Murine Sarcoma Virus: The Question of Defectiveness

Abstract. *Infection of mouse and rat cells by the murine sarcoma virus (Moloney isolate) showed two-hit kinetics for focus production in mouse cells but one-hit kinetics in rat cells. Antiserum added to cultures after infection suppressed focus formation in mouse cells but not in rat cells. These studies suggest that, in rat cells infected with murine sarcoma virus, leukemia virus is not needed for focus formation and that these foci result from proliferation of the transformed rat cell; in mouse cells, on the other hand, leukemia virus is needed as "helper," and focus formation requires spread of virus. The term "defectiveness" then, if used, should not be applied to RNA tumor viruses without qualification for the viral function studied and the cell system employed.*

Murine sarcoma viruses have been considered "defective" because they cannot produce foci of cell alteration in mouse cells without the presence of a murine leukemia virus as "helper" (*1*). During studies on the murine sarcoma virus, Moloney isolate (M-MSV), we observed distinct differences between the properties of the virus in mouse and rat tissue culture cells. These differences suggest a reconsideration of the term "defectiveness," when referring to the murine sarcoma viruses, and of the mechanism of the formation of foci of altered cells by sarcoma viruses in various tissue culture cell lines.

The normal rat kidney cell line (NRK) from Osborne-Mendel rats (*2*) was infected with a pool of Moloney sarcoma virus (*3*) which always contains Moloney leukemia virus in excess. The resulting infected cell line, designated NRK(M-MSV), released sarcoma virus as well as leukemia virus. Supernatant taken from these cells 2 days after planting was used in these experiments. Swiss mouse (NIH strain) embryo tissue culture (METC) cells, Fisher rat embryo tissue culture (RETC) cells, and NRK cells were prepared as previously described (*4*, *5*) and plated at 50 percent confluency 24 hours prior to inoculation of the virus. For the focus assays, cells were cultured in McCoy 5a medium with 5 percent inactivated (56°C for 30 minutes) calf serum and antibiotics (penicillin, streptomycin, and neomycin).

In quantitating leukemia virus, cultures were maintained 21 days in Eagle minimum essential medium containing 10 percent fetal calf serum, glutamine, and antibiotics (penicillin and streptomycin) (*4*, *6*). The virus titer was determined by the COMUL (complement-fixation for murine leukemia virus) test which measures virus infectivity by the induction, by murine leukemia viruses, of complement-fixing, group-specific viral antigen in tissue culture (*6*, *7*). The $TCID_{50}$ (tissue culture infective dose, 50 percent effective) titer per 0.4 ml is referred to as the COMUL titer. All cells were pretreated with diethylaminoethyl dextran (DEAE-D) in order to increase the efficiency of the assay (*8*).

The titer of M-MSV was assayed by focus formation (focus-forming units per 0.4 ml) in NRK cells by the method of Ting (*5*) and in mouse and rat embryo cells by the method of Hartley and Rowe (*1*). The leukemia virus in the M-MSV pool and the supernatant from the NRK(M-MSV) cell lines was titered by COMUL testing (*6*, *7*) and expressed as the COMUL titer.

Antiserum to M-MSV was produced by hyperimmunization of 2-month-old Osborne-Mendel rats with NRK(M-MSV) cells; the rats were bled from the tail vein and the serums pooled.

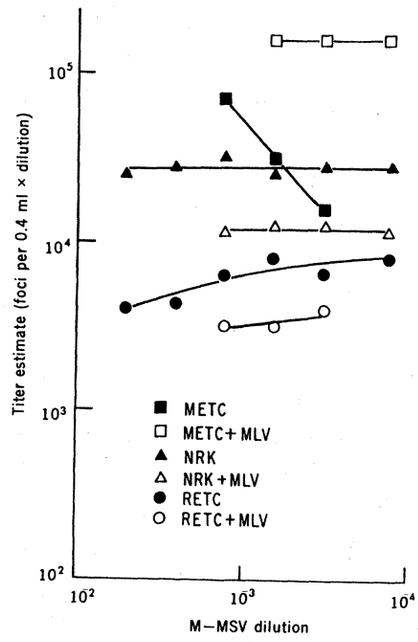

Fig. 1. Dose-response relations in titrations of M-MSV on mouse and rat cells, with and without helper virus.

