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ANTIVIRAL AND ANTIMETABOLIC ACTIVITIES OF POLY (7-DEAZAADENYLIC ACID) AND POLY(7-DEAZAINOSINIC ACID)

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Abstract—The antiviral and antimetabolic activities of poly(7-deazaadenylic acid) [poly(c^7A)]*, poly(7-deazainosinic acid) [poly(c^7I)] and their nucleosides 7-deazaadenosine (c^7A) and 7-deazainosine (c^7I) have been assessed in primary rabbit kidney (PRK) and mouse JLSV5 cells, the latter being a transformed, (Rauscher) murine leukemia virus-shedding cell line. The antiviral activity was monitored by investigating the effects of the compounds on the replication of vaccinia, herpes simplex or vesicular stomatitis virus in PRK or JLSV5 cells and by measuring endogenous oncornavirus production in JLSV5 cells. The inhibition of cellular RNA and DNA synthesis served as parameters of antimetabolic activity. With all compounds tested the minimum effective concentrations inhibiting virus replication coincided remarkably well with the minimum toxic concentrations impairing cellular RNA and DNA synthesis or normal cell morphology. These concentrations amounted to 0·3–0·6 μg/ml for c^7A and poly(c^7A), 3 μg/ml for c^7I and 10 μg/ml for poly(c^7I). At higher concentrations (≃ 70 μg/ml) poly(c^7A) and poly(c^7I) were also found to inhibit the *in vitro* DNA polymerase activity of (Moloney) murine leukemia virus. According to the data presented, poly(c^7A), poly(c^7I) and the corresponding monomers c^7A and c^7I cannot be considered as specific antiviral or antitumor agents.

The antibiotic 7-deazaadenosine (tubercidin, c^7A), a structural analog of adenosine in which CH replaces the nitrogen in position 7 of the aglycone (Fig. 1), and its deaminated derivative, 7-deazainosine (c^7I), have been shown to inhibit the growth of sarcoma and leukemia cells [1]. In addition, c^7A inhibits the growth of microorganisms (*Streptococcus faecalis*) [2], mouse L-cells [3], H.Ep No. 2 cells [4] and a variety of human tumor cells [5] at quite low concentrations (0·001–0·01 μg/ml). Deazaadenosine replaces adenosine in a number of metabolic reactions [2, 6], is incorporated into host cell RNA, and, upon intracellular conversion to the 2′-deoxy form, is also incorporated into host cell DNA [3]. Hence, it is not surprising that c^7A blocks the synthesis of DNA, RNA (and protein) in cell culture systems and that it inhibits the growth of both RNA and DNA viruses in these cells [3].

Deazainosine is 10- to 20-fold less effective than deazaadenosine in inhibiting Ehrlich ascites, sarcoma 180 and leukemia P-388 cells in mice [1]. It is also 10- to 20-fold less toxic than c^7A, as demonstrated in mice, rats and dogs [7]. Since c^7I, unlike c^7A, inhibits the growth of mouse L-cells only after a lag period of 24 hr [3], it has been assumed that c^7I requires intracellular conversion into c^7A (anabolites) in order to exert its biologic activity [1]. Deazaadenosine itself is not subject to deamination [2]. It has also been speculated that, depending on the rate at which cells do carry out the amination of c^7I to c^7A, deazainosine may have more selective toxicity for some cells (or tissues) than deazaadenosine [1, 7].

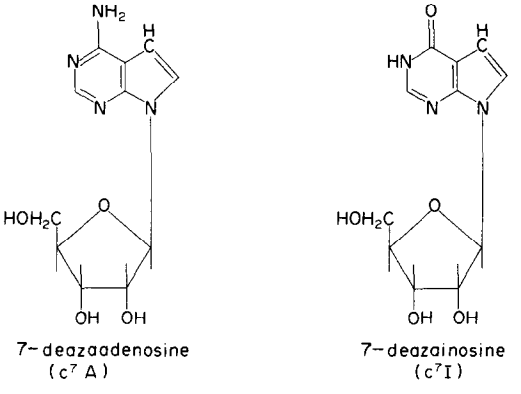

Fig. 1. Structural formulae of c^7A and c^7I.

