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Inhibition of Synthesis of Murine Leukemia Virus in Cultured Cells by Polyribonucleotides and Their 2'-*O*-Alkyl Derivatives

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SUMMARY

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Poly(adenylic acid) [poly(A)], poly(inosinic acid) [poly(I)], poly(uridylic acid) [poly(U)], and poly(cytidylic acid) [poly(C)] inhibit the synthesis of Moloney murine leukemia virus in cultured JLS-V9 cells. The potency of inhibition depends on the base composition; poly(I) is more potent than poly(A) and poly(U), and poly(C) yields only a marginal relative inhibition. 2'-*O*-Alkyl polynucleotides show an enhanced inhibitory potency relative to the parent polynucleotides, and this enhancement is more marked for pyrimidine than for purine polynucleotides. These polynucleotides do not affect the population growth rates of normal cells; thus inhibition of virus synthesis apparently is not due to any cytotoxicity of polynucleotides for normal cells. The following order of inhibitory potency is observed: poly(I) > poly(A) > poly(U) > poly(C); poly(2'-*O*-methyl-inosinic acid) [poly(Im)] > poly(2'-*O*-methyl-uridylic acid) [poly(Um)] > poly(2'-*O*-ethyl-adenylic acid) [poly(Ae)] > poly(Am) ≧ poly(2'-*O*-methyl-cytidylic acid) [poly(Cm)]; and poly(Im) > poly(I), poly(Am) > poly(A), poly(Um) > poly(U), poly(Cm) > poly(C).

INTRODUCTION

Single-stranded polynucleotides inhibit viral RNA-directed DNA polymerase or reverse transcriptase *in vitro* (1-9). Tuominen and Kenney (1) first noted the inhibition of DNA polymerase activity of Rauscher murine leukemia virus by polyribonucleotides. We have reported the inhibition of DNA polymerase activity of Moloney murine leukemia virus by several polyribonucleotides and their 2'-*O*-alkyl derivatives (2, 3). We found that polyinosinic acids were more potent than other polyribonucleotides and their 2'-*O*-methyl derivatives. Green and co-workers (9) studied the inhibition of partially purified avian virus DNA polymerase by 2'-*O*-alkyl polyribonucleotides. They noted poly(2'-*O*-methylinosinic acid) to be the most potent inhibitor of this enzyme. Similarly, Erickson and Grosch (5) reported the inhibition of avian and feline viral DNA polymerases by halogenated derivatives of poly(uridylic

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acid) and poly(cytidylic acid). Recently De Clercq *et al.* (8) noted the inhibition of oncornavirus DNA polymerase activities by 2'-azido derivatives of poly(U) and poly(C).

The activity of RNA-directed DNA polymerase apparently is required for infection and transformation of cells by these viruses (for recent reviews, see refs. 10 and 11). This is substantiated by the lack of infectivity of a mutant of Rous sarcoma virus deficient in RNA-directed DNA polymerase (12) and the temperature dependence of infectivity and DNA polymerase activity of temperature-sensitive mutants of Rous sarcoma virus (13). These considerations raised the possibility that inhibitors of RNA-directed DNA polymerase of RNA tumor viruses may also inhibit the replication of these viruses in cell culture and animal systems. In a preliminary report we noted that polyadenylic acids suppress the replication of murine leukemia virus in cell culture and spleen focus formation in mice (14). Recently Tennant *et al.* (15) reported the suppression of sarcoma development and death in newborn mice by poly(2'-methyladenylic acid). In their preliminary reports Tennant *et al.* (16) and Pitha *et al.* (17) also reported the inhibition of murine leukemia virus replication by polynucleotides, albeit at high concentrations (more than 100 μg/ml). In a subsequent report Tennant *et al.* (18) showed that poly(adenylic acid) and poly(2'-*O*-methyladenylic acid) have two concentration-dependent effects on MuLV[1] infection of cultured cells. At high concentration (100 μg/ml) poly(A) and poly(Am) inhibited the uptake of MuLV by cultured Swiss mouse embryo cells, and at low concentration (10 μg/ml) neither poly(A) nor poly(Am) affected the uptake of virus; poly(Am) did inhibit virus replication significantly. We have investigated the effect of polyribonucleotides on the synthesis of MuLV in cultured cells at relatively low concentrations, 10 μg/ml or less. To assess the impact of base composition and of 2'-*O*-substitution, we have studied several polynucleotides and their 2'-*O*-alkyl derivatives. We present here the results of our studies with poly(A), poly(Am), poly(Ae), poly(I), poly(Im), poly(U), poly(Um), poly(C), and poly(Cm).

