guinea pigs has been described as a relevant model for the infection in man (*19*). Therefore, this model was used to test the activity of PFA. Two days after inoculation with HSV-1, the skin was erythematous and small vesicles were present in the infected areas. At this stage of the infection treatment was initiated and continued for 3 days with two daily local applications of PFA, giving a total of six applications. On day 5 after infection, the area treated with PFA had healed (Fig. 1), while the area to which only solvent had been applied showed large coalescing vesicles. The areas treated only with solvent healed in 10 to 11 days. It should be stressed that PFA in this model has a therapeutic activity and successful treatment can start after symptoms are evident. Phosphonoacetic is also active in this animal model, but skin irritation occurs (*8, 20*).

Our experiments with PFA demonstrate the possibility of selecting specific antiviral compounds starting at the enzyme level. The apparently low toxicity and high antiherpes activity of PFA in cells, together with its therapeutic effect in an animal model, make PFA a candidate for clinical trials.

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Generation of New Mouse Sarcoma Viruses in Cell Culture

Abstract. *Endogenous nontumor-producing type C viruses from C3H mice were used to generate rapid, solid tumor-inducing variants in cell culture. The new mouse sarcoma viruses induce undifferentiated sarcomas with a short latency period upon inoculation into newborn NIH Swiss mice. Transforming viruses appear only transiently, at a time when the virus-infected cells show morphologic alterations; both before and after this time, transforming viruses cannot be detected. These results show that variants of endogenous type C virus which contain transforming genes (oncogenes) can arise during spread of the endogenous virus in fibroblast lines in vitro as well as in susceptible tissues in vivo.*

Type C virus genes are transmitted as part of the cellular DNA of many, and perhaps all, mammalian and avian species (*1, 2*). The released endogenous type C virus has usually been found to replicate relatively inefficiently and to be poorly oncogenic when tested in susceptible animal hosts (*3, 4*). Variant viruses that produce leukemia rapidly in mice have been readily selected both in vivo and in cell culture by selecting for the property of rapid viral replication (*5*). Some of the new variants also have properties of transforming viruses, producing transient morphological and growth-promoting effects on cells in culture (*5*).

The experiments described herein show that variants of endogenous nontransforming type C viruses can be obtained which directly transform cells in culture and induce rapid formation of solid tumors in vivo; this process comes about after replication of nontransforming mouse viruses in transformed mouse fibroblast cell lines. Two observations were of particular importance in the isolation of these new murine sarcoma viruses (MSV's). One was that the foci that were induced by the new variants initially showed only signs of minimal transformation when compared to foci induced by standard laboratory strains of MSV, such as the Moloney sarcoma virus (*6*). The other was that the morphological effects that accompany the adaptation to high-titer growth in a particular cell line are transient; immediate selection of virus released from such areas of transient transformation was needed in order to isolate a more stable, solid tumor-inducing variant.

We report here that we have isolated transforming viruses that rapidly produce sarcomas in animals and that these viruses can be generated entirely in cell culture. The results show that transient expression of transformed properties can yield stable transforming viruses.

We have described (*5*) rapid leukemia viruses with two different host ranges. One class grows well in mouse cell cul-

Table 1. Transient appearance of "transforming" virus from mouse cell cultures. The test cells were NIH/3T3. The infecting virus was R^+XC^+, which is ecotropic and replicates to a high titer. Spontaneously transformed, flat-growing NIH/3T3 cells were infected with R^+XC^+SL clone 3 virus that had been produced by SC-1 cells. Before and after morphological transformation of the infected NIH/3T3 culture, uncloned culture fluid was used for inoculation of newborn NIH Swiss mice. At the same time, the virus inoculum was tested for the presence of focus-forming activity as well as viruses of the xenotropic and MCF class.

Passage	Cell morphology	Release of transforming virus
0	Normal	0/12
1	Normal	0/10
3	Areas of crisscrossing	0/12
5	Crisscrossing plus rounded cells	3/7
11	Normal	0/8
20	Normal	0/12

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Table 2. Growth-stimulating effect of cloned virus on C3H/10T1/2 CL8 cells. The C3H/10T1/2 CL8 cells were infected at a multiplicity of infection of 2 to 5 polymerase-inducing units per cell with the respective virus stocks and seeded into 0.3 percent Noble agar within 24 hours after infection. Colony growth was measured 10 days after seeding and colony size determined by means of a measuring grid in the eyepiece of a Zeiss inverted microscope. The focus-forming titer of the mink focus-derived clone was 2.8×10^2 FFU/ml when tested for altered colony morphology in monolayer cultures. The polymerase-inducing titer (expressed as polymerase-inducing units, PIU) was 1.5×10^5 PIU/ml in this stock and 3 to 5×10^5 PIU/ml with the other two virus stocks. A colony of C3H/10T1/2 CL8 cells that is 0.13 mm in diameter contains approximately eight cells.