The studies presented herein were aimed at resolving the following questions:

(i) are poly(7-deazaadenylic acid) [poly(c^7A)] and poly(7-deazainosinic acid) [poly(c^7I)] equally effective in inhibiting virus replication as their monomer forms c^7A and c^7I?

(ii) If they are effective, is their antiviral activity due to a specific inhibition of one or another step in the viral growth cycle or merely the consequence

* Abbreviations used: c^7A, 7-deazaadenosine; c^7I, 7-deazainosine; poly(c^7A), poly(7-deazaadenylic acid); poly(c^7I), poly(7-deazainosinic acid); PRK, primary rabbit kidney; VSV, vesicular stomatitis virus; MuLV (Moloney), Moloney strain of murine leukemia virus; MEM, Eagle's minimal essential medium; PBS, Dulbecco's phosphate buffered saline; TCA, trichloroacetic acid; $CCID_{50}$, cell culture infecting dose-50 or virus dose infecting 50% of the cells cultures; PFU, plaque forming unit; T_m, melting temperature.

of an overall inhibition of cellular RNA and DNA synthesis?

(iii) Is the activity of poly(c^7A) and poly(c^7I) limited to cytolytic viruses, or are they also effective against oncogenic viruses (e.g. murine leukemia viruses)?

That poly(c^7A) and poly(c^7I) differ in biological activity from their corresponding nucleosides, would not be unexpected since (i) such polynucleotides may have altered biochemical properties (e.g. uptake, distribution,...) or (ii) being macromolecules, they might interact with cellular constituents in a completely different manner from the monomers.

In a previous study [8], poly(c^7A) was found to protect PRK cells against the cytopathic effect of vesicular stomatitis virus. It was also ascertained that this antiviral activity was not mediated by interferon production [8].

MATERIALS AND METHODS

Compounds. 7-Deazaadenosine (tubercidin, c^7A) was purchased from Calbiochem (Los Angeles, California). 7-Deazainosine was prepared from tubercidin by deamination with nitrous acid [9]. The preparation and properties of poly(c^7A) [8, 10] and poly(c^7I) [9] as well as the properties of their complexes with complementary polynucleotides have been described previously. Poly(c^7A) and poly(c^7I) were of high molecular weight as judged by sedimentation coefficient and by the fact that they were completely eluted from a Sephadex G-200 column (2·5 × 30 cm) in the void volume. The sources and characteristics of the other polynucleotides used herein have been reported elsewhere [8, 9]. Stock solutions of the compounds were prepared at 1 mg/ml in 0·1 M Tris–HCl, 0·2 M NaCl, pH 7·0 and stored at 4°.

Reagents. [^{3}H]uridine (26 Ci/m-mole), [*methyl*-^{3}H]thymidine (12 Ci/m-mole) and [^{3}H]dTTP ([*methyl*-^{3}H]thymidine-triphosphate) (sp. act. 21·6 or 30 Ci/m-mole depending on the batch) were purchased from the C.E.N. Radioisotopes Department, Mol, Belgium. The unlabeled deoxyribonucleoside triphosphates dATP, dCTP and dGTP, as well as calf thymus DNA, dithiothreitol, actinomycin D and cycloheximide were purchased from Sigma Chemical Co. (St. Louis, Mo.). Omnifluor was a product of NEN Chemicals, Frankfurt-am-Main, Germany and Carbopol (Carboxypolymethylene, Carbopol resin 934) was from B. F. Goodrich Co., Cleveland, Ohio. A stock solution of 4 mg/ml of Carbopol was prepared in PBS (Dulbecco's phosphate buffered saline) and stored at 4°.

Cell cultures. Primary rabbit kidney (PRK) cells and JLSV5 cells (a continuous cell line carrying Rauscher murine leukemia virus [11]) were grown to confluency in Eagle's minimal essential medium (MEM) (+ 10% normal or fetal calf serum) in cell culture tubes, 60-mm Falcon plastic petri dishes or 15-mm Linbro multiplates.