[1] The abbreviations used are: MuLV, murine leukemia virus; poly(Am), poly(2'-*O*-methyladenylic acid); poly(Ae), poly(2'-*O*-ethyladenylic acid); poly(Im), poly(2'-*O*-methylinosinic acid); poly(Um), poly(2'-*O*-methyluridylic acid); poly(Cm), poly(2'-*O*-methylcytidylic acid); PFU, plaque-forming units.

MATERIALS AND METHODS

Polynucleotides. Poly(A) (8.1 S), poly(I) (mol wt > 100,000), poly(U) (7.4 S), poly(C) (4.6 S), and oligo(dT)$_{12-18}$ were obtained from Miles Laboratories. Poly(Am) (7.0 S), poly(Ae) (9.0 S), poly(Im) (8.0 S), and poly(Cm) (5.5 S) were synthesized by polymerization of 2'-*O*-alkyl nucleoside diphosphates as described by Tazawa *et al.* (19). Poly(Um) (10.2 S) was obtained from P-L Biochemicals. Tritium-labeled thymidine triphosphate (50 Ci/mmole) was obtained from New England Nuclear Corporation. The concentration of polynucleotides in 0.01 M NaCl–0.01 M Tris-HCl (pH 7.2) was determined spectrophotometrically by using the following extinction coefficients for a 1 mg/ml solution: poly(A), 28.5 at 258 nm; poly(Am), 27.5 at 257 nm; poly(Ae), 26.5 at 257 nm; poly(I), 27.5 at 248 nm; poly(Im), 27.0 at 248 nm; poly(U), 30.0 at 260 nm; poly(Um), 29.5 at 260 nm; poly(C), 21.0 at 267 nm; poly(Cm), 18.5 at 268 nm.

Cells and virus. Mouse bone marrow-derived JLS-V9 cells (20) were grown as monolayers in plastic flasks, using RPMI-1640 culture medium containing 10% fetal calf serum. The Moloney strain of murine leukemia virus (MuLV$_M$) was obtained from cultured JLS-V9 cells chronically infected with this virus; the culture medium from such cells was harvested and, after being clarified by slow-speed centrifugation, was used as a virus inoculum for uninfected cells. This preparation of virus contained $7 \pm 1 \times 10^6$ plaque-forming units/ml when measured by XC assay (see below).

The RPMI-1640 culture medium, fetal calf serum, and calcium-magnesium-free phosphate-buffered saline [0.02 M sodium phosphate (pH 7.4)–0.15 M NaCl] were ob-

tained from Grand Island Biological Company.

Virus assays. The titer of $MuLV_M$ in a virus preparation was determined by two methods, XC plaque assay and virion-associated RNA-directed DNA polymerase assay. The XC plaque assays were performed essentially as described by Rowe *et al.* (21). Briefly, subconfluent monolayers of NIH 3T3 cells grown in Dulbecco's modified Eagle's medium (Grand Island Biological Company) were treated with DEAE-dextran, washed with phosphate-buffered saline, and inoculated with serial dilutions of the virus preparation. When the monolayer became confluent (4–6 days), they were ultraviolet-irradiated and overlayered with XC cells. Four days later the cultures were fixed and stained, and plaques due to syncytia formation were scored.

