# Simian Tumor Virus Isolate: Demonstration of Cytopathic Effects in vitro

Abstract. *Several cell lines that were derived from primates and inoculated with virus originally obtained from a spontaneous mammary carcinoma showed cytopathic effects characterized by multinucleation. These cytopathic effects appeared as early as 24 hours after inoculation. Multinucleated cells contained virus particles characteristic of the original virus isolate.*

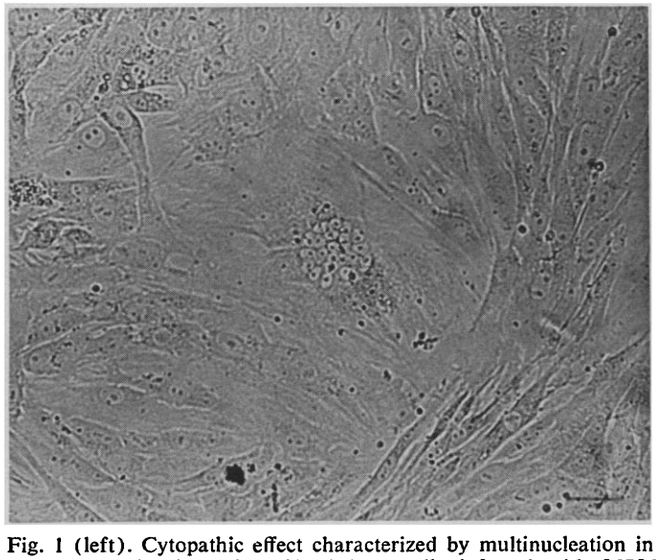
Recent studies in our laboratories demonstrated cytopathic effects (CPE) characterized by multinucleated cells in cell cultures that were derived from primates and inoculated with material containing Mason-Pfizer monkey virus (MPMV). The MPMV, originally isolated from a spontaneous mammary carcinoma of a rhesus monkey, had a morphology characteristic of known oncogenic RNA-type viruses (*1*). Until now the virus has been shown to replicate in several cell lines of primate origin with no apparent CPE (*2, 3*).

Twenty-one different primate cell lines were used for this study. Eighteen of these were derived from tissues removed from two fetal rhesus monkeys delivered by cesarean section. Undiluted virus inoculums consisting of 0.5 ml of MPMV in medium RPMI 1640 with 10 percent fetal calf serum were inoculated onto 1-day-old, low-passage cell cultures, were allowed to adsorb for 1 hour at room temperature, and were then overlaid with Eagle's minimum essential medium (MEM) containing 5 percent fetal calf serum and antibiotics (penicillin, 100 unit/ml; streptomycin, 100 $\mu$g/ml; fungizone, 2.5 $\mu$g/ml; and kanamycin, 200 $\mu$g/ml). Control cell cultures were inoculated with 0.5 ml of medium RPMI 1640 containing 5 percent fetal calf serum and were treated in a similar manner. All cultures were observed daily for the appearance of CPE. The results of these assays are summarized in Table 1. Positive CPE were repeatedly observed in cell lines from rhesus foreskin and lung and from human foreskin and were apparent as early as 24 hours after inoculation with several lots of high-titered virus. Attempts were made to induce CPE by inoculation of MPMV-infected NC37 cells onto several of the fetal rhesus cell lines. Cytopathic effects were apparent within 24 hours after inoculation of the MPMV-infected NC37 cells onto fetal foreskin and lung cell monolayers. These effects were also present 6 days after inoculation of tooth bud and heart cell cultures with the infected NC37 cells. One of the 21 cell lines tested, which showed no CPE, was mixed rhesus embryo cells. Jensen *et al.* (*2*) reported replication of MPMV in mixed rhesus embryo cell cultures with the absence of virus-related CPE.

The CPE repeatedly observed in foreskin and lung cultures after inoculation with MPMV were characterized by multinucleated cells containing as many as 18 nuclei (Fig. 1). Generally, the nuclei were clustered within the affected cells and were surrounded by a large cytoplasmic area. Similar multinucleated cells were reported in mouse mammary tumor virus (*4*) and feline syncytia-forming virus (*5*) infections. Cells infected with MPMV and stained with hematoxylin and eosin showed nuclei with varying degrees of degeneration characterized by basophilic intranuclear structures, as well as vacuolization in proximity to or encircling the affected nuclei (Fig. 2).