Colony size (mm)	Cells forming colonies (%)			
	Uninfected control	Mink cell plaque-forming clone*	Mink cell transformed focus clone†	MSV control
<0.13	100	96.0	92.0	19.0
0.13 to 0.26	<0.01	3.8	7.4	21.0
0.26 to 0.52	<0.01	<0.01	0.56	54.0
>0.52	<0.01	<0.01	0.027	6.2

*From Fig. 1A. †From Fig. 1B.

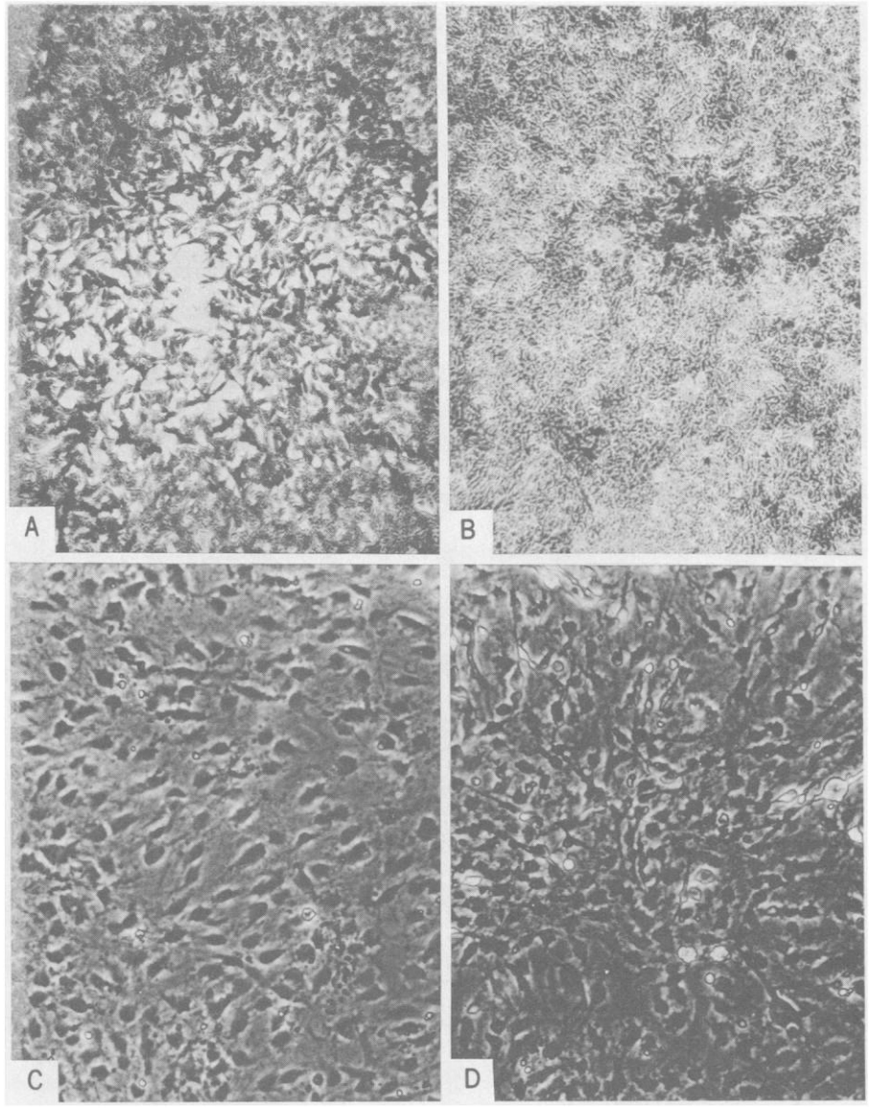

Fig. 1. Phase contrast microscopy of mink and C3H/10T1/2 CL8 cell lines infected with variants of endogenous type C viruses from mice. The mink lung cells were infected with spleen extract from a leukemic NIH Swiss mouse. This mouse had been inoculated with an XC plaque-cloned, rapid leukemia–inducing variant ($R^{++}XC^{+}RL$) of endogenous ecotropic C3H virus. The 10T1/2 cells were infected with the endogenous C3H virus after passage through spontaneously transformed NIH/3T3 cells. (A) Lytic plaque in mink cell monolayer. (B) Focus of morphologically altered mink cells. (C) Uninfected control, C3H/10T1/2 CL8 cells. (D) C3H/10T1/2 CL8 cells infected with culture fluid from NIH/3T3 cells infected with the virus selected for its ability to morphologically alter mouse cells (see text).

