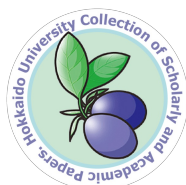




Title	Inhibitory Activity of (E)-5-(2-Bromovinyl)-2'-Deoxyuridine on the Salmonid Herpesviruses, Oncorhynchus masou Virus (OMV) and Herpesvirus salmonis
Author(s)	Kimura, Takahisa; Nishizawa, Toyohiko; Yoshimizu, Mamoru et al.
Citation	Microbiology and Immunology, 32(1), 57-65
Issue Date	1988
Doc URL	https://hdl.handle.net/2115/39476
Type	journal article
File Information	yoshimizu-58.pdf



Inhibitory Activity of (E)-5-(2-Bromovinyl)-2'-Deoxyuridine on the Salmonid Herpesviruses, *Oncorhynchus masou* Virus (OMV) and *Herpesvirus salmonis*

Takahisa KIMURA,^{*,1} Toyohiko NISHIZAWA,¹ Mamoru YOSHIMIZU,¹
and Erik De CLERCQ²

¹Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041, and

²Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

(Accepted for publication, September 9, 1987)

Abstract The highly potent and selective anti-herpesvirus agent, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVdU), was examined for its inhibitory effect on the salmonid herpesviruses *Oncorhynchus masou* virus (OMV) and *Herpesvirus salmonis* (*H. salmonis*). Minimum inhibitory concentrations (MIC) of BVdU for OMV and *H. salmonis* were 1.25 and 3.0 $\mu\text{g/ml}$, respectively; these values were equal to or higher than those obtained for acyclovir or cytarabine. OMV DNA polymerase activity was reduced in a dose-dependent fashion by BVdU 5'-triphosphate (BVdUTP) within the concentration range of 3 to 30 μM . However, BVdUTP could also be substituted for the natural substrate, TTP, in the OMV DNA polymerase assay. It is postulated that the inhibitory action of BVdU on the salmonid herpesviruses is more or less similar to that on other herpesviruses and resides with respect to the inhibition of the virus DNA polymerase activity as well as incorporation of BVdU into the viral DNA.

The fish herpesvirus *Oncorhynchus masou* virus (OMV) is the causative agent of infectious hepatitis in salmonid fish (11, 18). This virus also has oncogenic potential (12). The antiviral activity of acyclovir, 9-(2-hydroxyethoxymethyl)guanine (Acyclovir; ACV), against OMV and related *Herpesvirus salmonis* (*H. salmonis*) (20, 21) has been studied both *in vitro* and *in vivo*. ACV is inhibitory for both OMV and *H. salmonis in vitro* and also able to prevent OMV infection *in vivo* (13, 14).

(E)-5-(2-Bromovinyl)-2'-deoxyuridine (BVdU) is one of the most efficient anti-herpetic compounds described to date. It inhibits the replication of several mammalian herpesviruses such as herpes simplex virus type 1, varicella-zoster virus, suid herpesvirus type 1 and bovid herpesvirus type 1, at concentrations far below the cytotoxicity threshold. BVdU is specifically phosphorylated by virus-induced thymidine kinase and targeted at viral DNA synthesis (4-8, 10, 18).

We have examined the antiviral activity of BVdU against OMV and *H. salmonis in vitro*, based on minimum inhibitory concentration (MIC) and inhibition of viral replication. Compounds included as reference were ACV, 9- β -D-arabinofuranosyladenine (AraA), and 1- β -D-arabinofuranosylcytosine (AraC). BVdU was also

investigated for its effect on infectious hematopoietic necrosis virus (IHNV), and the toxicity of BVdU on the host (RTG-2) cells was assessed. OMV DNA polymerase was isolated and purified, and BVdU-5'-triphosphate (BVdUTP) was examined for its capacity to act as inhibitor or substrate (or both) of OMV DNA polymerase.

