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COMPOUNDS TESTED AGAINST PUNTA TORO VIRUS(U) UTAH STATE
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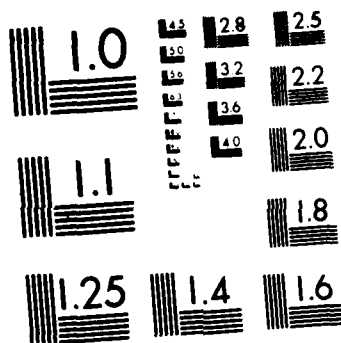
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Determination of the *In Vitro* and *In Vivo* Activity of Compounds Tested Against Punta
Toro Virus.

Annual Report

Robert W. Sidwell, Ph.D.

December 29, 1987

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Utah State University
Logan, Utah 84322

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→ This report describes the second year's studies using Punta Toro virus (PTV) infections in vitro and in vivo as test systems for evaluating anti-PTV compounds. Developmental Studies with Virus: → A large plaque-purified pool of Adames strain of PTV was studied to attempt to increase the lethal infectivity of the virus for C57BL/6 mice by passing the virus through mice, using pooled serum taken 2 days after infection to prepare a new PTV pool. This mouse-passaged virus, while more lethal to mice, did not cause uniform lethality at the various dilutions assayed. An investigation into using low multiplicity of infection introduced into LLC-MK₂ cells with the original PTV pool, then harvesting the virus as cell supernate 72 hr post-virus exposure provided a PTV pool which was more uniformly lethal to 3-week-old mice, the LD₅₀ being a 10^{-5.3} dilution when 0.2 ml was inoculated subcutaneously (s.c.). Studies into the Acceptability of Mice Used in Antiviral Studies: → Studies were run to confirm the acceptability of the C57BL/6 mice provided by Simonsen Laboratory which are used for our anti-PTV experiments. Extensive analysis of these mice for presence of potentially harmful pathogens indicated					
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the mice were acceptably free of such microorganisms. Comparative titrations of PTV in mice provided by other suppliers showed the Simonsen animals to be slightly more sensitive to the virus infection. **Preliminary Toxicity Assessments:** Preliminary assessments of toxicity of AVS compounds in 3-4 week-old mice were run on 30 compounds, using death and host weight change as parameters. **In Vitro Anti-PTV Experiments:** In vitro anti-PTV evaluations were performed on 295 AVS compounds in LLC-MK₂ cells with inhibition of viral cytopathic effect (CPE) as initial endpoint and reduction of virus titer (VTR) at maximum tolerated doses of initially active test compounds used as a confirmatory antiviral endpoint. Compounds considered CPE inhibitory to PTV were AVS 111, 136, 139, 257, 1089, 1754, 1976, 1978, 2296, 2301, 2543, 2700, 2811, 2980 and 3038. Of compounds evaluated for VTR, only AVS 111, 2301 and 2700 were not effective. Twenty-three AVS compounds inhibitory to Adames PTV in vitro were also evaluated against the Balliet strain of PTV. Compounds considered inhibitory to this virus in one or more tests were AVS 01, 52, 139, 149, 206, 215, 253, 257, 1089, 1159 and 1754. **In Vivo Anti-PTV (Hepatotropic) Experiments:** A total of 155 in vivo experiments were run to explore the anti-PTV effects of 30 AVS compounds. These experiments used the hepatotropic infection induced by s.c. inoculation of Adames PTV in 3 week-old C57BL/6 mice. Death and mean survival time were used as initial evaluation parameters; reduction in liver icterus, serum glutamic oxalic acid transaminase, serum glutamic pyruvic acid transaminase, serum virus and liver virus were also used in confirming in vivo anti-PTV experiments. Compounds considered inhibitory to PTV in mice were AVS 01, 02, 52, 79, 111, 360, 1754, 1767, 1778, 2149, 2776, 2777, 2880, 3587, 3588, 3589 and 3954. Compounds inhibitory to such PTV infections when used by oral gavage were AVS 01, 02, 206, 253, 1754, 1767, 1778, 2149, 2880 and 3934. **Effect of Viral Challenge Dose on Anti-PTV Activity of AVS02:** A single experiment was run to determine if altering PTV challenge dose would affect the in vivo antiviral activity of AVS 02. Relatively low dosages of the compound were effective against 1, 10, 100 or 1000 LD₅₀ (50% lethal dose) of the virus. **In Vivo Anti-PTV (Neurotropic) Experiments:** Seven compounds inhibitory to in vivo Adames PTV infections were also tested against intracerebrally injected Balliet PTV infections in 4-5 week-old mice. AVS 02, 206, 1754 and 2149 were considered effective. **Combination Chemotherapy Experiment:** The use of combinations of AVS01 and AVS2149 was studied vs in vivo Adames PTV infections in a series of experiments. Low, weakly effective doses of AVS2149 used in combination with usually ineffective doses of AVS01 were considered to have enhanced therapeutic effects against the infection.

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SUMMARY

This report describes our second year's studies using the Punta Toro virus (PTV) *in vitro* and *in vivo* models as test systems for evaluating anti-PTV compounds. Some further developmental work on the PTV model is also reported.

1. Developmental Studies on Adames PTV Pools: a) Influence of freezer temperature infectivity of stored PTV: Storage at continuously maintained -80°C and storage at -70°C (with almost daily temperature fluctuation due to opening of freezer) did not significantly affect PTV infectivity in C57BL/6 mice. b) Attempts to increase PTV infectivity in mice: Cell culture-adapted PTV passaged a single time through mice and harvested as a mouse serum pool on infection day 2 had an approximate $1.5 \log_{10}$ increased infectivity in C57BL/6 mice but was $1 \log_{10}$ less infectious in LLC-MK₂ cells. Large pools of PTV were subsequently prepared from mouse serum, but these large pools of virus while more lethally infectious, were highly erratic in inducing deaths in mice. c) Effect of Multiplicity of Infection (m.o.i.), time of harvest, and method of harvest on PTV infectivity: Several pools of PTV were prepared in LLC-MK₂ cells in which the above three parameters were examined. Three m.o.i.'s (0.01, 0.001, and 0.0001) were used; harvest times were 24, 48 and 72 hr post-infection; harvest methods included use of non-frozen supernatant fluid and use of frozen/thawed infected cells. The most infective virus was obtained using the lowest m.o.i. longest harvest time, and non-frozen supernate. These results correlate well with the characteristics of defective interfering virus particles. d) Preparation and titration of PTV pool with greater mouse infectivity: Using the most effective method found in c, above, a large pool of PTV was prepared and titrated in 3- and 4-week-old C57BL/6 mice. The pool was highly infectious to mice, the LD₅₀ being a $10^{-5.3}$ dilution when 0.2 ml was injected subcutaneously (s.c.), and a wider "window" of 100% lethality was seen.