tures, but not heterologous cell cultures, such as mink, rabbit, or human. These ecotropic viruses can also form typical syncytia in rat XC cells (*7*, *8*). The second class of rapid leukemia viruses replicates readily in heterologous cells as well as mouse cells and does not form syncytia with XC cells. They, too, can induce leukemia rapidly in newborn NIH Swiss mice. These dual tropic or amphotropic viruses (*9*, *10*) have many properties similar to those viruses designated MCF, isolated from the thymuses of the AKR mouse strain that shows a high incidence of leukemia (*11*, *12*). The ecotropic viruses produce a variety of morphological effects in cells they infect, ranging from rapid cell killing to foci of increased cell proliferation. The dual tropic viruses produce both of these effects in certain mouse embryo cell lines as well as in mink lung cells. Figure 1 shows an example of the lytic effects (Fig. 1A) as well as the transforming effect seen in mink cells (Fig. 1B).

When mouse tropic virus from a continuous line of nontransformed mouse cells, C3H/10T1/2 CL8 (*13*), was grown in other mouse cells, either the wild mouse cell line, SC-1 (*14*), or the NIH Swiss–derived line, NIH/3T3 (*15*), the virus was altered genetically to a replication-competent form (*4*), and during its subsequent spread transient transforming effects were seen (see Table 1). This phenomenon of transient transformation during spread of murine leukemia virus (MuLV) was reproducibly observed upon infection not only of NIH/3T3 but also of the two nontransformed mouse cell lines, C3H/10T1/2 CL8 (fibroblastic) and MC/3T3 (epithelioid) (*16*). The time period during which these morphological effects were most prominent varied with the virus strain as well as the host cell line and generally encompassed a short interval late during spread (80 to 95 percent viral group-specific antigen-positive cells) before the culture was homogeneously virus-positive. Transmission of culture fluid to nontransformed C3H/10T1/2 CL8 cells (Fig. 1C) during this time interval revealed the presence of focus-forming virus (Fig. 1D) at low titers. The development of these transformed foci required a longer period of time, between 9 and 14 days, than did focus formation induced by the laboratory strain of Moloney sarcoma virus, and the morphological alterations were less pronounced.

Upon inoculation of such culture fluid into newborn NIH Swiss mice, rapid formation of solid tumors occurred in the vicinity of the injection site (Table 1). The presence of focus-forming, solid tu-

Table 3. Tumorigenicity of plaque- and focus-derived variants of endogenous mouse type C virus from C3H/10T1/2 CL8 cells. Newborn NIH Swiss mice were inoculated intraperitoneally with 50 μl of virus stock. Mice were weaned at 4 weeks of age and observed daily for the development of tumors.

Virus clone derived from	Neoplasia		Incidence		Latency (months)	
	Leukemia	Solid tumor	Leukemia	Solid tumor	Leukemia	Solid tumor
Mink cell, lytic plaque	From lymphoma, well differentiated to stem-cell leukemia		5/12	0/12	3	
Mink cell, transformed focus		Sarcoma, undifferentiated		14/14		1
Mouse cell, transformed focus		Sarcoma, undifferentiated		3/7		2

mor-inducing virus in the culture fluid of the R^+XC^+ MuLV-infected NIH/3T3 cell line was limited to the time period during which the transient morphological effects were most apparent (passage 5 in the experiment from Table 1) and this activity was absent at earlier as well as later subculture generations. In addition to solid tumor-inducing virus, xenotropic MuLV and variants of the MCF class were also produced between subculture generations three and five.

Morphological transformation in the R^+XC^+ MuLV-infected NIH/3T3 cell culture as well as production of mouse cell focus-forming, solid tumor-inducing virus was transient; however, repeated cycles of cloning from such foci yielded a stock of virus with titers of transforming activity ranging from 10^2 to 10^3 focus-forming units (FFU) per milliliter. The nontransforming parent virus nevertheless remained in approximately a 1000-fold excess even in these stocks. Viruses that transform fibroblasts in culture can also be tested for their ability to induce cells to sustained growth in soft agar. In Table 2 we compare the ability of a "dual tropic" focus-forming virus cloned from a mink cell focus, as depicted in Fig. 1B, to induce C3H/10T1/2 CL8 cells to grow in soft agar with those of the mink plaque purified, dual tropic virus and a known mouse sarcoma virus (Moloney sarcoma virus). The results show that the new transforming virus is very different from the parental virus when tested for its ability to induce sustained cell division in soft agar, although still much less efficient than a laboratory strain like Moloney sarcoma virus. The uninfected cells and those infected with the unselected dual tropic virus were able to go through only a few rounds of cell division.