For the virion-associated RNA-directed DNA-polymerase assay, the virus preparation, such as culture medium harvested from infected cells, was clarified by slow-speed centrifugation (1000 × *g*, 5 min), layered on a column of 20% glycerol in 0.01 M Tris-HCl (pH 8.3)–0.15 M NaCl–0.01 M EDTA, and centrifuged at 100,000 × *g* for 1 hr in a Spinco SW 50.1 rotor (Beckman Instruments). The supernatant was discarded, the pelleted virus was suspended in a small volume of 0.01 M Tris-HCl (pH 8.3), and aliquots were used for determining the detergent-activated, virion-associated DNA polymerase activity. DNA polymerase assays were performed in a reaction mixture (50 or 100 μl) containing 0.05 M Tris-HCl (pH 7.9), 0.06 M NaCl, 0.001 M $MnCl_2$, 0.02 M dithiothreitol, 0.05% NP-40, 50:5 μM poly(A):oligo(dT) (molar ratio, 10:1), 50 μM [^{3}H]thymidine triphosphate (6600 cpm/pmole), and suspended pellet (25 or 50 μl). The mixture was incubated at 37° for 30 min, and the radioactivity incorporated into acid-precipitable material was determined as described before (3).

Polynucleotide treatment and virus infection of cultured cells. Duplicate monolayer cultures of JLS-V9 cells (1.6–1.8 × 10^6 cells/25-cm^2 plastic plate) were treated for 30 min with 2 ml of DEAE-dextran (10 μg/ml) in RPMI-1640 medium, washed with phosphate-buffered saline, and incubated for 2 hr with 2 ml of polynucleotide solution in RPMI-1640 medium. The control cultures received medium alone. Cultures were washed with phosphate-buffered saline and infected with $MuLV_M$ by incubating them for 1 hr with 2 ml of virus inoculum in RPMI-1640 medium plus 10% fetal calf serum (multiplicity of infection = 5–10 PFU/cell). Cultures were washed again with phosphate-buffered saline and incubated for 20 hr with 5 ml of polynucleotide solution in RPMI-1640 medium plus 10% fetal calf serum. The control cultures received medium without polynucleotide. Subsequently cultures were washed with phosphate-buffered saline and reincubated with fresh RPMI-1640 medium plus 10% fetal calf serum but without polynucleotide. Twenty hours later the culture medium was harvested and the titer of progeny virus in the medium was determined as described above. All operations were carried out at 37°.

RESULTS

The effect of poly(I) and poly(A) on the synthesis of $MuLV_M$ in cultured JLS-V9 cells is shown in Fig. 1. For this set of experiments, duplicate cultures of cells were treated with different concentrations of polynucleotide and inoculated with virus, and the culture medium was harvested as described in MATERIALS AND METHODS. The titer of progeny virus in the harvested medium from polynucleotide-treated and control cultures was determined by assaying for the virion-associated RNA-directed DNA polymerase activity catalyzed by poly(A):oligo(dT). It is apparent that increasing the concentration of these polynucleotides in the culture medium yielded a graded increase in the response in terms of inhibition of progeny virus synthesis relative to the controls. The slopes of the central two-thirds of the dose-response curves are about 0.58, which would be consistent with a situation in which 1 drug molecule is bound per receptor (22). The concentration of poly(I) yielding 50% inhibition of progeny virus synthesis under the experimental conditions was about 6 μg/ml, and that of poly(A)

appeared to be about 12 μg/ml.

To assess the relative inhibitory potency of various polynucleotides, we then investigated the effect of polynucleotides on progeny virus synthesis at a fixed concentration of polynucleotide (10 μg/ml) under experimental conditions similar to those just described. The results are presented in Tables 1 and 2. For these studies, the titer of virus (in the harvested medium) was determined both by XC plaque assay and by the RNA-directed DNA polymerase assay. Apparently the inhibition scored by XC plaque assay agreed with that scored by DNA polymerase, although there was a measurable difference between the two assays in the case of poly(A). In our hands the titer of virus estimated by DNA polymerase assays was more sensitive, precise, and reproducible than that estimated by XC plaque assays, and we depended more on the former than the latter assays for evaluating the relative inhibitory potency of the polynucleotides.

