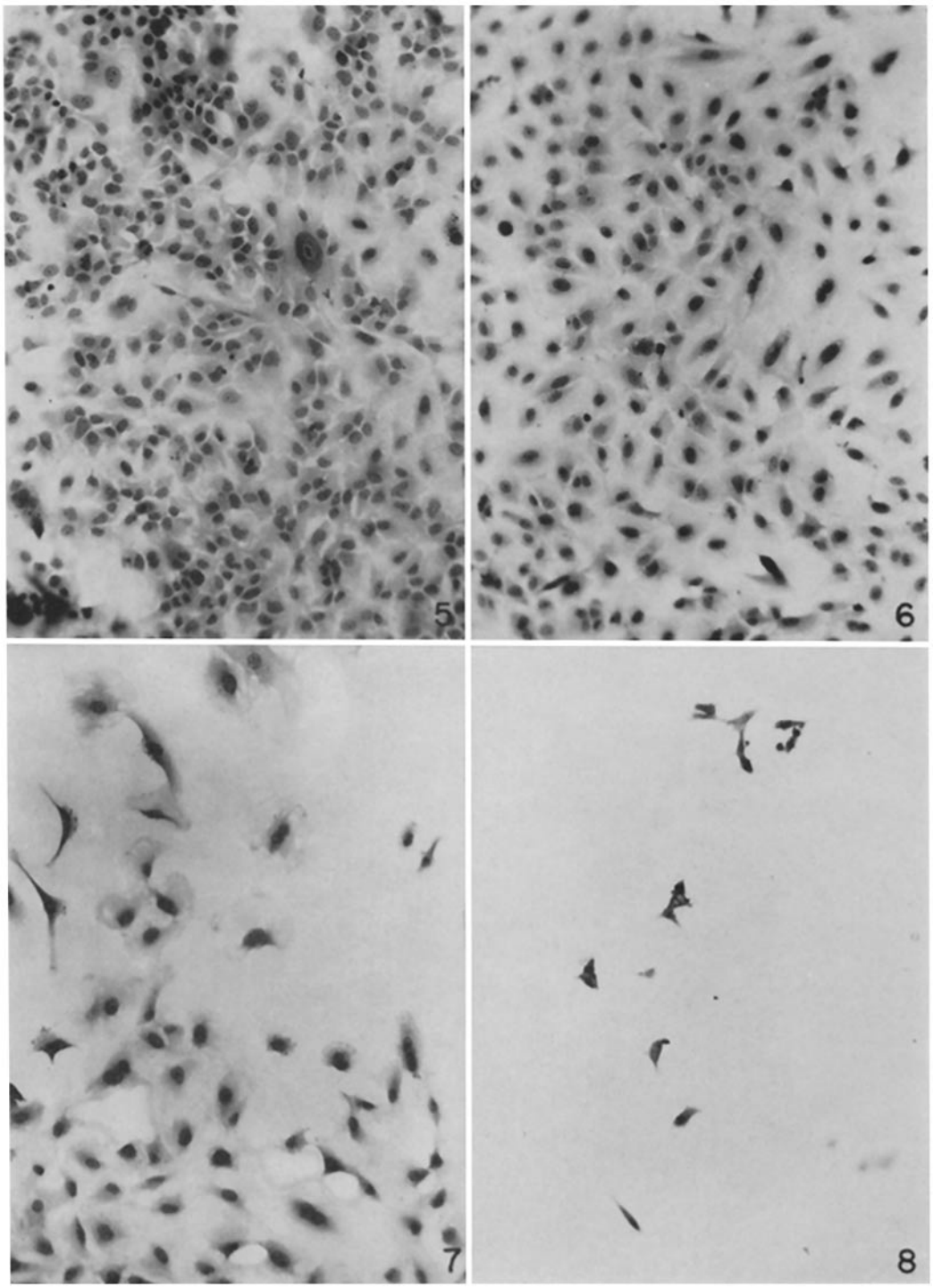
(Murphy and Armstrong: Variant of poliovirus, Type 2)

PLATE 42

FIGS. 5 and 6. Normal and infected cultures of Chang's line of parent conjunctival cells; virus multiplied in cells without induction of morphologic changes.

FIG. 7. Parent esophageal epithelial cell cultures showed a characteristic incomplete type of degenerative change. Cellular destruction was extensive although islands of resistant cells remained for periods of 3 to 4 weeks which were normal morphologically. The Maben line of adenocarcinoma cells reacted similarly to MEF virus infection.

FIG. 8. Clonal line B_2A_2 of Chang's strain of conjunctival cells infected with MEF virus underwent incomplete cellular destruction; however, all cells of the culture were similarly affected and islands (plaques) of cells with normal cytologic properties were not observed.



(Murphy and Armstrong: Variant of poliovirus, Type 2)