Virus neutralization tests were performed by mixing the immune or control serum with virus (1:1) for 30 minutes at 37°C before assaying in culture. In the focus formation inhibition assays, the calf serum of the standard medium was replaced by either normal or immune rat serum after 20 hours.

Figure 1 shows the results of the assay of the M-MSV pool in NRK, rat embryo, and mouse embryo cells. In mouse embryo cells the number of foci decreased with the square of the dilution factor, signifying two-hit kinetics as originally reported by Hartley and Rowe (*1*). When Moloney leukemia virus (MLV) was added at a predetermined helper concentration (10^{-3} dilution of a MLV pool with a COMUL titer of 10^6 in METC cells treated with DEAE-D), a one-hit relationship was present. More concentrated amounts of MLV, when added to the METC cells, inhibited focus formation. Less concentrated amounts (at 10^{-4} and 10^{-5} dilution) showed little or no helper effect.

In NRK and RETC cells, the assay of M-MSV showed one-hit kinetics. The addition of MLV as helper (10^{-1} and 10^{-2} dilution, respectively, of a MLV pool with a titer in rat cells of 10^4) had no effect on the kinetics of the system but the titers of focus-forming units were approximately 25 percent of the original titers. Less concentrated amounts of MLV had little effect on the titer in the rat cells and did not alter the one-hit curve.

The COMUL titer of this pool of M-MSV made from the 2-day-old supernatant of NRK(M-MSV) cells was 10^6 in METC cells and $< 10^3$ in both rat cells. The leukemia virus titer of the M-MSV pool measured by fluorescent staining of viral antigens (*9*) was $10^{3.7}$ for NRK and $10^{4.0}$ for rat embryo cells. Therefore, despite the greater sensitivity of the METC cells to the leukemia virus, the M-MSV gave two-hit kinetics in METC cells and one-hit in rat cells.

In order to determine if this difference in kinetics was limited to mouse embryo cells, we titrated the same pool of M-MSV in BALB/c 3T3 cells (*10*). Two-hit kinetics was also noted. The one-hit kinetics of M-MSV in NRK and RETC cells may therefore indicate a general property in all rat cells, whereas two-hit kinetics of M-MSV may be a characteristic in all mouse cells.

Thomas and his associates (*11*) have noted one-hit kinetics of M-MSV grown

in bovine cells. Titration of leukemia virus, however, was not included in their results, so excess helper virus may have been present. O'Connor and Fischinger (*12*) have also reported one-hit kinetics in mouse cells. They have attributed this result to a "competent" particle of M-MSV in which leukemia virus is present with sarcoma virus as an "interviral aggregation" (*13*).

A disparity in the ability of the leukemia and sarcoma viruses to adsorb to and propagate in mouse and rat cells is possible but unlikely as an explanation for these kinetic differences. The murine sarcoma virus MSV-0, which produces foci in rat cells only (*14*), adsorbs equally well to mouse and rat cells (*15*). The COMUL titer in rat cells is considerably lower than that in mouse cells. Moreover, the one-hit curve in NRK cells intersects the two-hit curve in mouse embryo cells (Fig. 1). One might speculate then that the "competency" of this pool of M-MSV in rat cells may be directly related to the differences in focus formation.

Hartley and Rowe (*1*) reported that in mouse embryo cells the number of foci induced by M-MSV is reduced three- to fivefold by the addition of antiserum to mouse leukemia virus to the assay plates 24 hours after infection. Our results with M-MSV in METC and BALB/c 3T3 cells confirm their report (Table 1); moreover, the mouse foci remaining are much smaller than usual. When medium containing antiserum that completely neutralized M-MSV was added to NRK and RETC plates 20 hours after M-MSV infection, there was no reduction in the number of foci produced by the pool (Table 1). Preincubation of the virus with the antiserum, however, removed all focus-forming virus from the inoculum. In both the neutralization and focus-suppression tests, added helper of a different type (Gross) did not affect the number of foci produced in rat and mouse cells in the presence of immune serums. These data indicate that both sarcoma and leukemia viruses are neutralized by the antiserums, and the M-MSV is not antigenically distinct (for example, MSV-0) from the Moloney leukemia virus associated with it.

