present in the other intimal cell line: under conditions similar to those described in Fig. 2 for experiments performed with cytosol labeled after extraction from the cell, inhibition by estradiol was 6 percent, by testosterone 5 percent, and by hydrocortisone 6 percent.

The vascular endothelial cell line used for these studies possesses an array of receptors for various hormones and neurotransmitters (*3*), a finding which suggests that, through their complex and finely regulated metabolic activity, endothelial cells play an essential role in the regulation of local circulation. In this respect, it is worth noting that the earliest detectable effect of estrogens on the uterus is increased blood supply and increased permeability of the vascular wall (*1*). Thus, the presence of estrogen receptors in endothelial cells seems to suggest that the response of the blood vessels of target organs to estrogens may be an important aspect of the mechanism through which these hormones regulate the cyclical functional variations of the reproductive system. As already noted, clinical observations suggest that the presence of a normal level of circulating ovarian hormones coincides with a lower degree of susceptibility to atherosclerosis. The finding that endothelial cells are potentially capable of responding to estrogens may stimulate attempts to elucidate the nature of the endothelial function which is regulated by these hormones; with this knowledge, one would be better able to evaluate which role a normal hormonal balance may play in delaying the development of the atherosclerotic lesion.

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References and Notes

1. J. Tepperman, in *Metabolic and Endocrine Physiology* (Year Book, Chicago, 1971), vol. 69, pp. 63–85.
2. W. B. Kannel, M. C. Hjortland, P. M. McNamara, T. Gordon, *Ann. Intern. Med.* **85**, 447 (1976); M. C. Hjortland, P. M. McNamara, W. B. Kannel, *Am. J. Epidemiol.* **103**, 304 (1976).
3. V. Buonassisi and C. Venter, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1612 (1976).
4. O. M. Lowry, N. S. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
5. Experiments involving the labeling of endothelial cell cultures with [^{3}H]estradiol for various lengths of time over a 3-hour period, and the separate extraction of the labeled receptor from the cytoplasm and from the nucleus, show that the amount of labeled receptor which is extractable from the nucleus increases with time. During the same period of time, the amount of labeled receptor in the cytoplasm tends to decrease. These results are consistent with the concept that the receptor-hormone complex forms in the cytoplasm and then appears in the nucleus.
6. A. J. Hsueh, E. J. Peck, J. H. Clark, *Nature (London)* **254**, 337 (1975).
7. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
8. Percentage of inhibition was determined by dividing the value of radioactivity bound to cytosol labeled in the presence of competing compound by the value obtained with cytosol labeled in the absence of competing hormone multiplied by 100. Percentage of inhibition is a measure of the fraction of specifically bound [^{3}H]estrogen which is displaced by unlabeled hormone. In the case when the competing compound is unlabeled estradiol, percentage of inhibition also represents the amount of radioactivity which is bound to specific estrogen receptors. The estradiol molarities used for these experiments slightly exceeded the level of hormone at which all the receptor sites are saturated. At higher estradiol molarities the amount of radioactivity which is not specifically bound increases because the level of estradiol at which all the receptor sites are saturated is largely exceeded. Therefore, the fraction of radioactive estradiol displaced by the unlabeled estrogen varies with the concentration of radioactive estradiol in the medium, being higher when the radioactive estradiol concentration in the incubation medium is lower.
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Trisodium Phosphonoformate, a New Antiviral Compound

Abstract. *Trisodium phosphonoformate selectively inhibits cell-free DNA polymerase activity induced by herpesvirus. The new inhibitor has an antiviral effect on herpes simplex virus types 1 and 2, pseudorabies virus, and infectious bovine rhinotracheitis virus in cell culture. It has a good therapeutic activity against cutaneous herpes simplex virus infection in guinea pigs.*

The search for antiviral agents has resulted in the discovery of compounds with activity against herpes simplex virus (HSV) infections, but only a few of these compounds have been submitted to clinical use. Herpes keratitis has been treated with idoxuridine (5-iodo-2′-deoxyuridine) and vidarabine (9-β-D-arabinofuranosyl adenine) (*1*, *2*). Herpes zoster (*3*) and herpes encephalitis (*4*) respond to treatment with vidarabine. However, the high cytotoxicity (*5*, *6*) of these compounds limits their usefulness. A less cytotoxic compound, phosphonoacetic acid (PAA), specifically inhibits DNA polymerase from different herpesviruses (*7*) and arrests herpesvirus infection in mice (*8*) and guinea pigs (*9*), but no clinical results with PAA have been reported.