2. Investigations into the Acceptability of Simonsen-Supplied C57BL/6 Mice: Due to some inconsistency of death in the C57BL/6 mice used in our early studies, studies were undertaken to determine that the inconsistency was not due to the mice. The mice were analyzed and certified to be free of 14 viruses, 14 bacteria, and 3 mycoplasma by the supplier and also by this University's Veterinary Diagnostic Laboratory. The 3-week-old mice occasionally exhibited a slight liver discoloration, which was determined to be a result of low iron content in the livers, an expected finding for newly weaned mice who had just begun a diet of solid food. C57BL/6 mice 3 weeks of age were purchased from Simonsen Laboratories, Charles River Laboratories and Harlan Sprague Dawley Laboratories, and our standard pool of PTV titrated in each. The LD₅₀ titers were: Simonsen mice: $10^{-2.8}$, Charles River mice: $10^{-2.3}$, Harlan mice: $10^{-2.2}$. These data indicated the mice supplied by Simonsen were suitable but a better virus pool was needed. This pool has now been prepared, as described in 1 above.

3. Effect of Adames PTV on Rectal Temperatures in Mice: Since a common manifestation of human phlebovirus infections is high temperature, we were interested in determining if peripheral inoculation of PTV to 3-week-old mice would affect the temperature of the animals. No increase in temperature was seen, but decreases occurred from infection days 3 through 6. This result was not unexpected, since hypothermia is expected due to metabolic acidosis resulting from underperfusion of tissues with blood as the animal becomes acutely ill. We have reported similar findings in influenza virus-infected mice.

4. Preliminary *In Vivo* Assessments of Toxicity: A total of 30 compounds have had some degree of preliminary assessment of toxicity in 3-4 week-old C57BL/6 mice, using death and host weight change as parameters.

5. *In Vitro* Evaluation of Test Compounds: A total of 295 AVS compounds were evaluated for *in vitro* anti-PTV activity against the Adames strain of virus. Confirming experiments were run on all considered positive in the initial test. Of these, 16 were considered to have positive anti-PTV activity as determined by CPE inhibition. These were AVS111, 136, 139, 257, 1089, 1754, 1976, 1978, 2296, 2301, 2543, 2700, 2811, 2980 and 3038. Virus titer reduction determinations were made at the maximum tolerated dose of a portion of these positive compounds. Only compounds AVS111, 2301, and 2700 were not effective by this parameter. A

total of 23 AVS compounds exhibiting inhibitory effects to the Adames PTV were also evaluated against the Balliet strain of virus. Those considered effective against the latter virus in at least one test were AVS01, 52, 139, 149, 206, 215, 253, 257, 1089, 1159, and 1754.

6. Initial *In Vivo* Hepatotropic PTV Evaluation of Compounds: A total of 30 compounds were subjected to our initial anti-PTV evaluation using death as endpoint in mice, and 16 confirming experiments were run using expanded parameters which included death, increased mean survival time, decreased liver score, reduced liver or serum virus and reduced serum glutamic oxalic acid transaminase (SGOT) and serum glutamic pyruvic acid transaminase (SGPT). Overall, 155 experiments were run on these compounds and on materials evaluated during 1986. In these experiments we often altered treatment regimen in an effort to demonstrate or improve activity and also to determine therapeutic indices (TI) for active substances. The new compounds evaluated included 9 compounds not considered to be immunomodulators (AVS02, 52, 65, 111, 167, 222, 272, 360, 2741, and 2742) and 20 materials characterized as immunomodulators (AVS1754, 1757, 1767, 1777, 1778, 2149, 2741, 2742, 2776, 2777, 2778, 2880, 3585, 3587, 3588, 3589, 3925, 3926, 3927, and 3934). The following were considered to have anti-PTV activity: AVS01, 02, 52, 79, 111, 360, 1754, 1767, 1778, 2149, 2776, 2777, 2880, 3587, 3588, 3589, and 3934. Seventeen experiments were run to evaluate the anti-PTV efficacy of 12 compounds administered by oral gavage. Compounds evaluated were AVS01, 02, 79, 206, 212, 253, 1754, 1767, 1778, 2149, 2880, and 3934. Compounds considered moderately or highly active when administered orally were AVS01, 02, 206, 253, 1754, 1767, 1778, 2149, 2880, and 3934.

7. Determination of Influence of PTV Dose on Antiviral Activity: A single experiment was run to determine if increasing or decreasing viral challenge would affect *in vivo* antiviral activity. AVS02, the 2',3',5'-triacetate of ribavirin, was evaluated in this study. Activity was seen using the lowest dose (31.3 mg/kg/day) of AVS02, when 1LD50 of PTV was used as challenge. However, when the virus challenge was increased to 10, 100, or 1000LD50, the lowest effective dose of AVS02 did not vary from 62.5 mg/kg/day, indicating this compound is effective against usually overwhelming virus infections.

8. Effect of AVS Compounds on Intracerebral (i.c.) Infections of Mice Induced by the Balliet Strain PTV: Seven compounds considered markedly effective against the Adames PTV infection in mice were tested further to determine their effect on i.c. infections induced in 4-5 week-old C57BL/6 mice by the Balliet strain of PTV. In these experiments, that treatment regimen considered most suitable for the Adames infection was used. Compounds tested were AVS02, 79, 206, 253, 1754, 1778, and 2149. Compounds AVS02, 206, 1754, and 2149 were considered to have an effect against this infection, with AVS2149 considered most active of those tested to date.

9. Overview of *In Vivo* Anti-PTV Activity of AVS Compounds: Each and every compound evaluated *in vivo* by us to date has been considered using all *in vivo* PTV experiment data. We feel Ribavirin (AVS01), ribavirin triacetate (AVS02), ribavirin carboxamidine (AVS206), MVE-2 (AVS1754), AM-3 (AVS1767), mannozym (AVS1778), ampligen (AVS2149), 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP, AVS2776), oxamisole (AVS2880) and the meta fluoro derivative of ABPP (AVS3588) to have the most promising activity for use vs PTV either alone or possibly in combination therapy experiments.

10. Effect of a Drug Combination on *In Vivo* PTV Infections: A series of experiments were run to determine if the combination of AVS01 (ribavirin), a recognized antiviral drug, and AVS2149, (ampligen), a known immune modulating substance, would have a greater *in vivo* anti-PTV effect than either substance used alone. The data indicate that the combination of a low, weakly effective dose of ampligen and similarly low, usually ineffective doses of ribavirin were highly active against the infection.