Viruses. Vaccinia, herpes simplex (type 1) and vesicular stomatitis virus (VSV) (Indiana strain) were propagated in embryonated eggs (chorioallantoic membrane), PRK cells or BSC-1 cells, respectively. Titers of these virus stocks, all determined in PRK cell cultures, were 10^7 (vaccinia), 10^6 (herpes simplex) and 10^6 (VSV) $CCID_{50}$ per ml. The Moloney strain of murine leukemia virus [MuLV (Moloney)] was supplied by Electro-Nucleonics Laboratories (Bethesda, Md.) and contained 10^{11}–10^{12} virus particles per ml of TNE buffer (0·01 M Tris–HCl, 0·1 M NaCl, 0·001 M EDTA, pH 7·0). All virus stocks were stored at −70°. Prior to use 8% glycerol was added to the MuLV (Moloney) virus suspension: this virus could be kept at 4° in the presence of 8% glycerol for several months without an appreciable loss of DNA polymerase activity.

Inhibition of PRK cell RNA and DNA synthesis. Confluent PRK cell monolayers in Falcon plastic petri dishes were exposed to different concentrations of the compounds (dissolved in MEM + 3% calf serum; 2 ml/petri dish) for 24 hr at 37°, washed with MEM, and then incubated for 30 min at 37° with either [*methyl*-^{3}H]thymidine (2 μCi/ml MEM per petri dish) or [^{3}H]uridine (1 μCi/ml MEM per petri dish). The cells were then washed with PBS and the plates successively immersed in a mixture of ethanol and acetic acid (2:1 v/v) and perchloric acid (stock solution of 70%, diluted 1/32 in distilled water) as described previously [12]. After the plates were broken into pieces and dissolved in the scintillation fluid (Omnifluor: 15 ml/petri plate), their radioactivity was determined in a (Packard) liquid scintillation spectrometer.

Inhibition of viral cytopathogenicity in PRK cells. Confluent PRK cell cultures in tubes were incubated with different concentrations of the compounds (in MEM + 3% calf serum, 1 ml/tube) for 24 hr at 37°, washed with MEM, and then inoculated with 100 $CCID_{50}$ (per tube) of either vaccinia, herpes simplex or vesicular stomatitis virus. Excess virus was removed after 1 hr. The cell cultures were replenished with MEM (+ 3% calf serum) (1 ml/tube) and further incubated at 37°. Cytopathogenicity was recorded as soon as it reached 100% in the control cell cultures (at 1–2 days for vesicular stomatitis virus, 2–3 days for vaccinia virus, 3–4 days for herpes simplex virus).

Inhibition of JLSV5 cell RNA synthesis. Confluent JLSV5 cell monolayers in Linbro multiplates were incubated with different concentrations of the compounds (in MEM + 10% fetal bovine serum, 1 ml/cup) for 24 hr at 37°, washed with MEM, and then treated with [^{3}H]uridine (2·5 μCi/ml MEM per cup) for 30 min at 37°. The cells were then successively washed with PBS, TCA (5%, at 4°), and denatured alcohol, and finally dissolved in 0·5 N NaOH (1 ml/cup). The samples were further diluted in (Packard) Dimilium Scintillation fluid (0·5 ml sample for 10 ml Dimilium) and their radioactivity determined in a (Packard) liquid scintillation spectrometer.

Inhibition of oncornavirus production by JLSV5 cells. Confluent JLSV5 cell monolayers in Falcon plastic petri dishes were exposed to selected concentrations of the compounds (in MEM + 10% fetal bovine serum, 3 ml/petri dish). [^{3}H]uridine (50 μCi/petri dish) was added immediately after addition of the compounds. After 24 hr incubation at 37° the supernatant fluid was collected and processed for sucrose gradient analysis as described previously [11]. Acid-insoluble radioactivity was determined with all fractions of the gradient. A characteristic C-type virus profile was witnessed with peak radioactivity at density 1·16 (g/cm^3). This peak radioactivity was used

Table 1. Effect of c^7A, c^7I, poly(c^7A) and poly(c^7I) on virus-induced cytopathogenicity in PRK cells