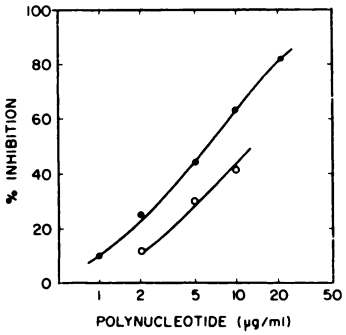

FIG. 1. *Effect of poly(I) and poly(A) on synthesis of Moloney murine leukemia virus in cultured JLS-V9 cells*

Duplicate monolayers of cells were treated with various concentrations of polynucleotide, or with culture medium alone, and infected with virus as described in the text. Culture medium from control and polynucleotide-treated cultures was harvested, and the titer of progeny virus in the medium was determined by assaying for the virion-associated RNA-directed DNA polymerase activity catalyzed by poly(A):oligo(dT). The results are expressed as percentage inhibition relative to the controls, which did not receive polynucleotide. ●——●, poly(I); ○——○, poly(A).

As the data presented in Table 1 show, the polynucleotides, at a concentration of 10 μg/ml, showed significant inhibition of progeny virus synthesis; the potency of inhibition evidently depended on their base composition. Poly(I) was more inhibitory than poly(A) as well as poly(U), yielding about 60% inhibition at 10 μg/ml. Poly(C) showed only marginal inhibition at this concentration.

The data showing the effect of 2′-*O*-alkyl polynucleotides on progeny virus synthesis are presented in Table 2. At a concentration of 10 μg/ml, poly(Im) yielded about

TABLE 1

Effect of polyribonucleotides on Moloney murine leukemia virus infection of cultured JLS-V9 cells

Monolayers of cultured cells were treated with DEAE-dextran (10 μg/ml, 30 min), washed with phosphate-buffered saline, and incubated for 2 hr in culture medium with or without polynucleotide (10 μg/ml). They were washed with phosphate-buffered saline and infected with Moloney murine leukemia virus (multiplicity of infection = 5–6 PFU/cell). The cultures were washed with phosphate-buffered saline and incubated for 20 hr in cultured medium with or without polynucleotide (10 μg/ml). They were washed again with phosphate-buffered saline and reincubated in fresh culture medium lacking polynucleotide. Twenty hours later the culture medium was harvested and the titer of progeny virus was determined by XC and DNA polymerase assays as described in the text.

Polynucleotide	XC plaque-forming assay		DNA polymerase assay	
	Activity	Inhibition	Activity	Inhibition
	PFU/ml × 10^{-4}	%	*pmoles/ml*	%
None	5.6 ± 1.0		34.9 ± 1.3	
Poly(A)	2.7 ± 0.6	51.4	20.7 ± 1.6	40.7
Poly(I)	2.1 ± 0.5	62.5	14.4 ± 0.9	58.7
Poly(U)	4.3 ± 1.0	23.2	27.3 ± 1.2	21.8
Poly(C)	4.9 ± 0.9	12.5	32.4 ± 1.3	7.2
Uninfected	0		0.6 ± 0.01	

TABLE 2

Effect of 2'-O-alkylated polyribonucleotides on Moloney murine leukemia virus infection of cultured JLS-V9 cells

Monolayers of cultured cells were treated with DEAE-dextran (10 μg/ml, 30 min), washed with phosphate-buffered saline, and incubated for 2 hr in culture medium with or without polynucleotide (10 μg/ml). They were washed with phosphate-buffered saline and infected with Moloney murine leukemia virus (multiplicity of infection = 8–10 PFU/cell). The cultures were washed with phosphate-buffered saline and incubated for 20 hr in cultured medium with or without polynucleotide (10 μg/ml). They were washed again with phosphate-buffered saline and reincubated in fresh culture medium lacking polynucleotide. Twenty hours later the culture medium was harvested and the titer of progeny virus was determined by XC and DNA polymerase assays as described in the text.