MATERIALS AND METHODS

Antiviral compounds. BVdU [(E)-5-(2-bromovinyl)-2'-deoxyuridine] was synthesized according to the method of Jones et al (10) and BVdUTP (BVdU 5'-triphosphate) was prepared as described by Allaudeen et al (1). AraA (9- β -D-arabinofuranosyladenine) was provided by Ajinomoto Co., Ltd. (Kawasaki, Japan). AraC (1- β -D-arabinofuranosylcytosine) was from Yamasa Co., Ltd. (Choshi, Japan) and ACV [acyclovir, 9-(2-hydroxyethoxymethyl)guanine] was obtained through the courtesy of the Wellcome Foundation Ltd. (London, England). BVdU, AraA and ACV were dissolved in double-distilled water to give a concentration of 1 mg/ml, and AraC to give a concentration of 0.5 mg/ml. After passage through a Millipore filter (0.45 μ m), they were stored at 0 C until use. BVdUTP was dissolved at a concentration of 1 mM in EtOH using max. = 296 and ϵ = 11,665. It was also stored at 0 C until use.

Viruses. *Oncorhynchus masou* virus (OMV, strain 00-7812) and *Herpesvirus salmonis* (*H. salmonis*) were used as representative of the pathogenic herpesviruses of fish. Infectious hematopoietic necrosis virus (IHNV, strain ChAb) was used as a reference. OMV was isolated from ovarian fluids of masou salmon caught in 1978 in Hokkaido (Japan). *H. salmonis* was kindly provided by Dr. K. Wolf of the National Fish Health Research Laboratories (West Virginia, U.S.A.) and IHNV was isolated from chum salmon (*Oncorhynchus keta*) in 1976 in Hokkaido (Japan). The viruses were propagated in RTG-2 or KO-6 cells as described below and stored at -80 C until use.

Cells. Rainbow trout (*Salmo gairdneri*) gonad cells (RTG-2) (19) were used throughout all experiments except for those aimed at isolating OMV DNA polymerase for which kokanee salmon (*O. nerka*) ovary cells (KO-6) (15) were used. The cells were maintained in Eagle's minimum essential medium (MEM, Gibco; Grand Island, N.Y., U.S.A.), supplemented with 10% fetal bovine serum (Gibco), 100 IU of penicillin and 100 μ g/ml of streptomycin (Sigma, St. Louis, Mo., U.S.A.), and incubated at 15 C.

Antiviral activity. Monolayers of RTG-2 cells, seeded in 24 well tissue culture plates (Falcon; Oxnard, Calif., U.S.A.), were infected with 100 TCID₅₀/ml of virus and then BVdU, ACV, AraA or AraC were added. The cells infected with OMV and IHNV were incubated at 15 C for 10 days. The cells infected with *H. salmonis* were kept at 10 C for 14 days. Then, the viral cytopathic effect (CPE) was determined on crystal violet staining. Antiviral activity was expressed as minimum inhibitory concentration (MIC) which was defined as the minimum concentration of the antiviral compound required to inhibit the viral CPE completely.

Effect of BVdU on replication of OMV. RTG-2 cells were seeded in 25 cm² tissue culture flasks (Falcon) at 8.0×10^5 cells/flask. After 3 days of incubation at 15 C, the RTG-2 monolayers were infected with 0.5 ml of OMV suspension containing 10^3 TCID₅₀/ml. After a 2 hr-adsorption period, BVdU was added at final concentrations of 0, 0.5, 1.0, and 2.0 μ g/ml, and the cell cultures were further incubated at 15 C. The virus titer in the cell culture fluid was assayed on RTG-2 cells by the 50% tissue culture infectious dose (TCID₅₀/ml) at selected time intervals (2, 4, 6, 8, 10, and 12 days after virus inoculation).

Effect of BVdU on RTG-2 cell growth. 10^6 RTG-2 cells were suspended in 5 ml of MEM in 25 cm² tissue culture flasks (Falcon). After incubation at 15 C for 3 days, BVdU (0, 0.5, 1.0, or 2.0 μ g/ml) was added to the cells, which were then further incubated at 15 C. Two, 7, and 12 days after the addition of BVdU, the cells were dispersed with trypsin and suspended in 5 ml of Hands' balanced salt solution (BSS, Gibco). The number of cells was determined with a hemocytometer.