Compounds†	Minimum inhibitory concentration* (μg/ml) Vaccinia virus	Herpes simplex virus	Vesicular stomatitis virus
c^7A	0·3	0·3	0·3 (1)‡
c^7I	3	3	3 (10)
Poly(c^7A)	0·3	0·3	0·3 (6)
Poly(c^7I)	>10	>10	10 (>10)

* Required to inhibit virus-induced cytopathogenicity by 50%.
† Added to the cells 24 hr before virus challenge.
‡ In parentheses: minimum toxic concentration (μg/ml) causing a microscopically visible alteration of the cells.

as a parameter for the endogenous production of extracellular oncornavirus particles by JLSV5 cells.

Inhibition of VSV multiplication in JLSV5 cells. Confluent JLSV5 cell monolayers in Linbro multiplates were incubated with different concentrations of the compounds (in MEM + 10% fetal bovine serum, 1 ml/cup) for 24 hr at 37°, washed with MEM, and then challenged with VSV at multiplicity of infection > 1. Excess virus was removed after 1 hr; the cells were washed and incubated for another 24 hr. The supernatant fluids were then harvested and virus yields were determined by plaque formation in L-929 cell cultures.

Inhibition of MuLV (Moloney) DNA polymerase activity. DNA polymerase activity was measured at 37° in 280 μl of an assay mixture that contained 40 mM Tris–HCl (pH 7·8), 50 mM NaCl, 4 mM $MnCl_2$, 1·6 mM dithiothreitol, 0·0125% (v/v) Triton X-100, 0·64 mM each of dATP, dCTP and dGTP, 0·035 mM [^{3}H]dTTP, 20 μg compound, 20 μl Carbopol stock solution [13] and 20 μl MuLV (Moloney) virus stock suspension. At various times 50-μl aliquots of the assay mixture were withdrawn, spotted onto Whatman GF/C glassfiber discs and tested for acid-insoluble radioactivity as described previously [13].

RESULTS AND DISCUSSION

Antiviral and antimetabolic activities of c^7A, poly(c^7A), c^7I and poly(c^7I) in PRK cells. As shown in Table 1, c^7A and poly(c^7A) suppressed vaccinia, herpes simplex and vesicular stomatitis virus-induced cytopathic effects in PRK cells at identical concentrations (0·3 μg/ml). Somewhat higher concentrations were needed for poly(c^7I) ($\geq$10 μg/ml) than for (c^7I) (3 μg/ml) to cause the same inhibition of virus-induced cytopathogenicity. In these experiments the compounds were added 24 hr before virus challenge. Almost identical results were obtained if the compounds were administered immediately after virus inoculation (data not shown).

The antimetabolic properties of the compounds, as monitored by determination of cellular RNA and DNA synthesis, are depicted in Figs. 2 and 3. As

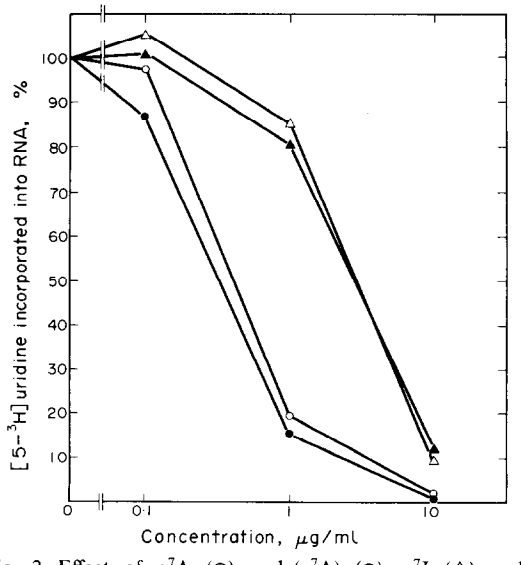

Fig. 2. Effect of c^7A (○), poly(c^7A) (●), c^7I (△) and poly(c^7I) (▲) on [^{3}H]uridine incorporation into RNA of PRK cells. [^{3}H]uridine incorporation was measured after the cells had been exposed to different concentrations of the compounds (as indicated in the abscissa) for 24 hr. Data represent mean values for four observations.