11. Publications and Presentations: One manuscript describing work performed on this project has been accepted for publication. Three presentations of these data have been made or are scheduled to be made.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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This report describes research accomplished in the second year of the project, "Determination of the In Vitro and In Vivo Activity of Compounds Tested Against Punta Toro Virus". Because of the extensiveness and diversity of the data obtained, the following report is divided into sections coinciding with the order set forth in the Summary.

I. DEVELOPMENTAL STUDIES ON ADAMES PUNTA TORO VIRUS MODEL.

Introduction

A major need in this project has been for a PTV virus pool which is stable and lethally infectious for mice. In the first year of this project, we produced a large PTV pool which was somewhat infectious to 3 week-old C57BL/6 mice, but the lethality induced often varied rather significantly from experiment to experiment. These data suggested: a) The virus pool had declined in titer upon freezing over 6 months at -70°C to -80°C . b) The virus pool had possible defective interfering viral particles or other factors which prevented an acceptable lethality from being produced. c) The virus pool lost titer due to passage in cells. This section describes experiments run to clarify the situation and to prepare a more lethally infective virus.

Materials and Methods

Virus: The Adames strain of PTV was provided by Dr. Dominique Pifat of USAMRIID. It was identified by Dr. Pifat as virus pool #215588, and had been safety tested by Dr. Pifat prior to being sent to us. This PTV was first isolated from the serum of A. Adames, an entomologist in the Darien Province of Panama in 1972. Passage history of the virus prepared in our laboratory and the confirmation of its identity were described in Section I of our Annual Report No. 1.

Cells: Cells used were Rhesus monkey kidney (LLC-MK₂), obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown in minimum essential medium (MEM; GIBCO Labs, Grand Island, NY) containing 5% fetal bovine serum (FBS; HyClone Labs, Logan, UT) and 0.1% NaHCO_3 without antibiotics. The cells were determined to be mycoplasma free.

Mycoplasma Testing: Mycoplasma was tested using the MycoTect™ system (GIBCO) as directed in the product use instructions. Indicator cell cultures used in each test positive and negative controls. The positive control cells were inoculated with ATCC 29052, *Mycoplasma hyorhinis* and with ATCC 25528, *Mycoplasma arginini*.

Mice: C57BL/6 mice at the ages indicated were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined at least 24 hr prior to use, caged in shoebox-style polycarbonate cages and fed Wayne Lab Blox Mouse Chow and tap water *ad libitum*. Room temperatures were maintained at approximately 73°F , with temperature and humidity recorded daily.

Results and Discussion

a) Influence of freezer temperature on infectivity of stored PTV: PTV was stored in two Kelvinator -80°C freezers. One freezer, maintained in our Laboratory Animal Research Center (LARC), was opened almost daily to add or remove samples. The second freezer was maintained in our virus laboratory as a back-up to the first freezer, and was seldom opened. The former freezer had an average temperature of -70°C , whereas the back-up freezer had an average temperature of approximately -80°C . To determine if the difference in freezer temperature may have affected PTV viability, virus from each freezer was quantified in parallel in mice from Simonsen to determine relative LD₅₀'s. The titration was performed by inoculating 0.2 ml of one-half $\log_{10}$ virus dilutions subcutaneously (s.c.) into 3 week-old mice. As seen in Table I-1, the LD₅₀ values did not vary appreciably, with the LARC-maintained PTV having a slightly greater titer ($10^{-2.8}$ compared to $10^{-2.5}$). A "window" of infectivity was also seen with both viruses, wherein only a specific virus dilution achieved an LD₉₀ in the mice. These data indicated the difference in freezer temperature did not affect the virus titer.

b) Attempts to Increase PTV Infectivity by Passage in Mice: We attempted to increase PTV infectivity by passage of our cell culture PTV pool through 3 week-old mice, harvesting the serum from these animals 2 days after virus inoculation to obtain a virus pool. Our previous work (Figure II-1, Annual Report No. 1) indicated PTV reaches titers of $>10^6$ 50% cell culture infectious doses (CCID50)/ml in serum from mice killed on day 2. The serum harvested in this preliminary experiment was titrated in parallel with the cell culture pool in both 3 week-old mice and in cells. The results (Table I-2) indicated the serum pool of virus has an LD50 of $10^{-4.0}$ in mice, although was one $\log_{10}$ less infectious in cells. This suggests the virus passed in mice has a greater infectivity for mice, possibly through elimination of many defective interfering (DI) particles we suspect are usually present in the cell culture pool.

A large pool of PTV was subsequently prepared in an identical manner in 300 3 week-old mice. This large pool of virus was titrated in mice, and found to be more infectious than the initial small pool prepared, but the deaths observed were erratic. Another pool was prepared in an identical manner in mouse serum, but in this experiment only serum from mice with a 2+ or greater liver score was used to prepare the pool. This virus pool also was more lethal to the mice, but also induced highly erratic deaths in the mice (Table I-3). At about this time, we found a new cell culture preparation of PTV to be acceptably lethal to mice (see I-c, to follow), so have not pursued further the animal passage of PTV as an attempt to increase virulence.

c) Effect of Multiplicity of Infection (m.o.i.), Time of Harvest, and Method of Harvest on PTV Infectivity: In an attempt to determine what factors might influence the production of infectious PTV in cells, an experiment was performed in which 0.01, 0.001 and 0.0001 m.o.i. of PTV were used to infect LLC-MK₂ cells. After the cells were infected, virus was harvested 24, 48 or 72 hr post-virus exposure; two methods of PTV harvest were used. These included use of non-frozen supernatant fluid and use of frozen/thawed cells. Virus from each of these m.o.i.'s, times of harvest, and methods of harvest were quantified initially in cell culture and later in 3 week-old C57BL/6 mice using death as endpoint.

The virus from the frozen/thawed cell pools was consistently 1 $\log_{10}$ lower in titer than that of supernate pools. for this reason, the frozen/thawed cell pools were not titrated in mice.

The lethality of the supernate pools is shown in Figure I-1, where each graph varies by harvest time, and in Figure I-2, where each graph varies by the m.o.i. The pool infected with the m.o.i. of 0.0001 had the lowest titer when harvested early, but overtakes and surpasses the titers of the other pools by the 72 hr harvest time. This pool was considered to provide the most virulent virus of all the pools tested. These data showing increased infectivity of cell passaged virus using low m.o.i. match well with those reports of Von Magnus (1) and Holland (2) for DI particles; we conclude that PTV has a tendency to produce DI particles, and are investigating this phenomenon further.

d) Preparation and Titration of PTV Pool with Greater Mouse Infectivity: Based on the results described in I-c, above, a large PTV pool was prepared in cells using 0.0001 m.o.i. viral inoculum, harvesting non-frozen supernate 72 hr after virus exposure. This virus was quantified in 3 and 4 week-old C57BL/6 mice by inoculating 0.1 or 0.2 ml s.c. into the mice and observing for death. The results are summarized in Table I-4. This virus pool was considered acceptably lethal to 3 week-old mice, especially if a 0.2 ml inoculum was used.