Purification of OMV DNA polymerase. Purification of OMV DNA polymerase was carried out by a modification of the method of Suzuki et al (18). KO-6 cells seeded in tissue culture flasks were infected with OMV (at a multiplicity of infection > 10) and incubated for 24 hr at 15 C. Cells were then suspended in an hypotonic buffer [50 mM Tris-HCl (pH 7.5), 200 mM KCl, 1 mM 2-mercaptoethanol, 20% (v/v) Triton X-100] and homogenized by a sonicator (Tomy UR-50) until complete destruction of the cells. Homogenates were centrifuged at 30,000 rpm for 120 min (Beckman SW50-1 rotor); the supernatant was dialyzed for 1-2 hr against buffer A [20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 10 mM 2-mercaptoethanol and 20% (v/v) glycerol], and applied to a EDTA-cellulose (Whatman, DE-52) column equilibrated with buffer A containing 20 mM KCl. OMV DNA polymerase was eluted from the column with a linear gradient of 20 mM (150 ml) to 500 mM (150 ml) KCl in buffer A at a flow rate of 20 ml/hr.

The active peak of OMV DNA polymerase was detected in the fractions containing 220 mM KCl. Fractions 50 through 67 were pooled and dialyzed against buffer A containing 20 mM KCl and then applied to a phosphocellulose (Whatman P-11) column equilibrated with buffer A containing 20 mM KCl. The DNA polymerase was eluted from the column with a linear gradient ranging from 50 mM (70 ml) to 650 mM (70 ml) KCl in buffer A at a flow rate of 10 ml/hr. The active peak of OMV DNA polymerase was detected in the fraction containing 420 mM KCl. Fractions 53 through 59 were pooled and dialyzed against a buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM 2-mercaptoethanol, 1 mM EDTA, 150 mM KCl and 60% glycerol, and stored at -20 C until use.

OMV DNA polymerase assay. The standard reaction mixture for OMV DNA polymerase activity contained 50 mM Tris-HCl (pH 7.8), 4 mM MgCl₂, 0.5 mM dithiothreitol (DTT), 70 μ M each of dATP, dGTP and dCTP, 7 μ M TTP, [methyl-³H]TTP or GTP (1.7 μ Ci/nmol), 2.5 μ g of activated DNA and 1.4 units of enzyme in a total volume of 25 μ l. To distinguish OMV DNA polymerase from cell DNA polymerase, 100 mM ammonium sulfate was added to the reaction mixture containing OMV DNA polymerase (18). One unit of enzymatic activity was defined

as the amount required to incorporate 1 nmol dNTP into DNA for 1 hr. The incubation was carried out at 25 C for 20 min. The reaction mixture was chilled and 20 μ l aliquots were transferred to DEAE-cellulose (Whatman DE-80) paper disks. The disks were washed six times with 5% Na_2HPO_4 and twice with ethanol, and then dried. The disks were then evaluated for radioactivity in a liquid scintillation counter.

RESULTS

Antiviral Effects of BVdU, ACV, AraC, and AraA

The antiviral effects of BVdU, ACV, AraC, and AraA were evaluated in RTG-2 cells infected with OMV, IHNV, or *H. salmonis*. The results were expressed by MIC, and are shown in Table 1. MIC values of BVdU, ACV, AraC, and AraA for OMV were 1.25, 1.25, 2.0, and >250 $\mu\text{g/ml}$, respectively. For *H. salmonis*, they were 3.0, 2.0, 2.0, and >250 $\mu\text{g/ml}$, respectively. For IHNV they were all >100 $\mu\text{g/ml}$. From these results, it was apparent that BVdU had an antiviral activity equal to or higher than ACV or AraC against the fish herpesviruses, whereas AraA had no effect whatsoever even at concentrations up to 250 $\mu\text{g/ml}$. None of the four compounds had any effect against the non-herpesvirus, IHNV, at a concentration up to 100 $\mu\text{g/ml}$.