These results demonstrate another marked difference between the interaction of M-MSV with mouse and rat tissue culture cells; they may explain in part the difference in kinetics observed. In mouse embryo cells, the reduction in number and size of foci by specific antiserum suggests that a continuous reinfection of nearby uninvolved mouse cells may be necessary for the ultimate appearance of recognizable mouse foci. One mouse cell infected and transformed cannot propagate independently and is overgrown by surrounding normal cells unless its progeny can infect and alter nearby cells; antiserum inhibits this reinfection.

Table 1. Tests for suppression of M-MSV foci in mouse and rat cells by the addition of immune serum before and after infection.

Percent reduction* of foci in cell lines			
METC	BALB/c 3T3	NRK	RETC
Preincubation†			
100	100	99	92
Immune serum added 20 hours after infection			
95	90	0	0

* $\left(1 - \frac{\text{Number of foci induced by virus plus immune serum}}{\text{Number of foci induced by virus plus control serum}}\right) \times 100\%$.

† Virus and serum incubated at 37°C for 30 minutes

Bather *et al.* (*16*) suggested this conclusion when transformed mouse cells did not form foci when transferred onto irradiated normal cells. The transformed NRK and RETC cells, on the other hand, have an inherent capacity to propagate independently. Under these conditions, once transformation occurs, reinfection is not necessary for ultimate focus formation. Antiserum, therefore, had no effect in the rat cells once adsorption and penetration of the virus had occurred. These data offer some explanation for the difficulties we and others have had in isolating and propagating transformed mouse embryo cells. Transformed rat cells, on the other hand, are very easy to isolate and grow.

The sarcoma virus in rat cells, in fact, may be very sensitive to competition from leukemia virus (for example, the reduction of foci in rat cells with addition of leukemia virus) (Fig. 1). In mouse embryo cells, on the other hand, sarcoma virus and leukemia virus are probably both needed for alteration. Propagation of the sarcoma virus or reinfection, or both, of nearby cells may also be directly dependent on the presence of a substantial but not overwhelming quantity of coinfecting leukemia virus.

Alternatively, rat cells may contain a latent helper virus. However, electron microscopy of NRK cells has revealed no virus particles (*14*); studies performed with radioactive uridine have shown no viral RNA particles released into the supernatant (*15*). Moreover, all complement-fixation testing of NRK and RETC control plates has been negative for the complement-fixing, group-specific antigen.

These studies, then, define two different phenomena occurring with murine sarcoma virus when infecting rat cell lines—one-hit kinetics and one-cell focus propagation. In rat cells, then, MSV cannot be considered defective for transformation.

The term defectiveness of RNA tumor viruses, in fact, has been used in two different contexts: (i) inability for a chick cell transformed by Rous sarcoma virus to "spawn infectious progeny without the intervention of a helper virus" (*17*); and (ii) inability of the murine sarcoma virus to initiate a focus in mouse embryo cells without the help of mouse leukemia virus (*1*).

The defectiveness of Rous sarcoma virus was brought into question by the studies of Dougherty and DiStefano (*18*), Vogt (*19*), and Weiss (*20*), who showed that the nonproducing Rous cells contained virus particles with biologic activity. The experience with MSV-0 in the murine system is very similar (*14*). The term defectiveness, then, if used, should not be applied to RNA tumor viruses without qualification for the viral function studied and the cell system employed. One might prefer, in fact, the term helper-dependent to describe the inability of sarcoma viruses to perform functions (for example, focus formation or propagation of parental virus) in certain specific cells without the presence of adequate leukemia virus as helper.

It is true, for example, that in heterologous systems (such as hamster and rat) nonproducing cells have been described for Rous sarcoma virus (*21–23*). These will produce progeny after cocultivation with normal chick cells, but in some cases the addition of an avian leukosis virus as helper is also required. Similar nonproducing cells result after MSV infection of hamsters in vivo (*24*) and rat cells in vitro (*25*).