Conclusions

- a) Slight freezer temperature fluctuation did not affect stored PTV titers.
- b) Passage of cell culture adapted PTV through mice appeared to increase lethality of harvested serum for mice, but preparation of a large pool yielded PTV causing erratic death patterns in mice.
- c) Low multiplicity of infection coupled with late harvest of infected supernate yielded virus having greatest lethality for mice.
- d) A large pool of PTV has been prepared which has acceptable high lethality for 3 week-old mice.

Literature Cited

1. Von Magnus, P. (1954) Incomplete forms of influenza virus. *Adv. Virus Res.* 2:59-79.
2. Holland, J.J. (1985) Generation and replication of defective viral genomes. pp. 77-99. In, Virology (B.N. Fields, ed.) Raven, New York.

Table I-1. Effect of Storage Condition on Infectivity of Punta Toro Virus in C57BL/6 Mice^a.

<u>Storage Location</u>	<u>Virus Dilution</u>	<u>% Survivors^d</u>	<u>Mean Surv. Time^e(days)</u>
Animal Facility ^b	10 ^{-0.5}	80	5.5
	10 ^{-1.0}	60	6.0
	10 ^{-1.5}	10	5.4
	10 ^{-2.0}	60	6.5
	10 ^{-2.5}	30	6.0
	10 ^{-3.0}	70	6.0
	10 ^{-4.0}	50	6.8
	10 ^{-5.0}	70	7.7
	10 ^{-6.0}	100	>21.0
	LD50: 10 ^{-2.8}		
Virus Laboratory ^c	10 ^{-0.5}	60	6.5
	10 ^{-1.0}	30	6.3
	10 ^{-1.5}	10	5.3
	10 ^{-2.0}	50	6.2
	10 ^{-2.5}	50	7.0
	10 ^{-3.0}	60	6.0
	10 ^{-4.0}	60	8.0
	10 ^{-5.0}	90	6.0
	10 ^{-6.0}	80	7.0
	LD50: 10 ^{-2.5}		

^aInfection: 0.2 ml of each virus dilution inoculated s.c. into 3-week-old (10-11 g) female mice.

^bAnimal facility-stored virus maintained at a temperature of ~-70°C, with freezer being open longer times than the virus laboratory-stored virus.

^cVirus laboratory-stored virus maintained at a temperature of ~-80°C, with less opening of the freezer.

^dMice held through 21 days.

^eAnimals dying on or before day 21.

Table I-2. Animal and Cell Culture Infectivity of Cell Culture and Serum-Prepared Punta Toro Viruses^a.

Virus Pool <u>Preparation</u>	Cell Culture <u>ID50</u>	C57BL/6 Mouse <u>LD50</u>
LLC-MK2 cells	10 ^{-7.7}	10 ^{-2.8}
C57BL/6 mouse serum	10 ^{-6.7}	10 ^{-4.0}

^aCell culture prepared: Twice plaque purified and a large pool prepared in LLC-MK2 cells as described in #1 of our 1st Annual Report. Serum prepared: Obtained from 10-11 g female C57BL/6 mice exsanguinated 2 days after subcutaneous inoculation with cell culture-prepared virus.

Table I-3. Animal Infectivity of Serum-Prepared Punta Toro Viruses.

<u>Virus Preparation</u>	<u>Virus Dilution</u>	<u>3 week-old % Survivors^a</u>	
Large Pool Preparation #1 (serum from all mice used)	10 ^{-1.0}	100	
	10 ^{-2.0}	40	
	10 ^{-3.0}	40	
	10 ^{-4.0}	20	
	10 ^{-5.0}	100	
	10 ^{-6.0}	100	
	10 ^{-7.0}	100	
LD50:		10 ^{-3.1}	
<u>Virus Preparation</u>	<u>Virus Dilution</u>	<u>3 week-old % Survivors^a</u>	<u>4 week-old % Survivors^a</u>
Large Pool Preparation #2 (serum from mice with 2+ livers)	10 ^{-1.0}	30	60
	10 ^{-1.5}	60	70
	10 ^{-2.0}	40	20
	10 ^{-2.5}	50	60
	10 ^{-3.0}	60	40
	10 ^{-3.5}	90	60
	10 ^{-4.0}	80	60
	10 ^{-4.5}	70	60
	10 ^{-5.0}	80	90
	10 ^{-5.5}	100	80
LD50:		10 ^{-2.5}	10 ^{-2.8}

^aMice held through 21 days.

**Table I-4. Animal Infectivity of PTV^a Prepared from Low m.o.i.-
Infected LLC-MK₂ Cells.**

<u>Virus Inoculum</u>	<u>Virus Dilution</u>	<u>3 week-old mice % Survivors^b</u>	<u>4 week-old mice % Survivors^b</u>
0.1 ml	10 ^{-1.0}	-	30
	10 ^{-1.5}	-	60
	10 ^{-2.0}	0	40
	10 ^{-2.5}	0	50
	10 ^{-3.0}	20	60
	10 ^{-3.5}	60	90
	10 ^{-4.0}	40	80
	10 ^{-4.5}	80	70
	10 ^{-5.0}	100	80
	10 ^{-5.5}	100	100
	10 ^{-6.0}	100	-
	LD50:	10 ^{-3.75}	10 ^{-2.47}
0.2 ml	10 ^{-1.0}	-	60
	10 ^{-1.5}	-	70
	10 ^{-2.0}	0	20
	10 ^{-2.5}	0	60
	10 ^{-3.0}	0	40
	10 ^{-3.5}	0	60
	10 ^{-4.0}	20	60
	10 ^{-4.5}	0	60
	10 ^{-5.0}	60	90
	10 ^{-5.5}	60	80
	10 ^{-6.0}	40	-
	LD50:	10 ^{-5.3}	10 ^{-2.75}

^aPool designated as PTA/4LLC 8-19-87.

^bMice held through 21 days.