Effect of BVdU on OMV Replication

Growth curves of OMV in the presence of various concentrations of BVdU (0, 0.5, 1.0, and 2.0 $\mu\text{g/ml}$) are shown in Fig. 1. In the control flask, the OMV titer in the cell culture fluid reached about 10^6 TCID₅₀/ml on the 14th day after infection. In the cell culture treated with BVdU at 0.5 $\mu\text{g/ml}$, the OMV titer reached about $10^{4.5}$ TCID₅₀/ml, and when BVdU was added at 1.0 $\mu\text{g/ml}$, the maximum virus titer achieved was about $10^{3.5}$ TCID₅₀/ml. On addition of 2.0 $\mu\text{g/ml}$ of BVdU, the maximum virus titer attained was only $10^{2.0}$ TCID₅₀/ml. It thus appeared that at 0.5 $\mu\text{g/ml}$, BVdU caused 96.8% inhibition of OMV replication, at 1.0 $\mu\text{g/ml}$ 99.6% inhibition, and 2.0 $\mu\text{g/ml}$ 99.99% inhibition. At the concentrations of BVdU (0.5, 1.0, and 2.0 $\mu\text{g/ml}$) which were found effective in suppressing virus infection (Table 1 and Fig. 1), BVdU had no inhibitory effect on growth of the host cells (data not shown).

Table 1. Effects of BVdU, ACV, AraC, and AraA on OMV, *H. salmonis* and IHNV infection in RTG-2 cells, expressed against 100 TCID₅₀/ml

	MIC ^{a)} ($\mu\text{g/ml}$)			
	BVdU	ACV	AraC	AraA
OMV	1.25	1.25	2.0	>250
<i>H. salmonis</i>	3.0	2.0	2.0	>250
IHNV	>100	>100	>100	>250

^{a)} Minimum inhibitory concentration.

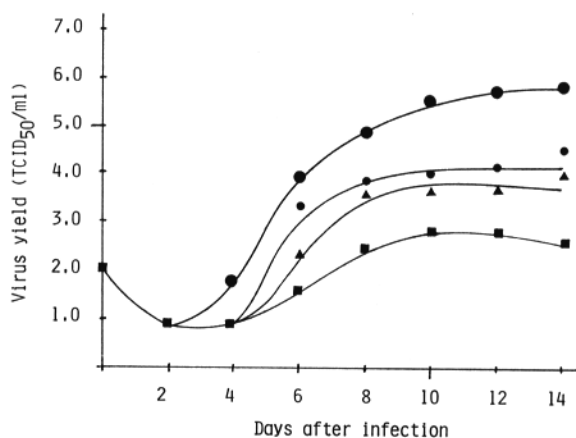


Fig. 1. Effect of different concentrations of BVdU on the growth of OMV in RTG-2 cells. ●, no drug; ○, BVdU 0.5 µg/ml; △, BVdU 1.0 µg/ml; ■, BVdU 2.0 µg/ml. Multiplicity of infection was 0.001. Each point means the average of four examinations.

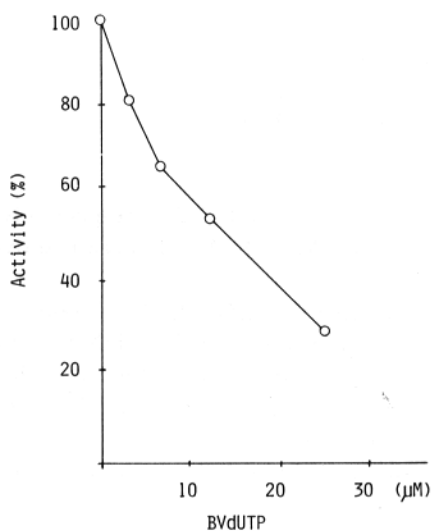


Fig. 2. Inhibitory effect of BVdUTP on OMV DNA polymerase activity. Remaining enzyme activity at concentrations of 3.13, 6.25, 12.5, and 25.0 µM of BVdUTP indicated by percentages of the control which did not contain BVdUTP.