In the homologous murine systems, however, unlike the avian system, the sarcoma viruses differ in the kinetics of focus formation and the process leading to focus formation. The dependency on leukemia virus for focus formation is not seen in any cell system susceptible to transformation by Rous sarcoma virus. This inability to produce foci in mouse cells without added helper virus may stem from a failure of MSV to enter or transform, or both. On the

other hand, because transformed mouse cells cannot propagate independently (unlike chicken and rat cells), leukemia virus may be needed for the propagation of sarcoma virus or the infection of nearby cells, or both, to produce a recognizable focus. Further work is needed to clarify this system.

These data indicate that the inability to initiate focus formation without leukemia virus as helper is not a general characteristic of the murine sarcoma virus but is specific for its action in mouse cells. In reference to transformation of rat cells, murine sarcoma virus resembles Rous sarcoma virus. Our observations point out the necessity of defining a sarcoma virus only in terms of its action in the particular host cell infected and the necessity of recognizing inherent differences in the ability of that cell itself to express this viral activity.

ROBERTSON PARKMAN
Laboratory of Biology,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

JAY A. LEVY*
Viral Carcinogenesis Branch,
National Cancer Institute

ROBERT C. TING
Laboratory of Biology,
National Cancer Institute

References and Notes

1. J. W. Hartley and W. P. Rowe, *Proc. Nat. Acad. Sci. U.S.* **55**, 780 (1966).
2. H. Duc-Nguyen, E. N. Rosenblum, R. F. Zeigel, *J. Bacteriol.* **92**, 1133 (1966).
3. The M-MSV isolate and tissue culture–adapted Moloney leukemia virus were kindly provided by Drs. J. Moloney, National Cancer Institute, and Janet W. Hartley, National Institute of Allergy and Infectious Diseases, respectively.
4. J. W. Hartley, W. P. Rowe, W. I. Capps, R. J. Huebner, *Proc. Nat. Acad. Sci. U.S.* **53**, 931 (1965).
5. R. C. Ting, *Virology* **28**, 783 (1966).
6. J. W. Hartley, W. P. Rowe, W. I. Capps, R. J. Huebner, *J. Virol.* **3**, 126 (1969).
7. Complement-fixation tests were conducted under the supervision of Horace C. Turner, National Cancer Institute.
8. H. Duc-Nguyen, *J. Virol.* **2**, 643 (1968).
9. W. P. Rowe, J. W. Hartley, R. E. Wilsnack, in preparation.
10. S. A. Aaronson and G. J. Todaro, *J. Cell Physiol.* **74**, 141 (1968).
11. M. Thomas, M. Boiron, Y. Stoytchkov, J. Lasneret, *Virology* **36**, 514 (1968).
12. T. E. O'Connor and P. J. Fischinger, *Science* **159**, 325 (1968).
13. ———, *J. Nat. Cancer Inst.* **43**, 487 (1969).
14. R. C. Ting, *J. Virol.* **2**, 865 (1968).
15. R. Parkman, unpublished observations.
16. R. Bather, A. Leonard, J. Yang, *J. Nat. Cancer Inst.* **40**, 551 (1968).
17. H. Hanafusa, T. Hanafusa, H. Rubin, *Proc. Nat. Acad. Sci. U.S.* **49**, 572 (1963).
18. R. M. Dougherty and H. S. DiStefano, *Virology* **27**, 351 (1965).
19. P. K. Vogt, *Proc. Nat. Acad. Sci. U.S.* **58**, 801 (1967).
20. R. Weiss, *Virology* **32**, 719 (1967).
21. R. J. Huebner, D. Armstrong, M. Okuyan, P. S. Sarma, H. C. Turner, *Proc. Nat. Acad. Sci. U.S.* **51**, 742 (1964).
22. P. S. Sarma, W. Vass, R. J. Huebner, *ibid.* **55**, 1435 (1966).
23. J. Svoboda, P. Chyle, D. Simkovič, I. Hilgert, *Folia Biol. (Prague)* **9**, 77 (1963).
24. R. J. Huebner, J. W. Hartley, W. P. Rowe, W. T. Lane, W. I. Capps, *Proc. Nat. Acad. Sci. U.S.* **56**, 1164 (1966).
25. J. A. Levy, unpublished observations.
26. We thank Dr. Wallace P. Rowe, National Institute of Allergy and Infectious Diseases, for his helpful discussion and advice in the preparation of this report.
* Address requests for reprints to J. A. Levy.