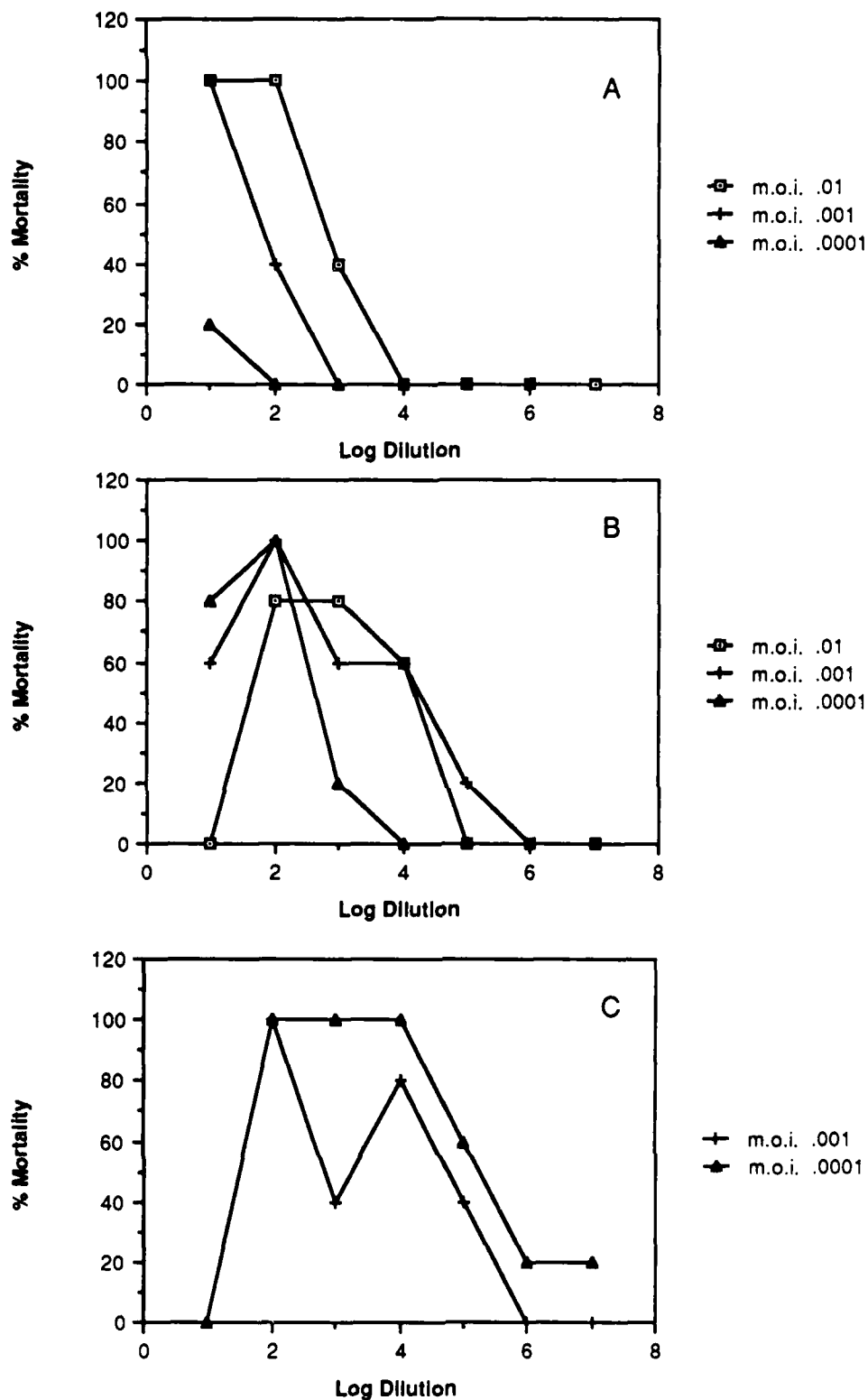


Figure I-1. Effect of m.o.i. and infected supernate harvest time on lethal infectivity of PTV in 3 week-old C57BL/6 mice. Infected supernate removed A) 24 hr post-virus exposure. B) 48 hr post-virus exposure. C) 72 hr post-virus exposure.

