*Proc. Nat. Acad. Sci. USA*
Vol. 71, No. 10, pp. 4241–4245, October 1974

# Reciprocal Inhibition of Mouse Leukemia Virus Infection by *Fv-1* Allele Cell Extracts

**(gene action/cell culture/virus host range/restriction mechanism)**

RAYMOND W. TENNANT, BONNIE SCHLUTER*, WEN-KUANG YANG, AND ARTHUR BROWN*

Carcinogenesis Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830; and * Department of Microbiology, University of Tennessee, Knoxville, Tenn. 37916

*Communicated by Alexander Hollaender, August 7, 1974*

**ABSTRACT** **Soluble extracts of mouse cells with *Fv-1*$^n$ or *Fv-1*$^b$ gene alleles specifically and reciprocally inhibit infection of B- or N-tropic mouse leukemia viruses in permissive cell cultures. NB-tropic virus infection was not inhibited by either cell extract. Extracts from *Fv-1*$^-$ cells did not inhibit infection by the three virus host-range types, but N- or B-tropic virus infection of *Fv-1*$^-$ cells was inhibited by extracts of the nonpermissive cells, and *Fv-1*$^{nb}$ cell extracts inhibited both viruses. The maximum degree of inhibition was 50–80% as determined by immunofluorescent or plaque assays, with extracts containing up to 500 μg/ml of nonpermissive cell protein. The inhibitor(s) is relatively unstable since activity is lost after 2 hr at 37° or 30 min at 56°. The inhibitor(s) was most effective if added 2 hr before or within 2 hr after infection, did not react with the virus directly, inhibit virus attachment, or inhibit the normal cell functions tested. These results indicate that nonpermissive mouse cells contain a product, possibly determined by the *Fv-1* gene, which inhibits some early postpenetration event(s) in leukemia virus infection.**

The mouse *Fv-1* locus consists of at least two alleles, *Fv-1*$^n$ and *Fv-1*$^b$, which reciprocally control the susceptibility of mice to N and B host-range types of murine leukemia viruses (1–3). Resistance is dominant and $F_1$ hybrids restrict both types of virus. The *Fv-1* gene is also expressed in cultured cells but the restriction is not absolute, and titration of the restricted virus yields two-hit kinetics (4–7). While cells of $F_1$ hybrids restrict both N- and B-tropic viruses, a class of viruses designated NB-tropic are not restricted by either allele. While most mouse strains have one or the other *Fv-1* allele, a cell line established from wild mice (SC-1) lacks the *Fv-1* gene and is sensitive to all three classes of virus (Dr. Janet Hartley, personal communication).

Studies on the mechanism of the *Fv-1* restriction have shown that the inhibition is intracellular (8–11). Analysis of the fate of virus in heterokaryons of permissive and nonpermissive cells indicated that the restriction functions at some early postpenetration step in infection (11). Since chemical activation of endogenous leukemia virus (12, 13) is not restricted by the *Fv-1* gene (14, 15), these results suggest that the restriction functions against some event leading to the formation of the DNA provirus that is intermediate in virus replication (16).

The studies described here are directed at the mechanism of the *Fv-1* restriction and show that extracts of nonpermissive cells reciprocally inhibit virus infection in permissive cells. The specificity of inhibition indicates that a product of the *Fv-1* gene inhibits an early event in exogenous virus infection.

Abbreviations: EMEM, Eagle's minimal essential medium; DEAE-D, DEAE-dextran; N-tropic, *Fv-1*$^b$ restricted; B-tropic, *Fv-1*$^n$ restricted; NB-tropic, not *Fv-1* restricted.

## MATERIALS AND METHODS

*Viruses.* N-, B-, and NB-tropic mouse leukemia viruses were obtained originally from Drs. W. P. Rowe and Janet Hartley, NIH. The N-tropic virus stock (Gross leukemia virus) was grown in cultures of NIH Swiss mouse embryo cells, as was the NB-tropic virus stock (Moloney leukemia virus); the B-tropic leukemia virus stock was prepared in BALB/c mouse embryo cells as described previously (17). The host-range specificity of the virus stocks was determined by titration on N- and B-type cells (11).