As pointed out by Cohen (*10*), a rational approach to viral chemotherapy should involve attempts to design compounds that interact specifically with the viral enzymes leaving the cellular enzymes unaffected. As part of a screening program for antiviral drugs, we have used cell-free HSV-1 DNA polymerase and influenza RNA polymerase and, as controls, cellular DNA and RNA polymerases to select specific HSV DNA and influenza RNA polymerase inhibitors (*11*).

One series of compounds investigated included analogs of pyrophosphate, which may interact with the binding of nucleoside triphosphates to polymerases (*12*). One of the first substances tested in this program was the trisodium salt of phosphonoformic acid (PFA), a compound originally synthesized in 1924 by Nylén (*13*) and structurally related to PAA. The crystal structure of this salt has been determined (*14*).

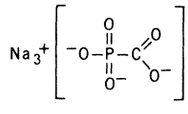

Table 1 shows the effect of PFA and, for comparison, PAA on several DNA and RNA polymerases. It is evident that HSV-1 DNA polymerase is inhibited at low concentrations by both PFA (*14a*) and PAA, and that the two compounds have comparable activities against several DNA polymerases. The preferential inhibition of HSV-1 DNA polymerase in

Table 1. Inhibition of cell-free polymerase activity

Polymerase	Concentration giving 50 percent inhibition (μ*M*)	
	PFA	PAA
HSV-1 DNA polymerase*	3.5	7.0
DNA polymerase α, calf thymus (25 U per assay)†	50.0	75.0
DNA polymerase α, calf thymus (2.5 U per assay)†	3.5	6.5
Micrococcus luteus DNA polymerase†	>500.0	>500.0
Escherichia coli DNA polymerase I†	>500.0	>500.0
Influenza RNA polymerase‡	20.0	300.0
RNA polymerase I, calf thymus†	>500.0	>500.0
RNA polymerase II, calf thymus†	>500.0	>500.0
Escherichia coli RNA polymerase	>500.0	>500.0

*Prepared and assayed according to Weissbach *et al.* (*21*). †Obtained and assayed as described earlier (*22*). ‡Prepared and assayed as described earlier (*22*), but Mn^{2+} was not used in the assay.

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comparison with DNA polymerase α from mammalian cells by PFA and PAA in cell-free systems is dependent on the enzyme concentration (*15*). This finding might explain the conflicting results published earlier concerning inhibition of DNA polymerase α by PAA (*16*). Influenza RNA polymerase is inhibited by PFA at a considerably lower concentration than by PAA. The slight effect of PAA on influenza RNA polymerase has not been reported earlier. The RNA polymerases I and II from mammlian cells are not affected nor are the prokaryotic RNA and DNA polymerases.

The inhibition of HSV-1 DNA polymerase and influenza RNA polymerase by PFA suggested that this compound might inhibit the multiplication of HSV and, at high concentration, influenza virus. Table 2 shows that plaque formation with all tested herpesviruses was reduced more than 90 percent by 100 μM PFA. There was no difference in inhibition of herpesviruses with PFA and PAA, nor did there seem to be any major difference in sensitivity to PFA between HSV-1, HSV-2, pseudorabies virus, or infectious bovine rhinotracheitis virus. Herpesviruses grown in African green monkey kidney, rabbit cornea, or calf kidney cells were all inhibited, indicating that the type of cell is not important for the antiviral effect. No virucidal effect of PFA on HSV-1 was observed (not shown). Vaccinia virus, which earlier has been reported to be sensitive to PAA (*17*) appeared to be significantly less sensitive to PFA. Adenovirus type 2 was slightly inhibited by PFA and PAA at 500 μM concentration. This may be due to inhibition of cellular DNA polymerase α. Phosphonoformic acid did not prevent poliovirus multiplication to any appreciable extent. Influenza virus replication was significantly inhibited by 500 μM PFA, but PAA was not inhibitory. This result correlates with the differential inhibition of influenza RNA polymerase by PAA and PFA (Table 1), although a higher concentration of PFA is required for plaque reduction.