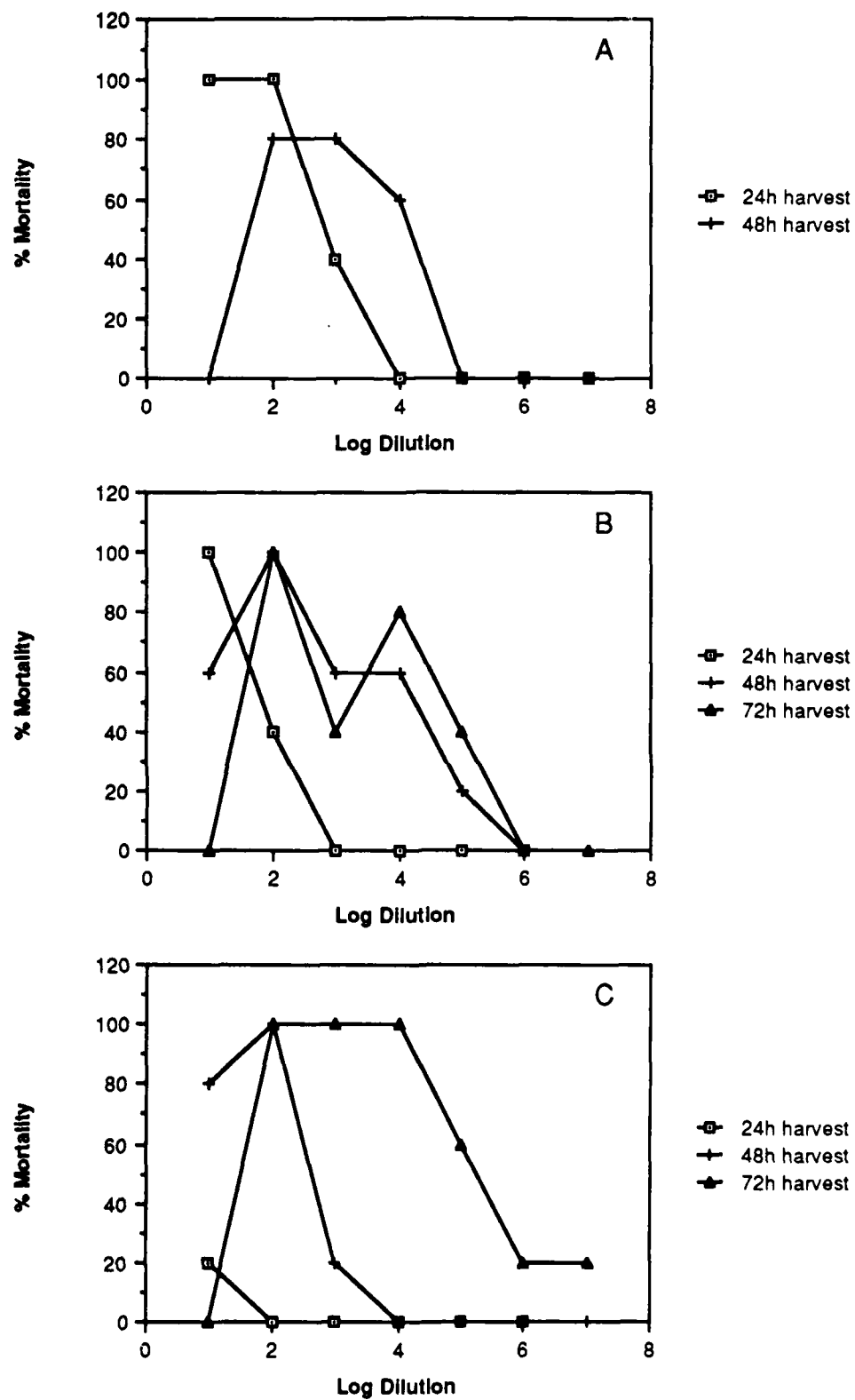


Figure I-2. Effect of m.o.i. and infected supernate harvest time of PTV in 3 week-old C57BL/6 mice. Cells infected with m.o.i. of A) 0.01, B) 0.001, C) 0.0001.

II. INVESTIGATIONS INTO THE ACCEPTABILITY OF SIMONSEN-SUPPLIED C57BL/6 MICE.

Introduction

The in vivo antiviral experiments run during the first year of this project were hampered to some degree by an erratic death rate in PTV-infected C57BL/6 mice supplied by Simonsen Laboratories. It was also noted that livers taken from normal control animals often had some discoloration amounting to approximately "2+" in some instances, and 10% liver homogenates prepared from these normal control mice often induced what appeared to be slight cytopathic effect in LLC-MK₂ cells. These data suggested that the mice we had been using may have been already infected with some other virus such as murine hepatitis. A series of steps were subsequently taken to determine if this was the case.

Materials and Methods

Mice: 3 week-old C57BL/6 mice were obtained from our regular supplier, Simonsen Laboratories, and also from Charles River Laboratories (Wilmington, MA) and Harlan Sprague Dawley Laboratories (Indianapolis, IN). All were quarantined and caged as described in Section I of this Report.

Microbiological Analysis: Diagnostic tests for identification of viral and bacterial isolates were achieved according to standard procedures. These were performed by Dr. James Russell of Simonsen Laboratories and by Dr. Ross A. Smart and associates at the Utah State University (USU) Veterinary Diagnostic Laboratory.

Steps Taken to Determine Mouse Acceptability: a) Simonsen Laboratory was alerted to our observations and asked to further check their C57BL/6 mouse colony for possible infection with viral and other microbiological agents. The following viruses were sought: Murine adeno, ectromelia, enzootic diarrhea of infant mice, GDVII, K, lactic dehydrogenase, lethal intestinal virus of infant mice, lymphocytic choriomeningitis, murine hepatitis, minute, pneumonia, polyoma, reo type 3, and Sendai. Tests were also run for the following bacteria: *Bacillus piliformis* (tyzzer's), *Citrobacter freundii*, *Corynebacterium kutscheri*, *Klebsiella pneumoniae*, *Pasteurella pneumotropica*, *Pasteurella multocida*, *Salmonella* sp, *Staphylococcus aureus* and pathogenic *Streptococcus* sp, *Mycoplasma pulmonis*, *arthritidis* and *neurolyticum* were also sought. Fungi included *Microsporum*, *Trichophyton*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*. Certain parasites common to mice were also assayed. These included *Myobia musculi*, *Myocoptes musculinus*, *Myocoptes rumbaesti*, *Aspicularis*, *Heterakis*, *Syphacia*, *Capillaria*, *Humenolepis*, *Coccidiosis*, *Giardia*, *Hexamita*, *Toxoplasma*, *Trichomonads* and *Hemobartonella*, sp.

b) Samples of the Simonsen C57BL/6 mice from three separate shipments were submitted for a full diagnostic workup to the USU Veterinary Diagnostic Laboratory.

c) Livers from normal mice exhibiting discoloration were examined by Dr. Arthur Mahoney, Professor of Nutrition and Food Sciences at USU.

d) Similar mice were purchased from Charles River and from Harlan Sprague Dawley Laboratories and our initial virus pool was titrated in these mice in parallel with the Simonsen mice. Virus inoculation was s.c. using 0.2 ml of each virus dilution.

e) Livers from normal mice from Simonsen, Charles River and Harlan Sprague Dawley were prepared as 10% homogenates in MEM and assayed in monolayers of LLC-MK₂ cells for possible CPE production.

Results and Discussion

a) Results of Simonsen diagnostic testing. The C57BL/6 mice used in our program were negative for all the pathogens tested. This was not surprising, in view of the methods Simonsen uses for carefully isolating each animal colony and screening all animals used as stock for their colonies.

b) USU Veterinary Diagnostic Laboratory results. No recognized gross alterations were seen in the mice examined. No microscopic alterations were seen in sections of heart, lung, liver, kidney, stomach and intestinal tract upon histopathologic examination. Lung and liver specimens were cultured on a variety of tissue cultures, with no cytopathogenic effect observed.

c) Nutrition and Food Science examination. Dr. Mahoney advised us that the slightly discolored livers he examined were probably low in iron content, an expected finding for newly weaned mice who had just begun a diet of solid food.

d) Comparative PTV titration in mice from three sources. The titration results are summarized in Table II-1. The results indicated the virus had similar LD50 values in each group of mice, although the LD50 in the Simonsen animals was better than the two other groups ($10^{-2.8}$ virus dilution compared to $10^{-2.3}$ and $10^{-2.2}$ in the mice from the other suppliers). It was apparent, however, that the LD90 virus dosage was attainable in only a rather narrow "window" of dilutions with this virus pool. The acceptable concentration in the Simonsen or Harlan Sprague Dawley mice was $10^{-1.5}$, with an LD90 not achieved in the Charles River animals. Thus any error in virus dilution of 0.5 $\log_{10}$ or greater could result in a marked difference in lethality to the mice. We therefore attribute the erraticism of death seen in early studies to probable slight errors in virus dilution by technicians.

e) Liver homogenate effects in LLC-MK₂ cells. 10% Liver homogenates of mice from all suppliers induced slight morphological alterations in LLC-MK₂ cells. We attribute this to possibly mild toxic effects from the liver material and not to a virus contaminant.