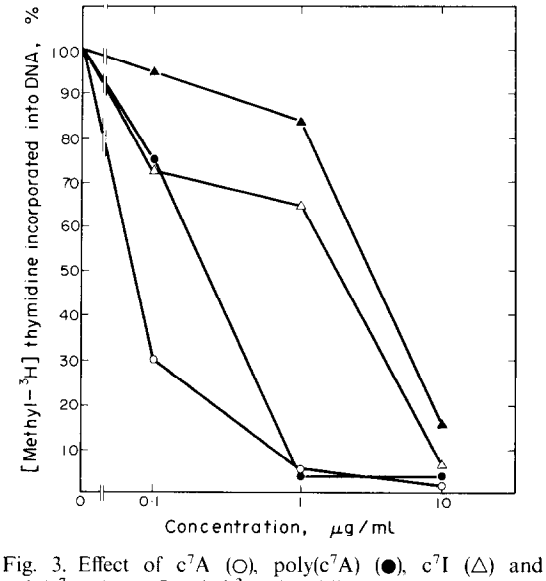

Fig. 3. Effect of c^7A (○), poly(c^7A) (●), c^7I (△) and poly(c^7I) (▲) on [*methyl*-^{3}H]thymidine incorporation into DNA of PRK cells. [*methyl*-^{3}H]thymidine incorporation was measured after the cells had been exposed to different concentrations of the compounds (as indicated in the abscissa) for 24 hr. Data represent mean values for two observations.

Table 2. Antiviral indexes of c^7A, c^7I, poly(c^7A) and poly(c^7I) in PRK cells

Compounds	Minimum virus-inhibitory concentration* (μg/ml) A	Minimum antimetabolic concentration† (μg/ml) B	Antiviral index B/A
c^7A	0·3	0·4	1·3
c^7I	3	3	1·0
Poly(c^7A)	0·3	0·3	1·0
Poly(c^7I)	10	3	0·3

* Required to inhibit virus-induced cytopathogenicity by 50% (data presented in Table 1).

† Causing an inhibition of RNA synthesis by 50% (calculated from data presented in Fig. 2).

demonstrated previously [3] in mouse L-cells, c^7A inhibted both RNA and DNA synthesis of PRK cells at concentrations as low as 0·1–1 μg/ml. Poly(c^7A) did not markedly differ from c^7A in inhibiting host cell RNA and DNA synthesis. Neither did poly(c^7I) significantly differ from c^7I, although both c^7I and poly(c^7I) were at least ten times less active than c^7A and poly(c^7A).

As summarized in Table 2, the minimum virus-inhibitory concentrations (required to prevent virus-induced cytopathogenicity by 50%) and minimum antimetabolic concentrations (required to suppress host cell RNA or DNA synthesis by 50%) of either c^7A, c^7I, poly(c^7A) or poly(c^7I) were remarkably similar. The antiviral indexes calculated from these data are close to or equal to 1 [for poly(c^7I), even lower than 1].

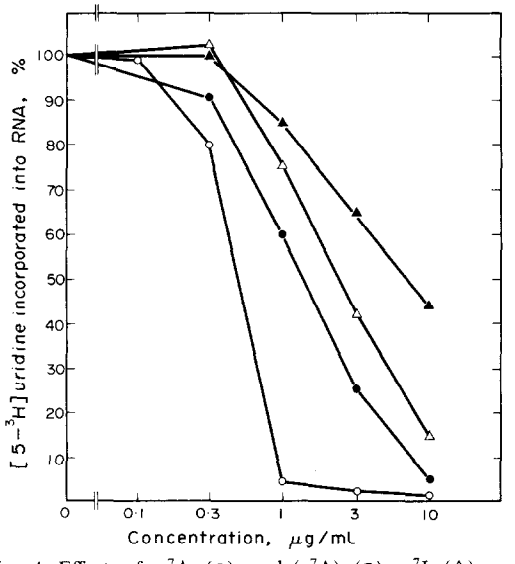

Fig. 4. Effect of c^7A (○), poly(c^7A) (●), c^7I (△) and poly(c^7I) (▲) on [^{3}H]uridine incorporation into RNA of JLSV5 cells. [^{3}H]uridine incorporation was measured after the cells had been exposed to different concentrations of the compounds (as indicated in the abscissa) for 24 hr. Data represent mean values for two observations.