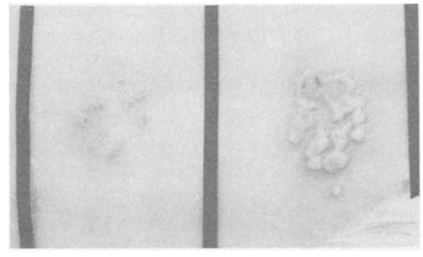

Fig. 1. A guinea pig weighing 350 g was infected on separate areas of the back with a high-titer HSV-1 strain (C 42) according to Hubler *et al.* (*19*). Treatment was initiated 48 hours after infection. The appearance at 5 days after infection is shown. The left infected area has been treated twice daily with a local application of 30 μl of 2 percent PFA in 0.1 percent Tween 80 and 10 percent glycerol in water, whereas the right infected area was treated with solvent only.

Table 2. Inhibition of plaque formation and cytopathogenic effects of PFA and PAA. The HSV-1 and HSV-2 were assayed by plaque titration (*23*). The HSV-1 8/11 had been subjected to repeated passage, the others were from patient isolates. Vaccinia was assayed by means of counting the plaques on HeLa cell monolayers. Pseudorabies and infectious bovine rhinotracheitis virus IBR were assayed by end-point titration on primary calf kidney cultures by standard procedures. Influenza WSN was plaque assayed on canine kidney (MDCK) cells (*24*). Adenovirus type 2 and poliovirus type 1 were from the Department of Microbiology, Biomedical Center, Uppsala, and were plaque assayed as described (*25, 26*). In all assays, PFA or PAA were included in the medium and added after virus adsorption. The inhibition was calculated by using the titers with and without drug. AGMK, African green monkey kidney; SIRC, rabbit kidney.

Virus	Cell	Percentage of inhibition			
		PFA		PAA	
		100 μM	500 μM	100 μM	500 μM
HSV-1 8/11	AGMK	95	>99.9	85	>99.9
HSV-1 8/11	SIRC		>99.9		>99.9
HSV-1 KJ502	SIRC	92			
HSV-1 90155	SIRC	95		94	
HSV-1 V8523	SIRC		>99.9		
HSV-1 V8663	SIRC		>99.9		
HSV-2 91075	SIRC	91		96	
HSV-2 B4327UR	SIRC	96			
HSV-2 V10183	SIRC		>99.9		
HSV-2 V12500	SIRC		>99.9		
Pseudorabies	Calf kidney	90	>99	90	>99
IBR	Calf kidney		>99.9		>99
Vaccinia	HeLa	14	24	26	81
Adenovirus type 2	KB	20	43	15	45
Influenza WSN	MDCK	0	97	0	10
Polio type 1	HeLa		21		39

Table 3. Comparison of antiviral and cellular effects of PFA, PAA, idoxuridine, vidarabine, and ribavirin. The inhibition of HSV-1 plaque formation was determined on AGMK cells (*23*) with the substances added to the medium after virus adsorption. Inhibition of cellular DNA, RNA, and protein synthesis was determined with HeLa cells after 18 hours of incubation with the drug (*27*). Cell proliferation was followed by counting HeLa cells in suspension culture with and without the drug for 48 hours (*27*).

Activity	Concentration giving 50 percent inhibition (μM)				
	PFA	PAA	Idoxuridine	Vidarabine	Ribavirin
HSV-1 plaque	10	20	5	15	25
Cellular DNA synthesis	1,000	1,000	30	70	2
Cellular RNA synthesis	>10,000	>10,000		500	13
Cellular protein synthesis	>10,000	>10,000	>500	>500	>500
Cell proliferation	>100				

The cellular toxicity of PFA and its inhibitory activity against herpesvirus multiplication has been compared to the corresponding activities of PAA, idoxuridine, vidarabine, and ribavirin as shown in Table 3. The concentrations yielding 50 percent reduction of HSV-1 plaque formation are somewhat similar, but the effects on cellular DNA synthesis differ considerably between these compounds. Both PFA and PAA are more than one order of magnitude less inhibitory to cellular DNA synthesis than the other compounds. PFA has no effect on RNA and protein synthesis at a concentration of up to 2.5 mM, and 100 μM PFA does not influence cell proliferation.