We have since prepared a more lethally potent virus pool, as described in Section I of this report, and we have had no problems with unacceptable deaths since the preparation of this virus.

Conclusions

The Simonsen C57BL/6 mice used in our PTV studies were considered acceptable for continues use in our chemotherapy studies.

Table II-1. Comparison of Punta Toro Virus Infectivity in C57BL/6 Mice from Different Suppliers^a.

<u>Supplier</u>	<u>Virus Dilution</u>	<u>% Survivors^b</u>	<u>Mean Surv. Time^c(days)</u>
Charles River	10 ^{-0.5}	20	6.5
	10 ^{-1.0}	90	7.0
	10 ^{-1.5}	50	5.8
	10 ^{-2.0}	40	8.7
	10 ^{-2.5}	60	9.3
	10 ^{-3.0}	70	7.3
	10 ^{-4.0}	100	>21.0
	10 ^{-5.0}	90	6.0
	10 ^{-6.0}	90	5.0
	LD50:	10 ^{-2.3}	
Simonsen	10 ^{-0.5}	80	5.5
	10 ^{-1.0}	40	6.0
	10 ^{-1.5}	10	5.4
	10 ^{-2.0}	60	6.5
	10 ^{-2.5}	30	6.0
	10 ^{-3.0}	70	6.0
	10 ^{-4.0}	50	6.8
	10 ^{-5.0}	70	7.7
	10 ^{-6.0}	100	>21.0
	LD50:	10 ^{-2.8}	
Harlan Sprague Dawley	10 ^{-0.5}	60	6.5
	10 ^{-1.0}	50	5.4
	10 ^{-1.5}	10	6.7
	10 ^{-2.0}	80	7.0
	10 ^{-3.0}	40	7.0
	10 ^{-4.0}	40	5.3
	10 ^{-5.0}	90	5.0
	10 ^{-6.0}	100	>21.0
	LD50:	10 ^{-2.2}	

^aInfection: 0.2 ml of each virus dilution inoculated s.c. into 3-week-old (10-11 g) female mice. Virus pool identified as PTVA/6LLC 9/9/86.

^bMice held through 21 days.

^cAnimals dying on or before day 21.

III. EFFECT OF ADAMES PUNTA TORO VIRUS ON RECTAL TEMPERATURES IN MICE.

Introduction

Since a common manifestation of phlebovirus (Rift Valley fever, sandfly fever) infections in man is high temperature, we were interested in determining if infections with the Adames PTV administered s.c. to 3 week-old mice would result in a similar temperature rise. This report describes an experiment run to determine this temperature change.

Materials and Methods

Mice: Female C57BL/6 mice were obtained from Simonsen Laboratories, and were caged and maintained as described in Section I of this Report.

Virus: The initial Adames PTV pool used in our first year's experiment was used for this study.

Experiment Design: Twenty mice were infected s.c. with PTV, then monitored daily from immediately prior to infection through 7 days or until death by use of a YSI42SC telethermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH).

Results and Discussion

The temperature findings are summarized in Figure III-1. No increase in temperature was seen, but significant decreases in temperature were seen from day 3 through day 6. The animals began dying on day 4, so temperatures taken after this time were of surviving mice and therefore not representative of the group as a whole. A total of 18 of the 20 infected mice (90%) died of the infection.

Such a temperature decline in these mice is not reflective of the phlebovirus infections seen in man. However, it should be pointed out that the animals received an overwhelming, lethal, infection in contrast to a usually less than lethal infection occurring naturally in man. Such a temperature decline is not surprising, since hypothermia is expected due to metabolic acidosis resulting from underperfusion of the tissues with blood as the animal becomes acutely ill. We reported a similar finding in influenza virus-infected mice (1).

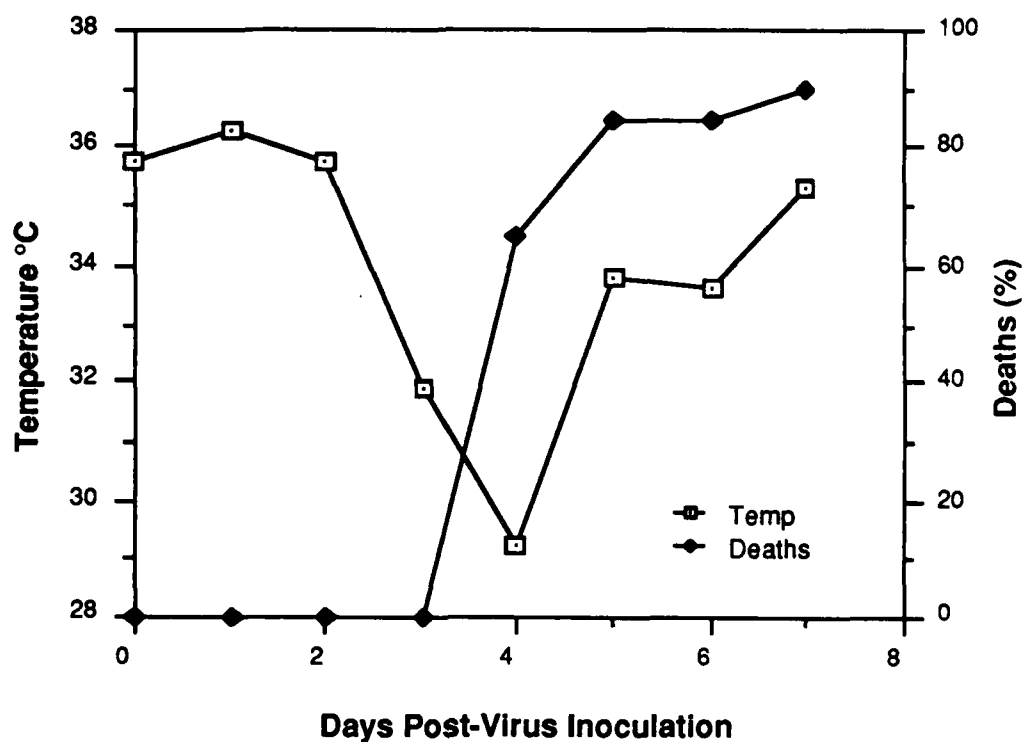
Conclusions

Peripheral PTV infections in mice which result in 90% lethality cause significant temperature beginning on infection day 3.

Literature Cited

1. Sidwell, R.W., J.H. Huffman, E.W. Call, H. Alaghamandan, P.D. Cook and R.K. Robins. Effect of selenazofurin on influenza A and B virus infections of mice. *Antiviral Res.* 6:343-353.