The CPE were demonstrable after inoculation of washed, disrupted, MPMV-infected foreskin cells onto uninfected foreskin cell monolayers. This serial passage of CPE would eliminate the possibility of a cytotoxic effect associated with MPMV material. Similarly, the CPE were observed in subcultured foreskin cells over a period of several months after inoculation. The CPE were demonstrated in MPMV-infected lung cell cultures, however, for periods up to 3 weeks following evidence of multinucleation, after which a slow regression of CPE was observed. Continued observation of the subcultures of the originally MPMV-infected lung cells periodically reveals small areas of multinucleation. Neutralization of the viral-induced CPE was effected by use of a 1 : 5 dilution of hyperimmune rabbit serum when virus preparations from three lots of MPMV were assayed on foreskin cells.

The MPMV-infected and uninfected cultures of monkey foreskin as well as a virus pellet concentrated by ultracentrifugation were fixed in glutaralde-



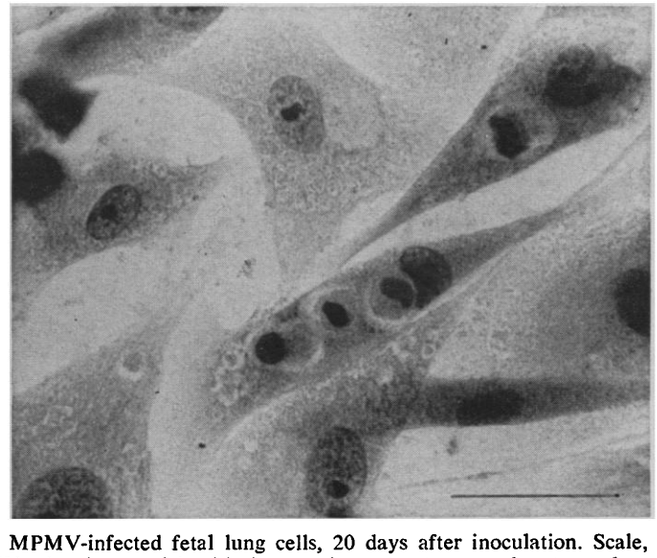


Fig. 1 (left). Cytopathic effect characterized by multinucleation in MPMV-infected fetal lung cells, 20 days after inoculation. Scale, 10 $\mu$m. Fig. 2 (right). Fetal lung cells infected with MPMV, showing nuclei with intranuclear structures and extranuclear vacuolization, 33 days after inoculation. Hematoxylin and eosin stain; scale, 10 $\mu$m.

Table 1. Development of CPE in primate cell lines inoculated with MPMV. The primate cell lines were inoculated with 0.5 ml of undiluted MPMV in medium RPMI 1640 containing 10 percent fetal calf serum. Positive CPE were characterized by areas of multinucleation. The numbers in parentheses are the number of days after inoculation. —, No CPE observed; ±, tentative identification of CPE; and +, definite CPE.

| Cell line | Monkey No. | Occurrence and onset of CPE (days) |
|---|---|---|
| | *Fetal rhesus* | |
| Brain | 4272, 4637 | — |
| Dura mater | 4272 | — |
| Bone marrow | 4272 | — |
| Testis | 4272 | — |
| Stomach | 4637 | — |
| Tooth bud | 4272 | ± (30) |
| Heart | 4637 | ± (6) |
| Kidney | 4637 | — |
| Spleen | 4637 | — |
| Adrenal | 4637 | — |
| Ovary | 4637 | — |
| Liver | 4637 | — |
| Eye | 4637 | — |
| Retina | 4637 | — |
| Spinal cord | 4637 | — |
| Mixed embryo | 4637 | — |
| Lung | 4272, 4637 | + (1) |
| Foreskin | 4272 | + (1) |
| | *Infant rhesus* | |
| Foreskin | 4860 | + (1) |
| | *Human* | |
| HEp-2 | | — |
| Foreskin* | | + (2) |

* From Flow Laboratories, Rockville, Maryland.

hyde, embedded in Epon, and examined by electron microscopy. Analysis of areas of multinucleation in MPMV-infected foreskin cultures demonstrated the presence of large numbers of intracytoplasmic, electron-dense, ring-shaped particles measuring 81 to 85 nm in diameter, resembling those particles described by Chopra and Mason (*6*). Examination of thin sections of the virus pellet revealed structures of similar size and morphology. No virus structures were observed in any of the uninfected cultures of foreskin examined.