Hence, these compounds cannot be considered as specific antiviral agents, since their inhibitory effects on viral growth (or viral cytopathogenicity) seem directly related to, and most probably accounted for by, an inhibition of cellular macromolecule synthesis.

Antiviral and antimetabolic activities of c^7A, poly(c^7A), c^7I and poly(c^7I) in JLSV5 cells. RNA synthesis in the tumor cell line JLSV5 was inhibited by c^7A, c^7I, poly(c^7A) and poly(c^7I) to approximately the same extent as RNA synthesis in the primary kidney cells (Fig. 4), c^7A and poly(c^7A) being consistently more effective than their deaminated analogues. Those concentrations of c^7A, poly(c^7A), c^7I and poly(c^7I) which brought about a 20–40% reduction of host cell RNA synthesis were selected for exploring an effect on extracellular oncornavirus production by JLSV5 cells. At the concentrations selected [0·3 μg/ml for c^7A, 1·0 μg/ml for poly(c^7A) and for c^7I, and 3·0 μg/ml for poly(c^7I)], the release of oncornavirus particles by JLSV5 cells, as measured by sucrose gradient analysis of the [^{3}H]uridine labelled particles, was not specifically suppressed. In fact, c^7A, c^7I, poly(c^7A) and poly(c^7I) inhibited host cell RNA synthesis and oncornavirus particle release to a similar extent (Table 3).

Next, VSV multiplication was determined in JLSV5 cells which had been exposed to different concentrations of the compounds. As illustrated in Fig. 5, c^7A and poly(c^7A) were equally effective but clearly more effective in reducing VSV yields than c^7I and

Table 3. Effect of c^7A, c^7I, poly(c^7A) and poly(c^7I) on oncornavirus production by JLSV5 cells

Compounds*	Dosis (μg/ml)	[^{3}H]uridine incorporation into host cell RNA (%)	[^{3}H]uridine incorporation into oncornavirus RNA (%)
c^7A	0·3	81	75·5
c^7I	1·0	75	69·3
Poly(c^7A)	1·0	60	76·7
Poly(c^7I)	3·0	65	81·0

* Added to the cells for 24 hr. [^{3}H]uridine added at the end (for measuring host cell RNA synthesis) or at the beginning (for measuring oncornavirus RNA synthesis) of this 24-hr period.

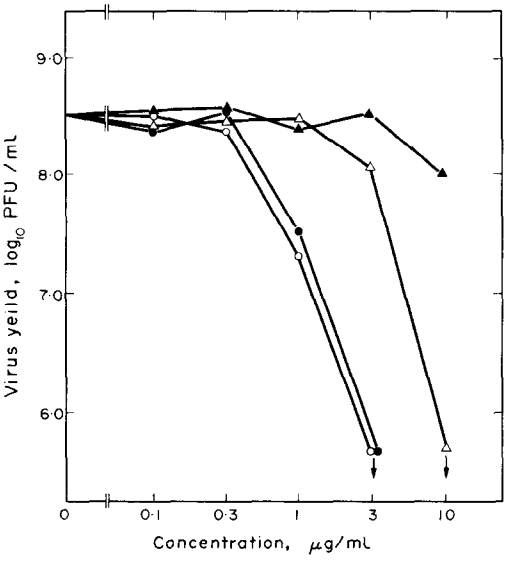

Fig. 5. Effect of c^7A (○), poly(c^7A) (●), c^7I (△) and poly(c^7I) (▲) on VSV replication in JLSV5 cells. Compounds added to the cells at different concentrations (as indicated in the abscissa) 24 hr before virus challenge. Minimum toxic concentrations, causing a microscopically visible alteration of the cells, were 1, 6, 3 and 10 μg/ml for c^7A, c^7I, poly(c^7A) and poly(c^7I), respectively.

poly(c^7I). Again, the minimum virus-inhibitory concentrations (required to suppress virus replication) and the minimum antimetabolic concentrations (reducing host cell RNA synthesis by 50%) were compared (Table 4). The antiviral indexes calculated from these data were close to 1. Poly(c^7A) showed a somewhat higher anti-viral index than the other compounds, but, it is clear that in JLSV5 cells, as noted above for PRK cells, the antiviral properties of the 7-deaza compounds are directly related to their detrimental effects on normal cell metabolism.