*Cell Cultures.* N-type cells were secondary cultures of NIH Swiss mouse embryo (Microbiological Assoc., Inc.) or NIH Swiss 3T3 cells (obtained from Dr. George Todaro, NIH); B-type cells were secondary cultures of BALB/c embryo cells or cultures of BALB/c 3T3 cells (obtained from Dr. Stuart Aaronson, NIH); SC-1 cells which are susceptible to all three virus host-range types were obtained from Dr. Janet Hartley, NIH. NB-type cells were secondary cultures of embryos from C57BL ♀ × C3H/Anf Cum mice ($BC3F_1$ strain, Cumberland View Farms, Clinton, Tenn.). All cells except SC-1 were grown in Eagle's minimum essential medium (EMEM) containing 10% fetal calf serum supplemented with 2 mM glutamine but without antibiotics; SC-1 cells were grown in McCoy's medium, supplemented as above. The genotypes of the cells used are: NIH Swiss mouse embryo and NIH Swiss 3T3; *Fv-1*$^{n/n}$; BALB/c mouse embryo and BALB/c 3T3, *Fv-1*$^{b/b}$; $BC3F_1$ mouse embryo, *Fv-1*$^{n/b}$; SC-1, *FV-1*$^{-/-}$.

*Cell Extracts.* Primary embryo cell cultures, grown in 100-mm plastic petri dishes were used when 85–100% of the cells were confluent. The medium was aspirated from five plates; the cells were removed by scraping them into 5 ml of EMEM and were then centrifuged at approximately 1000 × *g* for 5 min. The cells were resuspended in 1 ml of EMEM and were then disrupted for 3 min in a Raytheon sonic oscillator (200 W, 10 kilocycles). The sonicate was suspended in a total volume of 7 ml of EMEM and was centrifuged at approximately 800 × *g* for 3 min; the supernatant fluid, which generally contained 230–270 μg of cell protein per ml, was immediately applied to cells previously treated with DEAE-dextran (DEAE-D, 25 μg/ml) for 1 hr.

*Virus Assays.* Virus infection was assayed by modification of the XC-cell-plaque technique of Rowe *et al.* (18), or by immunofluorescent staining as we have described previously (17). In all cases the cells were treated with DEAE-D for 1 hr before infection.

*Assay of Radioactively Labeled Virus Adsorption.* [$^3$H]Uridine-labeled virus was prepared in cultures of AKR mouse embryo cells replicating an N-tropic endogenous virus, and

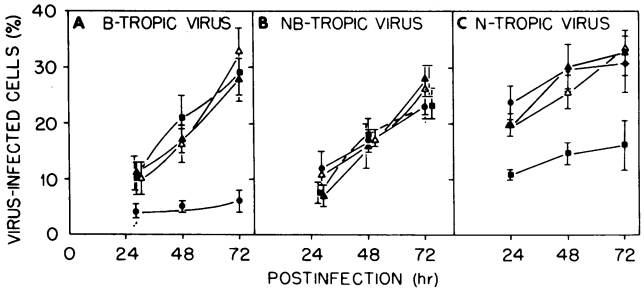


FIG. 1. Effect of cell extracts on virus infection. Secondary cultures of BALB/c or NIH Swiss mouse embryo cells were treated with DEAE-D for 1 hr, washed, and then treated with *Fv-1*$^{b/b}$, *Fv-1*$^{n/n}$, or *Fv-1*$^{-/-}$ cell extracts. After 2 hr, the cells were washed and infected with either B-tropic ($10^{5.3}$ PFU/ml), N-tropic ($10^{6.3}$PFU/ml), or NB-tropic virus ($10^{6.9}$ PFU/ml) for 2 hr. Fresh medium was added and coverslips were collected at 28, 48, and 72 hr, stained, and the percentage of infected cells was counted. ■, *Fv-1*$^{b/b}$ cell extract; ●, *Fv-1*$^{n/n}$ cell extract; ▲, *Fv-1*$^{-/-}$ cell extract; △, no extract. (A) B-tropic virus in BALB/c embryo cells; (B) NB-tropic virus in BALB/c embryo cells; (C) N-tropic virus in NIH Swiss embryo cells. The vertical bar through each point represents the 95% confidence interval for each observation.

partially purified as described previously (17). NIH Swiss 3T3 cells were incubated with the respective cell extract for 1 hr, treated with DEAE-D, washed, and the labeled virus (22 $\times$ $10^3$ cpm) was added. At the indicated intervals, the cells were washed and the amount of adsorbed radioactivity was determined as described elsewhere (17).