Polynucleotide	XC plaque-forming assay		DNA polymerase assay	
	Activity	Inhibition	Activity	Inhibition
	PFU/ml × 10^{-4}	%	*pmoles/ml*	%
None	46.0 ± 10		47.1 ± 2.9	
Poly(Am)	12.7 ± 3.0	72.3	13.6 ± 0.8	71.1
Poly(Ae)	9.0 ± 2.0	80.4	10.7 ± 1.3	77.3
Poly(Im)	5.7 ± 1.7	87.6	4.6 ± 0.7	90.2
Poly(Um)	7.0 ± 0.1	84.8	6.1 ± 0.3	87.0
Poly(Cm)	15.5 ± 2.5	66.3	12.8 ± 0.9	72.8
Uninfected	0.2 ± 0.02		0.17 ± 0.01	

90% inhibition of progeny virus synthesis as compared with about 60% inhibition observed for non-methyl poly(I): an increase of about 30% (Tables 1 and 2). Similarly, poly(Am) was about 30% more potent than poly(A). Poly(Ae) was even more potent than poly(Am). Thus it appears that 2'-*O*-methyl and 2'-*O*-ethyl derivatives of purine polynucleotides were about 30–40% more potent than the corresponding non-alkyl polynucleotides. 2'-*O*-Methyl derivatives of pyrimidine polynucleotides, on the other hand, were several times more potent than the corresponding non-methyl polynucleotides. Whereas poly(C), at 10 μg/ml, gave about 10% inhibition of progeny virus synthesis, poly(Cm) at the same concentration gave about 70% inhibition, a 7-fold enhancement of inhibitory potency. Similarly, poly(Um) was about 4 times more potent than poly(U) (Tables 1 and 2).

Since it is known that inhibitors of cell division also inhibit the synthesis of RNA tumor viruses (for recent reviews, see refs. 10 and 11), it was important to determine whether the inhibitory action of the polynucleotides might not be due to a general cytotoxicity of polynucleotides for the cultured cells used in this study. Therefore we studied the effects of some of the more potent polynucleotides [poly(I), poly(A), poly(Am), and poly(Ae)] on the growth rates of normal cultured cells. Representative data showing the effect of poly(I) on the growth rates of cultured JLS-V9 cells are shown in Fig. 2. For these studies, duplicate monolayers of cells were grown in the continuous presence of poly(I) (10 μg/ml) and replenished with fresh medium containing poly(I) every 24 hr. It is apparent that poly(I) had no significant effect on the population growth rates of normal cells. Similar results were obtained with other polynucleotides. When the viability of cultured cells was scored by the trypan blue exclusion test (23), no difference between the control and polynucleotide-treated cultures was found (data not shown). Since the cells were grown in the continuous presence of polynucleotides, these results also diminished the possibility that polymer degradation products might significantly affect the cell growth. In addition, when the cells were treated with polyadenylic acids in a manner similar to that used for the studies of their inhibition of progeny virus synthesis, but mock-infected, no difference between the growth of control and polynucleotide-treated cultures was noted (data not

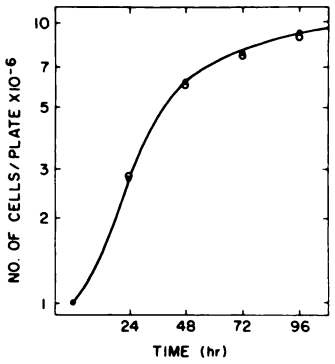

FIG. 2. *Effect of poly(I) on growth rates of cultured JLS-V9 cells*

Duplicate monolayers of cells were grown in the presence and absence of poly(I) (10 μg/ml) and replenished with fresh medium containing or lacking (controls) polynucleotide every 24 hr. Culture medium was removed at specified times, and cells were detached from the surface by mild trypsinization and counted. ●——●, control cultures; ○——○, poly(I)-treated cultures.

shown). Other workers have also reported that polyadenylic acids and polyuridylic acids, even at concentrations much higher than those used in this study (for example, 100–500 μg/ml), did not significantly affect the growth or viability of growing or stationary populations of cultured cells (16, 17).