A comparison of the results obtained in PRK cells (Table 2) with those obtained in JLSV5 cells (Table 4), reveals a remarkable similarity in the activities of the 7-deaza compounds, whether antiviral or antimetabolic. It is also clear that neither compound offers a specific advantage in tumor (JLSV5) cells as opposed to primary (PRK) cells.

Inhibitory effects of poly(c^7A) and poly(c^7I) on MuLV (Moloney) DNA polymerase activity. In a standard reaction mixture employed before [14] to assess the inhibitory effects of poly(2′-azido-2′-deoxyuridylic acid) and poly(2′-azido-2′-deoxycytidylic acid) on MuLV (Moloney) DNA polymerase activity, poly(c^7A) proved quite effective in inhibiting DNA synthesis at a concentration (*ca* 70 μg/ml) poly(A) failed to do so (Fig. 6a). This inhibitory effect was only partially reduced if poly(c^7A) had been complexed with poly(U) before addition to the reaction mixture. This is not entirely surprising since the complex may dissociate under the conditions used ([T_m of poly(c^7A). poly(U): 37° (0·15 M Na^+)] [8]).

Poly(I) and poly(c^7I) inhibited the MuLV (Moloney) DNA polymerase activity to the same extent (Fig. 6b). No inhibition was observed if poly(I) and poly(c^7I) had been complexed with poly(C) before addition to the reaction mixture, suggesting that the inhibitory effect specifically resided in the homopolymer structure. Poly(c^7I). poly (C) and poly(I). poly(C) are not expected to dissociate into its constituent homopolymers under the conditions used (T_m's: 49° and 67° (0·2 M Na^+), respectively [9]).

Additional experiments were carried out to ascertain whether the inhibitory effects of poly(c^7A) and poly(c^7I) on the *in vitro* DNA polymerase (or reverse transcriptase) activity of oncornaviruses were matched by a similar effect on the primo-infection of untransformed cells by these viruses. At 1 μg/ml (a concentration far beneath the concentration found inhibitory in the *in vitro* reverse transcriptase tests), poly(c^7A), poly(c^7I), as well as their mononucleosides failed to inhibit the development of infectious centers [14] in MO cells (an untransformed, contact-inhibited 3T3-type cell line derived from C3H mouse embryo) inoculated with (Moloney) murine sarcoma virus (data not shown). Cytotoxicity precluded the use of the compounds at concentrations higher than 1 μg/ml.

CONCLUSIONS

(1) In all tests, whether antiviral or antimetabolic, the activity of poly(c^7A) closely resembled or even equalled that of c^7A. Similarly, poly(c^7I) did not markedly differ in activity from c^7I. In accord with previous findings [1, 7], c^7A consistently surpassed c^7I in activity and the same relative potency was reflected by the corresponding polymers. The similarity in the activity of the 7-deaza nucleosides and their respective polymers may be interpreted to mean that poly(c^7A) and poly(c^7I) operate through their monomer forms, that they are degraded to mono (or oligo) nucleotides after they have been taken up by the cells, and that the degraded material is subsequently incorporated into cellular nucleic acids (as demonstrated before [3]).

Table 4. Antiviral indexes of c^7A, c^7I, poly(c^7A) and poly(c^7I) in JLSV5 cells

Compounds	Minimum virus-inhibitory concentration* (μg/ml) A	Minimum antimetabolic concentration† (μg/ml) B	Antiviral index B/A
c^7A	0·6	0·5	0·8
c^7I	3	2·5	0·8
Poly(c^7A)	0·6	1·5	2·5
Poly(c^7I)	10	7	0·7

* Causing a 3-fold reduction of VSV yield (calculated from data presented in Fig. 5).
† Causing an inhibition of RNA synthesis by 50% (calculated from data presented in Fig. 4).