24 November 1969; revised 12 January 1970

Arsenic in Detergents: Possible Danger and Pollution Hazard

Abstract. *Arsenic at a concentration of 10 to 70 parts per million has been detected in several common presoaks and household detergents. Arsenic values of 2 to 8 parts per billion have been measured in the Kansas River. These concentrations are close to the amount (10 parts per billion) recommended by the United States Public Health Service as a drinking-water standard.*

Considerable attention is being focused on the detrimental effects man has or can have on his environment. We report on the possible effects of some common household detergent products on water quality. In an investigation by emission spectrography of the trace element composition (Fe, Mn, Cr, Ni, Co, Zn, Sr, Li, SiO_2, and B) of three enzyme presoaks, three heavy duty enzyme detergents, one heavy duty detergent, and one detergent aid, we found continual spectrographic evidence of the presence of arsenic in most of the samples. Because the amount of arsenic was close to the detection limits of the spectrographic method, the more sensitive silver-diethyldithiocarbamate method was used (*1*) for the quantitative determination.

Waste waters of these detergent products can easily enter the water system and therefore contribute to water pollution. We analyzed detergent samples, water from the Kansas River, and water entering and leaving the water and sewage treatment plants in Lawrence, Kansas; the concentrations of arsenic in many of the detergent products were high enough to pose a pollution problem and a potential health hazard to people using them constantly.

A problem of serious water pollution also exists (Tables 1 to 3). The U.S. Public Health Service gives tolerances of 10 ppb (recommended) and 50 ppb (mandatory) of arsenic in drinking water (*2*). We have calculated the concentrations of arsenic to be expected in tubs of typical washers of 10-, 30-, and 60-gallon capacities (1 gallon = 3.8 liters) (Table 2). Especially important are the high amounts of arsenic in two presoaks. When used as directed, the arsenic concentration of the different household laundry aids greatly exceeds that recommended for drinking water. While a "tub of suds" is not used for drinking, the danger clearly exists that arsenic can be absorbed through unbroken skin. Another side effect of arsenic is the possibility of skin rashes and other types of contact dermatitis skin reactions in sensitive people (*3*). For example, the presence of arsenic at 50 ppm inhibits the healing of wounds (*4*). The medical literature reveals remarkably little about the long-term effects of such contact with arsenic. There is also evidence of the accumulation of arsenic in the livers of mammals (*5*). The fixing of arsenic in human hair after the use of arsenic-containing detergents was reported as early as 1958 (*6*). Arsenic is added to the system by the use of detergents in everyday wash chores. This usage contributes to the amount of arsenic in river waters. In areas of repeated usage, this concentration (3 to 8 ppb for the Kansas River) can be expected to rise in the near future with continued use of detergent products containing arsenic. Arsenic is a cumulative poison which builds up slowly in the body. According to some medical sources, long-term arsenosis may not be detectable for 2 to 6 years or longer. To our knowledge, no previous data on arsenic concentrations in the Kansas River are available.

To ascertain whether the arsenic was being added in the water-usage cycle, we measured the arsenic concentrations at different points in the water-distribution system (Table 3). Blind sets of

Table 1. Concentrations of arsenic in certain detergents and presoaks. Abbreviations are: EP, enzyme presoak; HDED, heavy duty enzyme detergent; DA, detergent aid; HDD, heavy duty detergent; and SD, single determination.

Detergent type		Arsenic concentration (ppm)	
		Average	Range
A.	EP	34	31–43
B.	HDED	32	SD
C.	HDD	9	8–10
D.	HDED	15	SD
E.	HDED	41	38–45
F.	EP	7	6–9
G.	DA	2	1–3*
H.	EP	59	51–73

* Lower limit of detection.