*Measurement of Cell DNA Synthesis and Growth.* Secondary Swiss mouse embryo (*Fv-1*$^{n/n}$) cells were plated and, after growth for 18 hr, the cells were treated with DEAE-D for 1 hr, washed, and the respective cell extract added for 2 hr. The cells were then washed and given complete medium. At the indicated intervals three cultures per group were labeled with [$^3$H]thymidine (1 $\mu$Ci/ml, 2 Ci/mM) for 1 hr and the amount incorporated (trichloroacetic acid insoluble) was determined as described previously (17). Protein concentration of each sample was determined by the Lowry method (19). Cell counts were determined on duplicate cultures at each interval.

## RESULTS

*Effect of Extracts of Nonpermissive Cells on Virus Infection.* In order to determine if a product of the *Fv-1* gene can inhibit virus infection, nonpermissive cells were disrupted and permissive cells were incubated with the soluble fraction prior to infection. In the first experiment, extracts were prepared from approximately $10^7$ *Fv-1*$^{n/n}$, *Fv-1*$^{b/b}$, and *Fv-1*$^{-/-}$ cells. *Fv-1*$^{b/b}$ cells on coverslips were treated with DEAD-D, incubated with each of the extracts for 2 hr, and then infected with B- or NB-tropic virus. Cultures were collected at 24, 28, and 72 hr after infection and the percentage of virus-infected cells assayed by fluorescent antibody staining. The results (Fig. 1A and B) show that the number of B-tropic virus-infected cells in cultures treated with *Fv-1*$^{n/n}$ extract, was reduced by 60–80% compared to cells treated with *Fv-1*$^{b/b}$ or *Fv-1*$^{-/-}$ extracts or mock-treated. None of the cell extracts inhibited infection by NB-tropic virus. In another experiment, *Fv-1*$^{n/n}$ cells, treated with the three extracts, were infected with N-tropic virus (Fig. 1C), and virus infection was inhibited only in cultures treated with *Fv-1*$^{b/b}$ extract. The percentage of infected cells was determined from at least two samples per group, and at least 1000 cells were counted per sample. One determination was on coded samples and 95% confidence intervals were calculated. These results show that treatment of permissive cells with extracts of nonpermissive cells specifically and reciprocally inhibits only the virus susceptible to the *Fv-1* allele.

The experiments were repeated, and the effect of the extracts was tested by determining the amount of virus replication by the XC-cell plaque assay. The results of these experiments (Table 1) also show that the effect of the extracts was specific for the virus restricted by the *Fv-1* allele. The replication of N- and B-tropic virus dilutions were proportionately inhibited by the nonpermissive extract but the replication of NB-tropic virus was not significantly decreased. The degree of inhibition in the plaque titration experiments was comparable to that seen with the fluorescent antibody assay, which measures the number of infected cells; in neither case was the inhibition complete. However, the *Fv-1* restriction is not absolute in cell cultures, since high doses of the restricted virus can overcome the gene effect, and infection fits a two-hit pattern (2, 6). The nature of the two-hit response is not understood and is a complex phenomenon. The cell extracts proportionately reduced infection by virus dilutions but did not convert the infection to a two-hit pattern, however, the specificity of the inhibition by nonpermissive cell extracts indicates that the effect is a specific property of the nonpermissive cell extract.

The effect of the cell extracts, including *Fv-1*$^{n/b}$ cells, were tested also on the infection of N-, B-, and NB-tropic viruses in *Fv-1*$^{-/-}$ cells in a combined experiment (Table 2). The *Fv-1*$^{b/b}$ extract significantly inhibited only N-tropic virus, and the *Fv-1*$^{n/n}$ extract, only B-tropic virus. The $F_1$ hybrid cell extract (*Fv-1*$^{n/b}$) inhibited both N- and B-tropic viruses, but no extract inhibited NB-tropic virus. Infection was inhibited by 40–50% and occurred only with extracts of cells which have the *Fv-1* allele which restricts the appropriate virus host-range type. These results, therefore, confirm the specificity of the extract effect for the pattern of *Fv-1* gene restriction established *in vivo* and in cell culture (3–6).