Inhibitory Effect of BVdUTP on OMV DNA Polymerase

OMV DNA polymerase purified by DEAE-cellulose and phosphocellulose chromatography exhibited a specific activity of 683 units/mg, and its activity was assayed on addition of different concentrations of BVdUTP to the reaction mixture. When added at 3.13, 6.25, or 12.5 µM, BVdUTP reduced the activity of

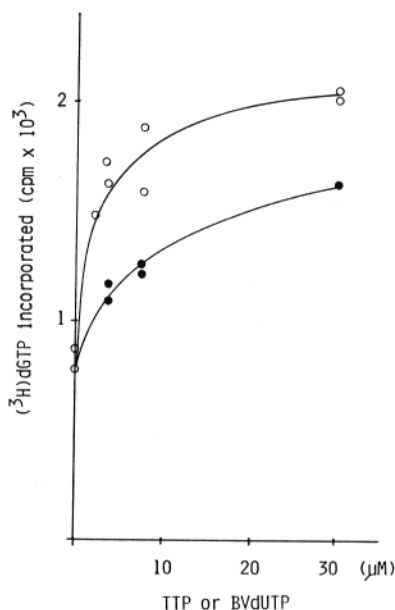


Fig. 3. Utilization of BVdUTP as a substrate for OMV DNA polymerase. ○, incorporation of [³H]dGTP into DNA at different concentrations of TTP as substrate. ●, incorporation of [³H]dGTP into DNA when BVdUTP was substituted for TTP.

OMV DNA polymerase by 18.7, 34.7, and 45.2%, respectively (Fig. 2). When added at 25 μM, BVdUTP suppressed OMV DNA polymerase activity by 71.2%.

Utilization of BVdUTP as Substrate for OMV DNA Polymerase

The amounts of DNA synthesized by OMV DNA polymerase, utilizing either BVdUTP or TTP as substrate, are depicted in Fig. 3. The amounts of DNA synthesized increased with increasing concentrations of TTP or BVdUTP in the reaction mixture. At the saturating concentration of 30 μM, the amount of DNA synthesized with BVdUTP as substrate was about 75% the amount of DNA synthesized with TTP as substrate.

DISCUSSION

BVdU is one of the most efficient antiherpesvirus compounds known to date. It is particularly active against HSV-1 and VZV (4-6). However, HSV-2 is about 200 times less susceptible to BVdU than HSV-1. BVdU is an average of five to ten times effective than ACV against HSV-1, but against HSV-2 it is about 25 times less effective than ACV (4-6).

In this study, the antiviral effects of BVdU against the fish pathogenic herpesviruses OMV and *H. salmonis* were determined *in vitro* and compared with those

of ACV, AraC, and AraA. The MICs of AraA for both OMV and *H. salmonis* were all $>250 \mu\text{g/ml}$; thus AraA had no activity against these viruses. MICs of BVdU, ACV, and AraC for OMV were 1.25, 1.25, and $2.0 \mu\text{g/ml}$, and those for *H. salmonis* was 3.0, 2.0, and $2.0 \mu\text{g/ml}$, respectively. BVdU, ACV, and AraC were almost equally active against OMV and *H. salmonis*. None of the four compounds had any activity against the rhabdovirus, IHNV. At concentrations (0.5, 1.0, and $2.0 \mu\text{g/ml}$) which did not impair growth of the host cells, BVdU caused a concentration-dependent reduction in OMV yields (Fig. 1), suggesting that its anti-OMV activity was specific.

The MICs of BVdU, AraC, and ACV for OMV and *H. salmonis* were substantially higher than those reported previously for BVdU, AraC, and ACV against HSV-1 (5). As far as BVdU is concerned, phosphorolysis by pyrimidine nucleotide phosphorylases (8) may contribute to premature degradation of the compound and, therefore, an increased antiviral effect may be achieved by replenishing the cell culture medium with fresh BVdU at regular time intervals.