Positive CPE were demonstrated for each of 15 separate lots of MPMV assayed on fetal foreskin cells. When the appearance of CPE (multinucleation) was used as an index of infection, the infectivity titer of several lots of virus was determined by inoculating foreskin cultures with decimal dilutions of virus and staining the monolayers with neutral red (0.1 percent) 6 days after inoculation. When individual foci were counted, a linear relation was made between the virus dilution and the number of foci of multinucleated cells. For example, when replicate foreskin cultures were inoculated with dilutions ($10^{-2}$, $10^{-3}$, $10^{-4}$) of virus from lot A, the average numbers of foci recorded were 509, 54, and 15, respectively. The titer was expressed as focus-forming units (FFU) per milliliter. This assay was found to give reproducible titers when duplicate titrations were run on a single lot of virus (for example, $1.4 \times 10^8$ and $2 \times 10^8$ FFU/ml). With one lot of high-titered virus assayed, a second morphologic change was observed which might possibly be indicative of a malignant transformation. At 24 to 48 hours after inoculation, those cultures that received undiluted inoculums were characterized by areas of clustered, rounded cells loosely adhering to the culture surface. This morphologic alteration, commonly correlated with viral transformation, appeared to be associated with a proliferative cellular response. These areas of transformation were similar to those described in transformation studies reported by Baluda (*7*), Manaker and Groupe (*8*), and Rabotti *et al.* (*9*). The cultures that received inoculums containing a $10^{-1}$ dilution of the seed virus were found to have foci of rounded, highly refractile cells, as well as a large number of areas of multinucleation. At higher dilutions, including a dilution of $10^{-5}$, only multinucleation was observed. Over a 16-day period after inoculation no clustering of cells was observed in the higher dilutions as was observed in those cultures inoculated with undiluted material and material diluted $10^{-1}$.

The findings presented thus provide a specific CPE that can be associated with the presence of MPMV and allow for a more rapid means of MPMV detection and quantitation than is presently available.

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**References and Notes**

1. H. C. Chopra, *Proc. Amer. Cancer Res.* **11**, 16 (1970).
2. E. M. Jensen, I. Zelljadt, H. C. Chopra, M. M. Mason, *Cancer Res.* **30**, 2388 (1970).
3. H. C. Chopra, I. Zelljadt, E. M. Jensen, M. M. Mason, N. J. Woodside, *J. Nat. Cancer Inst.* **46**, 127 (1971).
4. E. Y. Lasfargues and M. R. Murray, in *Cancer Cells in Culture*, H. Katsuta, Ed. (Univ. of Tokyo Press, Univ. Park Press, Tokyo, Japan, 1968), p. 231.
5. A. J. Hackett, A. Pfiester, P. Arnstein, *Proc. Soc. Exp. Biol. Med.* **135**, 899 (1970).
6. H. C. Chopra and M. M. Mason, *Cancer Res.* **30**, 2081 (1970).
7. M. A. Baluda, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 415 (1962).
8. R. R. Manaker and V. Groupe, *Virology* **2**, 838 (1956).
9. G. F. Rabotti, J. C. Landon, T. W. Pry, L. Beadles, J. Doll, D. P. Fabrizio, A. J. Dalton, *J. Nat. Cancer Inst.* **38**, 821 (1967).
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## Disposition of Morphine in Man

Abstract. *The disposition of morphine was investigated by means of radioimmunoassay after a single intravenous dose (10 milligrams per 70 kilograms) was administered to 10 adult normal male subjects who had not received other drugs for 2 weeks preceding the study. A multiphasic decline in serum concentrations of morphine occurred. Detectable blood concentrations of morphine, or of a metabolite, or of both persisted for 48 hours after a single intravenous dose.*

Although morphine is considered one of our oldest and most efficacious drugs, the details of its disposition have not been adequately established and quantified in man. The principal reason for this has been the insensitivity of the methods available for measuring morphine. The recent development of an extremely sensitive procedure for morphine determination by radioimmunoassay (*1*) afforded the opportunity to perform these studies.

Ten normal adult, white male volunteers (aged 21 to 23 years) who had not received any drugs for 2 weeks preceding the study were given morphine sulfate (10 mg/70 kg, intravenously) at 9 a.m. Blood specimens (2 ml each) were drawn at varying intervals thereafter. The serums were separated and kept frozen until they were assayed within 1 week after venipuncture. The radioimmunoassay of morphine was performed by the method of Spector and Parker (*1*). Rabbit serum, the source of the antibody, was diluted 1 : 200; 0.1 ml of the diluted antiserum was incubated at 4°C for 24 hours with 10 μl of the volunteer's serum. To this mixture, [$^3$H]dihydromorphine (4000 count/min) and normal rabbit serum (0.1 ml) were added. The total volume