A direct comparison of the effect of PFA on herpesvirus DNA and cellular DNA synthesis in infected cells was made as follows. African green monkey kidney cells infected with HSV-1 were labeled with [^{3}H]thymidine from 2 to 16 hours after infection, and viral and cellular DNA were separated on cesium chloride gradients (*18*). In the presence of 100 μM PFA, viral DNA synthesis was reduced by 85 percent and cellular DNA synthesis by 15 percent, showing a selective effect of PFA on viral DNA synthesis in the infected cell.

Cutaneous herpesvirus infection in

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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References and Notes

1. H. E. Kaufman, *Proc. Soc. Exp. Biol. Med* **109**, 251 (1962).
2. D. Pavan-Langston and H. C. Dohlman, *Am. J. Ophthalmol.* **74**, 81 (1972).
3. R. J. Whitley, L. T. Ch'ien, R. Dolin, G. J. Galasso, C. A. Alford, Jr., Eds., and the Collaborative Study Group, *N. Engl. J. Med.* **294**, 1193 (1976).
4. R. J. Whitley, S.-J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford, and the Collaborative Study Group, *ibid.* **297**, 289 (1977).
5. J. I. McGill, H. P. Williams, J. R. McKinnon, A. D. Holt-Wilson, B. R. Jones, *Trans. Ophthalmol. Soc. U.K.* **94**, 542 (1974).
6. A. H. Ross, A. Julia, C. Balakrishnan, *J. Infect. Dis.* **133** (Suppl.), A192 (1976).
7. J.C.-H. Mao, E. E. Robishaw, L. R. Overby, *J. Virol.* **15**, 1281 (1975).
8. S. R. B. Harris and M. R. Boyd, *J. Antimicrob. Chemother.* **3** (Suppl. A), 91 (1977).
9. S. Alenius and B. Öberg, *Arch. Virol.*, in press.
10. S. S. Cohen, *Science* **197**, 431 (1977).
11. E. Helgstrand and B. Öberg, in "Proceedings of the 10th International Congress of Chemotherapy, September 1977," *Current Chemother.* (1978), p. 329.
12. S. S. Leinbach, J. M. Reno, L. F. Lee, A. F. Isbell, J. A. Boezi, *Biochemistry* **15**, 426 (1976).
13. P. Nylén, *Chem. Ber.* **57B**, 1023 (1924).
14. R. R. Naqui, P. Wheatley, E. Foresti-Serantoni, *J. Chem. Soc.* **1971A**, 2751 (1971).
14a. After this report was submitted we learned that the effect of PFA on herpesvirus was independently reported by J. M. Reno, L. F. Lee, J. A. Boezi, *Antimicrob. Agents Chemother.* **13**, 188 (1978).
15. B. Eriksson and B. Öberg, in preparation.
16. J. Hay *et al.*, *J. Antimicrob. Chemother.* **3** (Suppl. A), 63 (1977).
17. L. R. Overby, R. G. Duff, J.C.-H. Mao, *Trans. N.Y. Acad. Sci.* **284**, 310 (1976).
18. G. M. Aron, D. J. M. Purifoy, P. A. Schaffer, *J. Virol.* **16**, 498 (1975).
19. W. R. Hubler, Jr., T. D. Felber, D. Troll, M. Jarratt, *J. Invest. Dermatol.* **62**, 92 (1974).
20. S. Alenius, Z. Dinter, B. Öberg, *Antimicrob. Agents Chemother.*, in press.
21. A. Weissbach, S.-C. L. Hong, J. Aucker, R. Muller, *J. Biol. Chem.* **248**, 6270 (1973).
22. B. Eriksson *et al.*, *Antimicrob. Agents Chemother.* **11**, 946 (1977).
23. P. M. Ejercito, E. D. Kieff, B. Roizman, *J. Gen. Virol.* **2**, 357 (1968).
24. J. Bentley and E. A. Wickham, *Arch. Gesamte Virusforsch.* **33**, 234 (1971).
25. L. Philipson, *Virology* **15**, 263 (1961).
26. J. J. Holland and L. C. McLaren, *J. Bacteriol.* **78**, 596 (1959).
27. A. Larsson, K. Stenberg, B. Öberg, *Antimicrob. Agents Chemother.* **13**, 154 (1978).
28. We thank E. Lycke, A. Svedmyr, S. Gronovitz, and C. Källander for the HSV strains; Z. Dinter for the pseudorabies, IBR, and vaccinia; and R. Krug for the influenza WSN virus.

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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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