*Dose-Response Titration of Cell Extracts.* Extracts of *Fv-1*$^{b/b}$ and *Fv-1*$^{-1-}$ cells were prepared and the protein concentration was assayed by the Lowry method (19). N-type cells were treated with DEAE-D, washed, incubated with dilutions of each extract for 2 hr, washed again, and then infected with N-tropic virus. The number of infected cells was assayed 42 hr after infection, and the results (Fig. 2) show that only the *Fv-1*$^{b/b}$ extract inhibited infection. The maximum inhibition was 50–60% in cells treated with approximately 150–550 $\mu$g of *Fv-1*$^{b/b}$ cell protein per $10^6$ cells and fell to approximately 10% with 10 $\mu$g of extract protein. The plateau between 150–550 $\mu$g suggests that the amount of inhibitor was either not sufficient to restrict the high virus challenge used (28% infection in control cells), that only a fixed proportion of the cells was able to take up the inhibitor, or that some fraction of the virus is insensitive to the effect of the extract.

*Stability of the Inhibitory Factor(s).* The extracts were generally prepared from confluent primary embryo cultures of the respective cell types collected within 1 week of plating. Preliminary evidence suggests that extracts of cultures held over 2 weeks are less effective. The maximum degree of inhibition was seen when the extracts were freshly prepared and added within 2 hr prior to infection.

TABLE 1. *Mean Virus PFU per culture treated with cell extracts*

| Virus dilution ($Log_{10}$ of reciprocal of dilution) | Infected cells | Cell extract | | | |
|---|---|---|---|---|---|
| | | *Fv-1*$^{-/-}$ | *Fv-1*$^{b/b}$ | *Fv-1*$^{n/n}$ | None |
| *B-tropic* | BALB/c embryo | | | | |
| 0.5 | | TNC | | TNC | TNC |
| 1.0 | | TNC | | 65 | TNC |
| 1.5 | | 54(0) | | 24(40) | 40 |
| 2.0 | | 20(0) | | 14(30) | 20 |
| 2.5 | | 7(0) | | 0(100) | 4 |
| 3.0 | | 5(0) | | 0(100) | 3 |
| *B-tropic* | BALB/c embryo | | | | |
| 2.0 | | 28(0) | 22(0) | 9(50) | 18 |
| 2.5 | | 10(0) | 10(0) | 5(45) | 9 |
| 3.0 | | 4(0) | 1(50) | 1(50) | 2 |
| *N-tropic* | NIH Swiss embryo | | | | |
| 3.0 | | TNC | 64 | | TNC |
| 3.5 | | 79(0) | 14(78) | | 64 |
| 4.0 | | 32(0) | 4(85) | | 26 |
| *N-tropic* | NIH Swiss embryo | | | | |
| 3.0 | | | 66 | | TNC |
| 3.5 | | | 26(59) | | 64 |
| 4.0 | | | 15(42) | | 26 |
| *NB-tropic* | BALB/c embryo | | | | |
| 3.5 | | 22(0) | | 20(0) | 20 |
| 4.0 | | 8(0) | | 6(0) | 6 |
| 4.5 | | 4(0) | | 2(50) | 4 |
| *NB-tropic* | NIH Swiss embryo | | | | |
| 4.0 | | 25(17) | 29(3) | | 30 |
| 4.5 | | 11(0) | 9(0) | 10(0) | 7 |

Secondary cultures of BALB/c or NIH Swiss embryo cells were treated with DEAE-D, washed, and incubated with the respective cell extract for 2 hr. After washing, the cells were infected for 2 hr at 37°. After 72 hr, the cultures were UV-irradiated (1800 ergs/mm$^2$) and overlayed with uninfected cells of the same type. After 72 hr, the cells were irradiated again and overlayed with XC cells. The cultures were fixed 3 days later, stained, and the PFU were counted in duplicate cultures at each dilution.

TNC, too numerous to count.

Numbers in parenthesis represent percent inhibition.

The inhibitor effect of fresh extracts was destroyed by heating at 60° for 0.5 hr and was stable for only 2 hr when held at 37°. When held at 4°, the inhibitory effect was stable for 4 hr, but frozen and thawed extracts lost the inhibitory effect. These results show that the inhibitor(s) is relatively unstable in crude extracts.