To test the growth-stimulating potential of the new transforming viruses in animals, newborn NIH Swiss mice were inoculated intraperitoneally with 50 μl of culture fluids. The transforming, dual tropic virus induced large, progressively growing solid tumors within 4 weeks in 100 percent of the inoculated animals (Table 3). The tumors developed in the abdominal cavity close to the injection site. In some animals they were multifocal. All of the tumors, on histological examination, were diagnosed as undifferentiated sarcomas. Animals inoculated at birth with the transforming variant of the ecotropic virus similarly produced sarcomas in three of seven animals within a 2-month period. The viruses released by the tumor cells, in turn, were able to transform 10T1/2 cells in culture in the case of the ecotropic virus, and both mouse and mink cells in the case of the dual tropic virus.

Upon reinoculation of newborn NIH Swiss mice with virus that was produced by several tumor-derived cell lines in culture, the majority of the inoculated mice developed leukemia with an incidence and latency period typical of the helper MuLV from which the focus-forming virus had been derived. Rapid formation of solid tumors occurred only in 8 to 20 percent of the inoculated animals. The established tumor cell lines were stably transformed as determined by transplantation in NIH Swiss mice. These cells continued to release virus capable of inducing solid tumors.

Although type C viruses from rodents are numerous and have been frequently isolated, those viruses able to transform fibroblasts in vitro and to produce sarcomas rapidly upon inoculation are quite rare in the mouse. The Moloney sarcoma virus was obtained from rhabdomyosarcomas induced in BALB/c mice by injecting high titers of leukemia virus (*6*). Other transforming viruses of mice, such as the Abelson variant of Moloney leukemia virus (*17, 18*), the Friend spleen focus-forming virus (*19*) and the FBJ osteosarcoma virus (*20*) all have been derived from animals rather than cell cultures. The Moloney sarcoma-specific sequences of the viral RNA have been found to be closely related to genes already present in normal mouse cellular DNA (*21*). Similarly, the chicken sarcoma virus (*22, 23*) and rat sarcoma viruses (*24*) have specific sequences, the majority of which are present in the cell DNA of the species of origin. In the avian system, recent studies have shown that the transformation-associated sequences of the sarcoma viruses are different from those found in a carcinoma-inducing virus, MH_2, and in a rapid leukemia-inducing virus, MC29, but that all are present in normal cellular DNA (*25*).

The new viruses described here, selected for their ability to transform fibroblasts, differ genetically from Kirsten as well as Moloney sarcoma virus. This was determined in nucleic acid hybridization experiments with the respective sarcoma virus-specific DNA probes (*21, 24*), the new sarcoma virus RNA, and cellular RNA from tumor tissue. Nucleic acid probes to the sarcoma-specific sequences of Moloney sarcoma virus (*21*) and Kirsten sarcoma virus (*24*) show that the newly isolated sarcoma viruses described here are unrelated to either of the known murine sarcoma viruses.

The data from a number of laboratories make it clear that there are several distinct families of virogenes in normal cellular DNA as well as several different potentially transforming genes (oncogenes). The technique outlined herein should make it possible to generate viruses that contain new distinct, defined transforming genes from cell cultures.

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Growth Inhibition of Transformed Cells with Succinylated Concanavalin A

Abstract. *Succinylated concanavalin A reversibly inhibits the growth of SV40 transformed mouse 3T3 cells and thus causes an accumulation of the cells in the G_1 phase of the cell cycle. In a soft substrate (methylcellulose) succinylated concanavalin A also restores in transformed cells the growth behavior typical of untransformed cells.*

Morphological, biochemical, and functional changes of the cell surface are associated with neoplastic transformation both in vivo and in vitro. Lectins, multivalent proteins with carbohydrate specificities, have been used to probe and perturb the surfaces of a wide variety of cell types (*1*). A preferential agglutinability with lectins was one of the early indications that surfaces of transformed cells differed from those of the untransformed parent cells (*2, 3*). With the aim of altering and possibly readjusting the cell surface architecture, we have grown cells in the presence of succinylated concanavalin A (succinyl-Con A) a nontoxic, nonagglutinating derivative of the jack bean lectin concanavalin A (Con A).

It was reported (*4*) that succinyl-Con A reversibly inhibits the growth of untransformed mouse fibroblasts (3T3) through an interaction with cells in the mitotic and early G_1 phases of the cell cycle. During these phases, many untransformed cells assume several of the cell surface characteristics of their transformed derivatives (*5, 6*). We report here that succinyl-Con A reversibly inhibits the growth of SV40 virus transformed 3T3 mouse cells and thus causes an accumulation of cells in the G_1 phase, thereby inducing a growth phenotype similar to that of the untransformed parent cell.