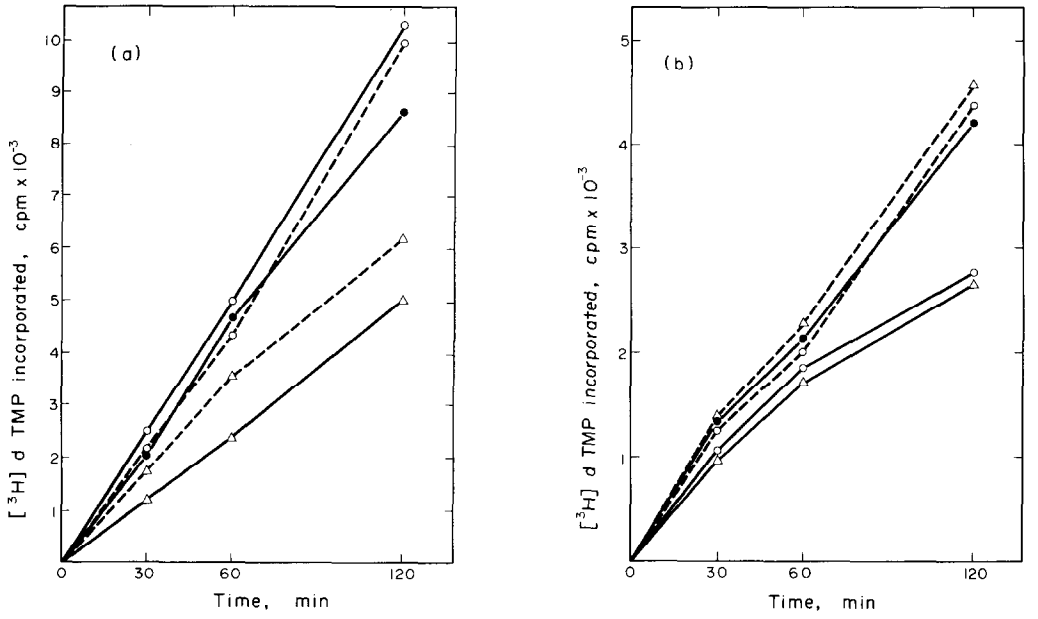

Fig. 6a. (a) Effect of poly(c^7A) (Δ——Δ), poly(c^7A)·poly(U) (Δ- - - -Δ), poly(A) (O——O), and poly(A)·poly(U) (O- - - -O) on MuLV (Moloney) DNA polymerase activity (control: ●——●). Standard assay mixture as described in Materials and Methods. Final concentrations of the polymers: 20μg/280 μl. (b) Effect of poly(c^7I) (Δ——Δ), poly(c^7I)·poly(C) (Δ- - - -Δ), poly(I) (O——O), and poly(I)·poly(C) (O- - - -O) on MuLV (Moloney) DNA polymerase activity (control: ●——●). Standard assay mixture as described in Materials and Methods. Final concentration of the polymers: 20 μg/280 μl.

(2) On the basis of our *in vitro* cell culture data, poly(c^7A), poly(c^7I), c^7A and c^7I cannot be proposed as specific antiviral or antitumor agents since they inhibited virus growth and host cell macromolecular synthesis in both primary (untransformed) and continuous (transformed) cells to the same extent. Although poly(c^7A) and poly(c^7I) might be considered as depot forms of c^7A and c^7I, they do not appear to offer specific advantages over the monomers.

(3) In parallel with their inhibitory activity on host cell RNA synthesis, poly(c^7A), poly(c^7I), c^7A and c^7I suppressed the production of (Rauscher murine leukemia) virus particles by JLSV5 cells. At relatively high concentrations, which could not be reached in cell cultures because of the inherent cytotoxicity of the compounds, poly(c^7A) and poly(c^7I) also inhibited the *in vitro* DNA polymerase activity of Moloney murine leukemia virus.

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