*Effect of Nonpermissive Cell Extracts on Virus Attachment.* It is possible that the inhibitory effects of nonpermissive cell extracts could be due to irreversible binding or destruction of virus during attachment to treated cells. Therefore, we tested the effect of cell extract on the attachment of [$^3$H]-uridine-labeled N-tropic virus. The rate and extent of attachment was not affected by nonpermissive or permissive cell extract at 37° (Fig. 3), or when virus was allowed to adsorb at 4° followed by shift to 37°, the latter indicates that the extracts are specific for some postattachment event during infection.

*Effect of Nonpermissive Cell Extracts on Virus In Vitro.* We also tested the effect of treatment of virus with nonpermissive cell extracts by incubating virus and extract together for 20 min at 25° after which the mixture was diluted 1:10 in medium before incubating with cells. The nonpermissive extracts had no effect on infection of permissive cells, showing that the inhibitor(s) did not react with the viruses directly.

*Effect of Nonpermissive Cell Extracts on Permissive Cells.* Since RNA tumor-virus infection is dependent upon certain cellular functions (20–22), we tested whether the nonpermissive cell extracts had any effect on the growth or DNA

TABLE 2. *Percent of virus-infected cells in Fv-1*$^{-/-}$ *cultures treated with cell extracts*

| Virus | *Fv-1*$^{b/b}$ | *Fv-1*$^{n/n}$ | *Fv-1*$^{n/b}$ | *Fv-1*$^{-/-}$ | None |
|---|---|---|---|---|---|
| B-tropic | 34 ± 4(10.5) | 22 ± 5(42.1) | 18 ± 3(52.6) | 36 ± 4(5.3) | 36 ± 3 |
| N-tropic | 14 ± 2(46.2) | 35 ± 5(0) | 16 ± 3(38.5) | 28 ± 5(0) | 26 ± 3 |
| NB-tropic | 79 ± 2(0) | 79 ± 4(0) | 78 ± 3(0) | 74 ± 3(3.9) | 77 |

*Fv-1*$^{-/-}$ cells were pretreated with DEAE-D for 1 hr at 37°. After the cells were washed and incubated with extract for 2 hr, the cells were washed prior to viral infection, washed again 2 hr after virus addition, and medium was added. Coverslip cultures were collected 28 hr after infection and the number of infected cells was determined by immunofluorescent cell counts on at least 1000 cells per group.

Numbers in parenthesis represent percent inhibition.

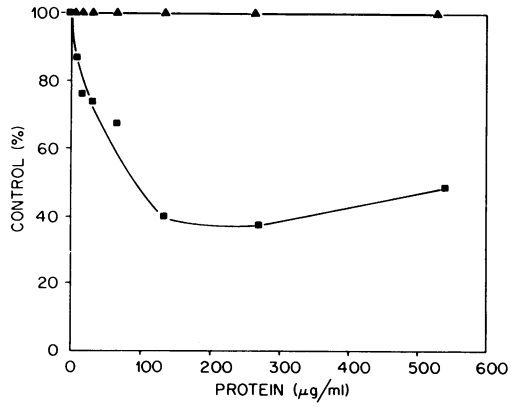


FIG. 2. Response of N-tropic virus to varying concentrations of cellular extract. Secondary cultures of NIH Swiss mouse embryo cells, which had been pretreated with DEAE-D for 1 hr and washed, were exposed to various dilutions of either *Fv-1*$^{b/b}$ or *Fv-1*$^{-/-}$ cell extract for 2 hr. Cells were then washed and infected with N-tropic virus ($10^{6.3}$ PFU/ml). After 2 hr the cells were again washed and fresh medium was added. Coverslips were collected 42 hr after infection, stained with fluorescent antibody, and the percentage of infected cells was counted. Protein concentration of the extracts was determined by the Lowry method. ■, *Fv-1*$^{b/b}$ cell extract; ▲, *Fv-1*$^{-/-}$ cell extract.