When grown on a solid substratum in Dulbecco's modified Eagle's medium containing either low (2 percent) or high (10 percent) calf serum concentrations, SV40 3T3 cells characteristically grow to high cell densities until nutrient depletion or medium acidity, or both, result in cessation of growth followed by cell death. In the presence of succinyl-Con A, however, SV40 3T3 cells stop growing at low cell densities and maintain their density for several days (Fig. 1A).

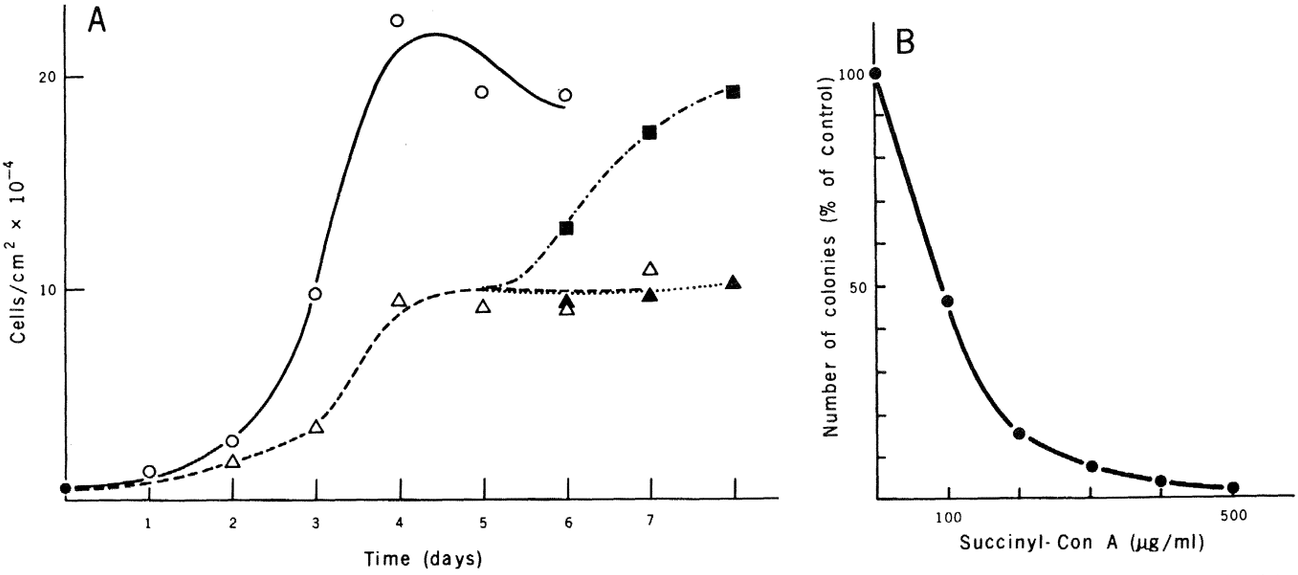

Fig. 1. (A) Inhibition of growth on plastic to high cell densities. The SV40 3T3 cells (6×10^4) were plated into 35-mm Falcon plastic dishes containing 2 ml of Dulbecco's modified Eagle's medium (DME) (4.5 g per liter of glucose) plus 10 percent calf serum (CS). After 24 hours the medium was changed to DME + 2 percent CS with (△) or without (○) 400 μg of succinyl-Con A per milliliter. To keep the inhibited cultures viable for up to 10 days the medium was changed to DME plus 0.1 percent CS with 400 μg of succinyl-Con A per milliliter on day 5 (▲). The cells were released from growth inhibition by changing the medium to DME plus 0.1 percent CS with 10 m*M* α-methyl-D-mannoside on day 5 (■). Cells were harvested with a buffered trypsin solution and counted in a Coulter counter. (B) Inhibition of anchorage-independent growth. The cells were subcultured into DME plus 10 percent CS, 1.2 percent methylcellulose (4000 count/sec, Dow Chemical), and succinyl-Con A as indicated over a 4-ml layer of DME plus 10 percent CS, succinyl-Con A, and 0.9 percent agar in a 6-cm Falcon plastic tissue culture dish; 1 and 2 weeks after subculturing, 4 ml of fresh DME containing 10 percent CS and 1.2 percent methylcellulose were added to each dish; 3 weeks after subculturing the colonies were counted; each point is the total of four dishes; 100 percent represents 2590 colonies.

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