The selectivity of BVdU as an antiherpes compound is essentially based on two virus-induced enzymes, thymidine (Thd) kinase and DNA polymerase (7). The HSV-1-induced dThd kinase converts BVdU successively to its 5'-mono- and 5'-diphosphate, which is then further converted by cellular kinase(s) to the 5'-triphosphate (BVdUTP). BVdUTP selectively inhibits the activities of HSV-1 and VZV DNA polymerase (1, 22) and it can also serve as a substrate for the synthesis of viral DNA (2, 16). This mechanism of action is similar to that proposed for ACV (3, 9, 17).

The interaction of BVdU with OMV DNA polymerase was also investigated in the present study. OMV DNA polymerase activity was reduced by 71.2% if $25 \mu\text{M}$ BVdUTP was added to the reaction mixture (Fig. 2). Therefore an investigation was performed to determine if BVdUTP was incorporated into DNA as a substrate by OMV DNA polymerase. When TTP was substituted by BVdUTP, DNA synthesis was reduced only by about 25% (Fig. 3). It was suggested that under these conditions BVdUTP was incorporated into the DNA instead of TTP, but the incorporative ratio of BVdUTP was only 25% of that of TTP. Thus BVdUTP not only inhibits DNA polymerase activity but also acts as a substrate for OMV DNA synthesis. In this sense its interaction with OMV DNA synthesis is considered to be similar to that previously established for other herpesvirus such as HSV-1 and VZV.

The antiviral effect of BVdU on fish herpesviruses *in vivo* is a subject of future studies.

The authors are grateful to Dr. Satoru Suzuki and Dr. Mineo Saneyoshi (Faculty of Pharmaceutical Science, Hokkaido University, Sapporo, Japan) for their technical support and advice.

This study was supported in part by grant No. 58400001 from the Ministry of Education, Science and Culture, Japan, and a grant from the Japan Fisheries Resource Conservation Association.