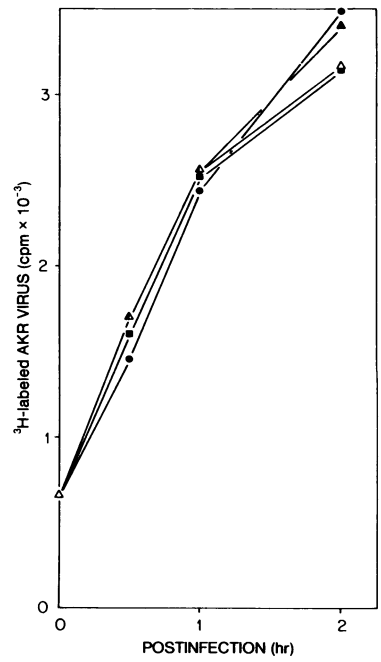


FIG. 3. Effect of cell extracts on adsorption of $^3$H-labeled AKR virus. NIH 3T3 cells were treated with extract for 1 hr at 37°. Cells were washed and then treated with DEAE-D for 1 hr. After a second wash, the cells were incubated with $^3$H-labeled AKR virus ($10^{4.3}$ cpm) at 37°. At the indicated intervals, the cells from duplicate plates were washed three times, scraped into 1 ml of phosphate buffered saline (pH 7.4), and counted in 10 ml of *Aquasol*. ■, *Fv-1*$^{b/b}$ cell extract; ●, *Fv-1*$^{n/n}$ cell extract; ▲, *Fv-1*$^{-/-}$ cell extract; △, no extract.

synthesis of permissive cells. Permissive cells (N-type) were treated with extracts of *Fv-1*$^{b/b}$ or *Fv-1*$^{n/n}$ cells or no extract, and the rate of DNA synthesis was tested by pulse-labeling with [$^3$H]thymidine and the rate of cell growth was measured by protein assay and cell counts. Since there was no significant inhibitory effect of either cell extract on these parameters (Fig. 4A, B, and C), the inhibitory effect is not due to a prolonged or transitory effect on cell functions required for virus expression.

*Effect of Nonpermissive Cell Extracts on the Course of Virus Infection.* In the previous experiments, the nonpermissive cell extracts were added just prior to infection and the maximum inhibition of infection was generally between 60 and 80%. To test the effect of the extracts on later steps in the infection cycle, cells were treated at intervals between 2 hr before and 8 hr after infection (Fig. 5). When assayed by immunofluorescence, inhibition was maximum (75%) when the nonpermissive cell extract was added 2 hr before infection and was between 45 and 60% when added within 2 hr after infection. A similar pattern was seen by plaque assay, and in both assays the degree of inhibition fell to 20 and 25% by 8 hr after infection. These results indicate that the inhibitor(s) is specific for some relatively early event in virus infection.

## DISCUSSION

The results we have presented show that mouse cells with a nonpermissive *Fv-1* gene allele contain a product or products which can inhibit infection of permissive cells by specific mouse leukemia virus host-range types. Since a limited number of mouse strains with the *Fv-1* locus were tested, assignment of the inhibitory effect specifically to this locus must be tentative, but the specificity of the results obtained make the association highly suggestive. Some of the experiments described were confirmed independently in our laboratory with coded samples, and all fluorescent cell counts were done on at least two samples per group, at least 10 samples per field, and 95% confidence intervals were calculated for each group. The nature of the inhibitor(s) has not yet been determined but the results suggest that it is released from nonpermissive cells by sonic disruption, can be taken up by permissive cells, and inhibits some relatively early event in virus infection. Since the nonpermissive cells were not exposed to exogenous virus before disruption, and showed no evidence of spontaneous endogenous virus activation, it appears that the inhibitor(s) is present or continuously synthesized by the cells.

The specificity of the inhibitor(s) for the virus host-range types naturally restricted *in vivo* and in cell culture, shows an effect consistent with a product of the *Fv-1* gene. The *Fv-1* gene has been assigned to linkage group VIII (23), and resistance to N- or B-tropic virus leukemogenesis *in vivo* is dominant (1–3, 24). Cells from resistant mice also restrict virus infection in cell culture (4, 5), and the cells of $F_1$ hybrid mice restrict both virus host-range types (5). The gene does not restrict the chemical activation of endogenous leukemia virus of the nonpermissive host, but does restrict perpetuation of the activated virus (5, 14, 15). In cell culture, the resistance is not absolute and high virus concentrations can overcome the restriction (3, 4, 7).