DISCUSSION

The results presented in this report show that single-stranded polyribonucleotides can specifically inhibit the synthesis of murine leukemia virus in cultured cells. The relative inhibitory potency of polynucleotides depends on their base composition and 2′-*O*-substitution. The following order of potency of inhibition was obtained: poly(I) > poly(A) > poly(U) > poly(C); poly(Im) > poly(Um) > poly(Ae) > poly(Am) ≧ poly(Cm); and poly(Nm) > poly(N). These results can be summarized by three generalized statements portending testable structure-activity hypotheses. (a) Depending on the functional groups on the ring [see item (b)], purine polynucleotides may be more potent than pyrimidine polynucleotides. Although not strictly comparable, the greater potency of poly(I) over poly(U) and of poly(A) over poly(C) supports this notion. (b) Polynucleotides possessing a ring O=C—N—H functional group may be more potent than polynucleotides lacking this function [poly(I) > poly(A) and poly(U) > poly(C)]. Alternatively, acidic polynucleotides may be more potent than basic polynucleotides. (c) 2′-*O*-Alkyl polynucleotides are more potent than the parent non-alkyl polynucleotides [e.g., poly(Am) > poly(A)], and 2′-*O*-ethyl derivatives are more potent than 2′-*O*-methyl derivatives [poly(Ae) > poly(Am)].

The physicochemical basis of these observations is not yet clear. The explanation(s) for these results obviously must reside in the solution properties of polynucleotides, largely governed by their constituent bases, and in their interaction with a molecular target(s) or receptor(s). One could suggest, for example, that the higher potency of purine polynucleotides relative to pyrimidine polynucleotides may be related to the greater resonance energy, along with stacking interactions, of purine than of pyrimidine bases. The total resonance energy as well as resonance energy per electron in general is greater in purines than in pyrimidines (23), and purine oligo- or polynucleotides in general show greater stacking interactions than pyrimidine oligo- or polynucleotides (24–28). One could also suggest that the ring function, O=C—N—H, interacts with the molecular target(s) or receptor(s).

The higher potency of 2′-*O*-alkyl polynucleotides relative to non-alkyl polynucleotides is presumably due to their greater relative resistance to thermal or nucleolytic degradation (19) and, hence, their half-life in biological fluids. It is also possible that the 2′-oxygen function plays a role in the interaction of polynucleotide with its receptor(s) such that the substituents which increase the charge (electron) density at this position also increase the interaction. This might explain both the higher potency of 2′-*O*-methyl polynucleotides relative to non-methyl polynucleotides, and the higher potency of ethyl than of methyl polynucleotides. By virtue of the inductive effects, the ethyl group can be expected to increase the charge density at the 2′-oxygen to a degree greater than the

methyl group. It is clear that a precise molecular explanation(s) for our results must await a better understanding of the physicochemical properties of the single-stranded polynucleotides as well as identification of the molecular target(s) or receptor(s) of polynucleotide inhibition. These targets could be activities specifically associated with the infecting virus but could also include those cellular activities which may be directly or indirectly involved in virus infection.

Lack of any effect of polynucleotides on normal cell growth would suggest that the molecular target(s) of polynucleotides is not the normal cellular biosynthetic activities but those activities which are specific for or modified by virus infection. Among these activities, a likely target is the virion-associated RNA-directed DNA polymerase. This likelihood is supported by several observations. (a) Polynucleotides are more effective as inhibitors when added prior to or just after the virus infection; this suggests that an event early in virus replication is affected (16–18). (b) Polynucleotides do not inhibit the replication of those lytic RNA viruses which do not require the activity of RNA-directed DNA polymerase (17, 18). (c) The relative order of inhibitory potency of polynucleotides for murine leukemia viral DNA polymerase *in vitro* in general correlates with their relative inhibitory potency for virus replication in cell culture (2, 3).

In summary, our studies show that single-stranded polynucleotides are potent inhibitors of virus replication in cell culture and that their inhibitory potency depends on their base composition and 2′-*O*-substitution. The inhibition apparently is not due to any general cytotoxic effects of polynucleotides on normal cell growth. The target of inhibition remains to be defined, although it seems more likely to be a function associated with virus replication than a function operative in normal cell growth.