REFERENCES

- 1) Allaudeen, H.S., Cheng, M.S., Bertino, J.R., and De Clercq, E. 1981. On the mechanism of selective inhibition of herpesvirus replication by (E)-5-(2-bromovinyl)-2'-deoxyuridine. *Proc. Natl. Acad. Sci. U.S.A.* **78**: 2698-2702.
- 2) Allaudeen, H.S., Cheng, M.S., Lee, J.J., De Clercq, E., and Prusoff, W.H. 1982. Incorporation of E-5-(2-halovinyl)-2'-deoxyuridine into deoxyribonucleic acids of herpes simplex virus type 1 infected cells. *J. Biol. Chem.* **257**: 603-606.
- 3) Coen, D.M., and Schaffer, P.A. 1980. Two distinct loci confer resistance to acycloguanosine in herpes simplex virus type 1. *Proc. Natl. Acad. Sci. U.S.A.* **77**: 2265-2269.
- 4) De Clercq, E., Descamps, J., De Somer, P., Barr, P.J., Jones, A.S., and Walker, R.T. 1979. (E)-5-(2-Bromovinyl)-2'-deoxyuridine: a potent and selective antiherpes agent. *Proc. Natl. Acad. Sci. U.S.A.* **76**: 2947-2951.
- 5) De Clercq, E., Descamps, J., Verhelst, G., Walker, R.T., Jones, A.S., Torrence, P.F., and Shugar, D. 1980. Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. *J. Infect. Dis.* **141**: 563-574.
- 6) De Clercq, E., Descamps, J., Ogata, M., and Shigeta, S. 1982. *In vitro* susceptibility of varicella-zoster virus to E-5-(2-bromovinyl)-2'-deoxyuridine and related compounds. *Antimicrob. Agents Chemother.* **21**: 33-38.
- 7) De Clercq, E., and Walker, R.T. 1984. Synthesis and antiviral properties of 5-vinylpyrimidine nucleoside analogs. *Pharmac. Ther.* **26**: 1-44.
- 8) Desgranges, C., Razaka, G., Rabaud, M., Bricaud, H., Balzarini, J., and De Clercq, E. 1983. Phosphorylation of (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) and other 5-substituted-2'-deoxyuridines by purified human thymidine phosphorylase and intact blood platelets. *Biochem. Pharmacol.* **32**: 3583-3590.
- 9) Elion, G.B. 1983. The biochemistry and mechanism of action of acyclovir. *J. Antimicrob. Chemother.* **12** (Suppl. B): 9-17.
- 10) Jones, A.S., Verhelst, G., and Walker, R.T. 1979. The synthesis of potent antiherpes virus agent, E-5-(2-bromovinyl)-2'-deoxyuridine and related compounds. *Tetrahedron Lett.* **45**: 4415-4418.
- 11) Kimura, T., Yoshimizu, M., and Tanaka, M. 1981. Studies on a new virus (OMV) from *Oncorhynchus masou*. I. Characteristics and pathogenicity. *Fish Pathol.* **15**: 143-147.
- 12) Kimura, T., Yoshimizu, M., and Tanaka, M. 1981. Studies on a new virus (OMV) from *Oncorhynchus masou*. II. Oncogenic nature. *Fish Pathol.* **15**: 149-153.
- 13) Kimura, T., Suzuki, S., and Yoshimizu, M. 1983. *In vitro* antiviral effect of 9-(2-hydroxyethoxymethyl)guanine on the fish herpesvirus, *Oncorhynchus masou* virus (OMV). *Antiviral Res.* **3**: 93-101.
- 14) Kimura, T., Suzuki, S., and Yoshimizu, M. 1983. *In vivo* antiviral effect of 9-(2-hydroxyethoxymethyl) guanine on experimental infection of chum salmon (*Oncorhynchus keta*) fry with *Oncorhynchus masou* virus (OMV). *Antiviral Res.* **3**: 103-108.
- 15) Lannan, C.N., Winton, J.R., and Fryer, J.L. 1984. Fish cell lines: establishment and characterization of nine cell lines from salmonids. *In Vitro* **20**: 671-676.
- 16) Mancini, W.R., De Clercq, E., and Prusoff, W.H. 1983. The relationship between incorporation of E-5-(2-bromovinyl)-2'-deoxyuridine into herpes simplex virus type 1 DNA with virus infectivity and DNA integrity. *J. Biol. Chem.* **258**: 792-795.
- 17) Schnipper, L.E., and Crumpacker, C.S. 1980. Resistance of herpes simplex virus to acycloguanosine: role of viral thymidine kinase and DNA polymerase loci. *Proc. Natl. Acad. Sci. U.S.A.* **77**: 2270-2273.
- 18) Suzuki, S., Kimura, T., and Sancyoshi, M. 1986. Characterization of DNA polymerase induced by salmon herpesvirus, *Oncorhynchus masou* virus. *J. Gen. Virol.* **67**: 405-408.
- 19) Wolf, K., and Quimby, M.C. 1962. Established eurythermic line of fish cells *in vitro*. *Science* **135**: 1065-1066.
- 20) Wolf, K., Hermans, R.L., Darlington, R.W., and Taylor, W.G. 1975. Salmonid viruses: test of species susceptibility, suggestions for isolation, geographic distribution and control measures. *Fish Health News* **4**: 6.

- 21) Wolf, K., Darlington, R.W., Taylor, W.G., Quimby, M.C., and Nagabayashi, T. 1978. *Herpesvirus salmonis*: characterization of a new pathogen of rainbow trout. J. Virol. **27**: 659-666.
- 22) Yokota, T., Konno, K., Shigeta, S., and De Clercq, E. 1984. Comparative inhibition of DNA polymerase from varicella zoster virus (TK+ and TK-) strains by (E)-5-(2-bromovinyl)-2'-deoxyuridine 5'-triphosphate. Mol. Pharmacol. **26**: 376-380.

(Received for publication, May 6, 1987)