The properties of the inhibitor(s) extracted from nonpermissive cells are consistent with the functions of the *Fv-1* gene outlined above. The inhibitor(s) was present only in cells with the nonpermissive allele and was effective only against the host range type naturally restricted. At the concentrations tested, the extracts did not completely inhibit infection but the effect was of relatively long duration. Virus was not inactivated by incubation with nonpermissive cell extracts and there was no inhibition of virus attachment by treatment of the cells. The inhibitor(s) also had no effect on cell DNA synthesis and multiplication. Exogenous virus in-

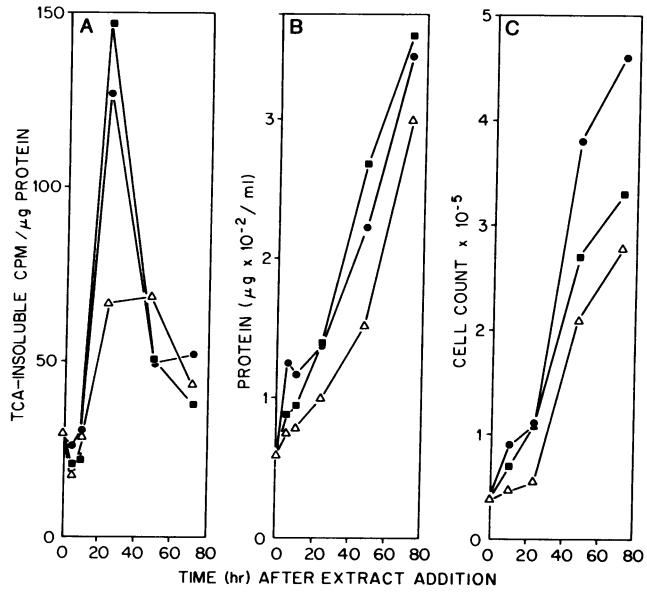


FIG. 4. Effect of cell extracts on cell growth and DNA synthesis. Eighteen-hour secondary cultures of NIH 3T3 cells were treated with DEAE-D for 1 hr at 37°. After washing once, the extract was added and the cells were incubated for 2 hr. Cells were washed again and complete medium was added. Cells were pulse-labeled for 1 hr with [$^3$H]thymidine (1 μCi/ml; specific activity 2.0 Ci/mM). Trichloroacetic acid (TCA)-insoluble counts and protein concentrations were determined from triplicate samples taken at 0, 5, 10, 24, 48, and 72 hr. (A) Trichloroacetic acid-insoluble counts per μg of protein; (B) protein concentration; (C) cell counts., ■, *Fv-1$^{b/b}$* cell extract; ●, *Fv-1$^{n/n}$* cell extract; △, no cell extract.

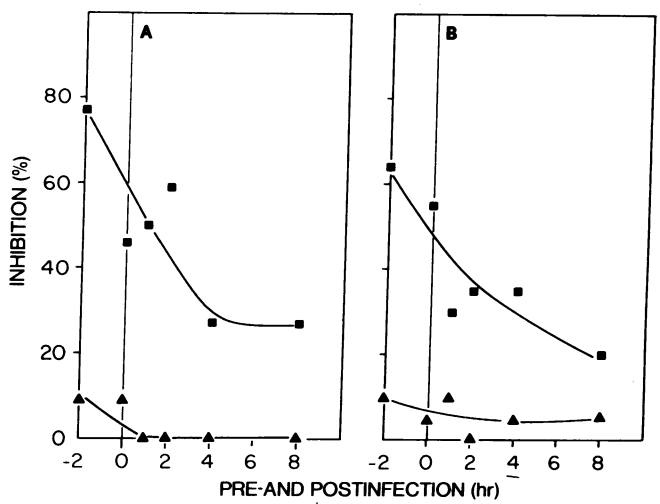


FIG. 5. Effect of cell extract added before and after viral infection of cells. After DEAE-D treatment for 1 hr at 37°, the NIH 3T3 cells were washed and infected with N-tropic virus (A, $10^{6.3}$ PFU/ml; B, $10^{3.3}$ PFU/ml) for 2 hr at 37°. Cells were washed, medium was added, and the cells were reincubated at 37°. Extracts were added at −2, 0, 1, 2, 4, and 8 hr before and after infection. After a 2-hr incubation, the cultures were washed and medium was added. Coverslips for fluorescent-antibody staining were collected 42 hr after infection (A). The modified XC test (B) was performed as described in Table 1. ■, *Fv-1$^{b/b}$* cell extract; ▲, *Fv-1$^{-/-}$* cell extract.