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REFERENCES

1. Tuominen, F. W. & Kenney, F. T. (1971) *Proc. Natl. Acad. Sci. U. S. A.*, 68, 2198–2202.
2. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1974) *Biochem. Biophys. Res. Commun.*, 59, 608–615.
3. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) *Mol. Pharmacol.*, 11, 421–426.
4. Erickson, R. J., Janik, B. & Sommer, R. G. (1973) *Biochem. Biophys. Res. Commun.*, 52, 1475–1482.
5. Erickson, R. J. & Grosch, J. C. (1974) *Biochemistry*, 13, 1987–1993.
6. Abrell, J. W., Smith, R. G., Robert, M. S. & Gallo, R. C. (1972) *Science*, 177, 1111–1113.
7. Srivastava, B. I. S. (1973) *Biochim. Biophys. Acta*, 335, 77–84.
8. De Clercq, E., Billiau, A., Hobbs, J., Torrence, D. F. & Witkop, B. (1975) *Proc. Natl. Acad. Sci. U. S. A.*, 72, 284–288.
9. Green, M., Gerard, G. F., Grandgenett, D. P., Gurgo, C., Rankin, A. M., Green, M. R. & Cassell, D. M. (1974) *Cancer*, 34, 1427–1428.
10. Temin, H. M. (1974) *Adv. Cancer Res.*, 19, 48–104.
11. Green, M. & Gerard, G. F. (1974) *Prog. Nucleic Acid Res. Mol. Biol.*, 14, 187–334.
12. Hanafusa, H. & Hanafusa, T. (1971) *Virology*, 43, 313–316.
13. Verma, I. M., Mason, W. S., Drost, S. D. & Baltimore, D. (1974) *Nature*, 251, 27–31.
14. Arya, S. K., Carter, W. A., Alderfer, J. L. & Ts'o, P. O. P. (1975) *Mol. Pharmacol.*, 11, 501–505.
15. Tennant, R. W., Hanna, M. G., Jr. & Farrelly, J. G. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, 71, 3167–3171.
16. Tennant, R. W., Kenney, F. T. & Tuominen, F. W. (1972) *Nat. New Biol.*, 238, 51–53.
17. Pitha, P. B., Teich, N. M., Lowy, D. R. & Pitha, J. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, 70, 1204–1208.
18. Tennant, R. W., Farrelly, J. G., Ihle, J. N., Pal, B. C., Kenney, F. T. & Brown, A. (1973) *J. Virol.*, 12, 1216–1225.
19. Tazawa, I., Tazawa, S., Alderfer, J. L. & Ts'o, P. O. P. (1972) *Biochemistry*, 11, 4931–4937.
20. Wright, B. S., O'Brien, P. A., Shibley, G. P., Mayyasi, S. A. & Lasfargues, J. C. (1967) *Cancer Res.*, 27, 1672–1677.
21. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1970) *Virology*, 42, 1136–1139.
22. Goldstein, A., Aronow, L. & Kalman, S. M. (1974) *Principles of Drug Action, Ed. 2*, pp. 82–96, Wiley, New York.
23. Phillips, H. J. & Terryberry, J. E. (1957) *Exp.*

Cell Res., 13, 341–347.
24. Pullman, B. & Pullman, A. (1963) *Quantum Biochemistry*, pp. 209–210, Interscience, New York.
25. Ts'o, P. O. P., Melvin, I. S. & Olson, A. C. (1963) *J. Am. Chem. Soc.*, 85, 1289–1296.
26. Cantor, C. R. & Tinoco, I., Jr. (1965) *J. Mol. Biol.*, 13, 65–77.
27. Yang, J. T. & Samejima, T. (1969) *Prog. Nucleic Acid Res. Mol. Biol.*, 9, 223–300.
28. Rottman, F. & Heinlin, K. (1968) *Biochemistry*, 7, 2634–2641.