fection requires the function of viral reverse transcriptase and the formation of a DNA provirus (16), but reverse transcriptase does not appear to be required for activation (17). Since nonpermissive cell extracts were most effective only when added immediately before or after infection, it is possible that the inhibition involves some event leading to the formation or integration of the provirus intermediate, and we are now testing the effect of nonpermissive cell extracts on virion reverse transcriptase activity. This interpretation is supported by the fact that viral protein synthesis was inhibited in the treated cell cultures that had been assayed by immunofluorescence with broad reacting antibody. It is not clear from these studies whether the extracts act directly or induce an inhibitor for some specific property of the sensitive virus. The existence of cell gene products of such reciprocal specificity, which dominantly restrict virus host-range types intracellularly, is unique in oncorna virus systems. It is also important to determine if similar inhibitors for other host-range types of RNA tumor viruses exist, since this type of restriction may be important in determining the consequences of tumor virus expression.

This research was sponsored jointly by the Virus Cancer Program of the National Cancer Institute and the U.S. Atomic Energy Commission under contract with Union Carbide Corporation, and by NIH Grant T0I-AI-00435 to the University of Tennessee.

1. Lilly, F. (1970) *J. Nat. Cancer Inst.* **45**, 163–169.
2. Odaka, T. & Yamamoto, T. (1962) *Jap. J. Exp. Med.* **32**, 405–413.
3. Lilly, F. & Pincus, T. (1973) *Advan. Cancer Res.* **17**, 231–277.
4. Hartley, J. W., Rowe, W. P. & Huebner, R. J. (1970) *J. Virol.* **5**, 221–225.
5. Pincus, T., Hartley, J. W. & Rowe, W. P. (1971) *J. Exp. Med.* **133**, 1219–1233.
6. Pincus, T., Rowe, W. P. & Lilly, F. (1971) *J. Exp. Med.* **133**, 1234–1241.
7. Yoshikura, H. (1973) *Int. J. Cancer* **11**, 739–746.
8. Eckner, R. J. (1973) *J. Virol.* **12**, 523–533.
9. Huang, A. S., Besmer, P., Chu, L. & Baltimore, D. (1973) *J. Virol.* **12**, 659–662.
10. Krontiris, T. G., Soeiro, R. & Fields, B. N. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2549–2553.
11. Tennant, R. W., Myer, F. E. & McGrath, L. (1974) *Int. J. Cancer*, in press.
12. Lowy, D. R., Rowe, W. P., Teich, N. & Hartley, J. W. (1971) *Science* **174**, 155–156.
13. Aaronson, S. A., Todaro, G. J. & Scolnick, E. M. (1971) *Science* **174**, 157–159.
14. Stephenson, J. R. & Aaronson, S. A. (1972) *J. Exp. Med.* **136**, 175–184.
15. Stephenson, J. R. & Aaronson, S. A. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 2798–2801.
16. Temin, H. M. & Baltimore, D. (1971) *Advan. Virus Res.* **17**, 129–186.
17. Tennant, R. W., Farrelly, J. G., Ihle, J. N., Pal, B. C., Kenney, F. T. & Brown, A. (1973) *J. Virol.* **12**, 1216–1225.
18. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1971) *Virology* **42**, 1136–1139.
19. Lowry, O. H., Rosebrough, N. T., Farr, A. R. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
20. Bader, J. P. (1969) in *The Biochemistry of Viruses*, ed. Levy, H. B. (Marcel Dekker, New York), pp. 293–329.
21. Yoshikura, H. (1968) *Exp. Cell Res.* **52**, 445–450.
22. Ihle, J. N., Kenney, F. T. & Tennant, R. W. (1974) *J. Virol.* **14**, 451–456.
23. Rowe, W. P., Humphrey, J. B. & Lilly, F. (1973) *J. Exp. Med.* **137**, 850–853.
24. Rowe, W. P. & Hartley, J. W. (1972) *J. Exp. Med.* **136**, 1286–1301.