Figure III-1. Effect of Punta Toro Virus Infection on Rectal Temperature in 9-11 g C57BL/6 Mice



IV. PRELIMINARY *IN VIVO* ASSESSMENT OF TOXICITY.

Introduction

Before compounds submitted to us can be evaluated for *in vivo* PTV activity, information is needed regarding the approximate LD50 of those compounds as determined using the same treatment schedule to be used in the antiviral experiments. This report summarizes the LD50 data generated either from preliminary toxicity assessment experiments or from data derived from use of concomitantly run toxicity controls in actual PTV experiments. Since some compounds submitted to us are immune modulating materials their most effective dose is often remote from the maximum tolerated dose. In such cases, we are usually instructed by USAMRIID personnel on the doses to use in *in vivo* PTV experiments and we seldom have a need to determine an LD50 dose. Some data regarding these immunomodulating compounds is also included in this section, however, to provide information for others desiring to use such compounds.

Materials and Methods

Compounds: All compounds were submitted to us by Technassociates, Inc. (Rockville, MD). The compounds were weighed and dissolved or suspended in vehicles considered most appropriate for the compound. These vehicles were physiological saline for injection, sterile water for injection, or 4% carboxy methylcellulose in physiological saline.

Animals: C57BL/6 mice 3-4 weeks of age were obtained from Simonsen Laboratories. They were quarantined, housed and maintained as described in Section I.

Toxicity Assessments: Mice were injected with varying 2-fold dilutions of each compound according to the indicated treatment regimens. All were weighed immediately prior to treatment and again 18 hr after the final treatment to determine if normal weight gain occurred. In preliminary toxicity studies, the mice were held a total of 14 days. When used as parallel toxicity controls in PTV studies, the animals were held a total of 21 days. Five mice were used at each dosage level. The volume administered was 0.01 ml/g of body weight. Parameters for evaluation included weight change, obvious signs of distress such as diarrhea, prostration, or tremors, and death which was noted daily. The LD50 dose was calculated by the Reed-Muench method (1).

Results and Discussion

The toxicity determinations, expressed as approximate LD50 values, are summarized in Table IV-1. Data on a total of 30 compounds are shown. In some cases ">" values are shown because we as yet have not achieved a lethal dose. Values shown as "~" were estimated based on the observation that slightly lower doses were lethal, but to less than 50% of the animals, or treatment with the lower dose caused marked weight loss in the animals, suggesting the MTD dose had essentially been reached.

Conclusions

Approximate LD50 values were obtained for 30 AVS compounds.

Literature Cited

1. Reed, L.J. and H. Muench. (1938) A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493-497.

Table IV-1. Preliminary Toxicity Evaluations of AVS Compounds^a

<u>Compound (AVS No.)</u>	<u>Name</u>	<u>Treatment Schedule</u>	<u>Treatment Route</u>	<u>Approximate LD50 (mg/kg/day)</u>
02	Ribavirin triacetate	bid x 5 once only	s.c. s.c.	850 >1000
52	Thioformycin B	tid x 5	s.c.	~800
65	Formycin B	bid x 5	s.c.	1500
79	9-β-ribofuranosylpurine -6-thiocarboxamide	qd x 5 bid x 5 tid x 5 bid x 5	s.c. i.p. i.p. p.o.	300 150 150 >200
111	Tiazofurin	bid x 5	s.c.	~3000
147	Enviroxime	bid x 5	s.c.	~3000
167	Glycerhetic acid	bid x 5	s.c.	~2000
206	Ribavirin carboxamidine	bid x 5 bid x 5	s.c. p.o.	~3000 ~2000
212	Suramin	tid x 5	s.c.	~300
253	Selenazofurin	qd x 5 bid x 5	s.c. p.o.	~500 ~500
272	3-Deazaguanine	bid x 5	s.c.	~400
1754 ^c	MVE-2	once only once only	i.p. p.o.	250 ~300
1767 ^c	AM-3	bid x 5 bid x 5	s.c. i.p.	3000 ~600
1777 ^c	Streptonigrin	bid x 5	s.c.	0.3
1778 ^c	Mannozyim	bid x 5	s.c.	200
2149 ^c	Ampligen	bid x 5	i.p.	>25
2741	1-(β-D-ribofuranosyl)-1,2,4-triazole-3 -(1,4,5,6-tetrahydropyrimidine)•HCL	bid x 5	s.c.	>500
2742 ^c	1-(β-D-ribofuranosyl)-1,2,4-triazole-3 -(5-hydroxy-1,4,5,6-tetrahydro -pyrimidine)•HCL	bid x 5	s.c.	>500
2776 ^c	2-Amino-5-bromo-6-phenyl-4(3H)- pyrimidinone (ABPP)	qd x 3 once only	i.p. i.p.	~500 ~500
2777 ^c	2-Amino-5-iodo-6-phenyl-4(3H)- pyrimidinone (AIPP)	qd x 3 once only	i.p. i.p.	~500 >400
2778 ^c	2-Amino-5-bromo-6-methyl-4(3H)- pyrimidinone (ABMP)	qd x 3	i.p.	~600
2880 ^c	Oxamisole	qd x 3 bid x 3 bid x 5	i.p. i.p. p.o.	~50 ~50 ~50

<u>Compound (AVS No.)</u>	<u>Name</u>	<u>Treatment Schedule</u>	<u>Treatment Route</u>	<u>Approximate LD50 (mg/kg/day)</u>
3585 ^c	Neurotropin	once only	i.p.	>400
3587 ^c	2-amino-5-chloro-6-phenyl- 4(3H)-pyrimidinone	qd x 3 once only	i.p. i.p.	500 600
3588 ^c	Meta fluoro of ABPP	qd x 3 once only	i.p. i.p.	>400 >400
3589 ^c	5-chloro-2,3-difluorophenyl- 4(3H)-pyrimidinone	qd x 3 once only	i.p. i.p.	>400 >400
3925 ^c	du Pont A2222-1	once only	i.p.	~200 ^b
3926 ^c	du Pont A2227-1	once only	i.p.	75
3927 ^c	du Pont A754-a	once only	i.p.	180
3934 ^c	Gel32	qd x 7	p.o.	>300

^a3-4 week-old C57BL/6 mice.

^bToxicity possibly due to DMSO/Tween 80 vehicle used in initial concentration.

^cConsidered possible immunomodulators.

V. IN VITRO EVALUATION OF TEST COMPOUNDS AGAINST PUNTA TORO VIRUS.

Introduction

The initial phase of our anti-PTV testing program is the in vitro evaluation of compounds submitted to us. In the initial tests, inhibition of cytopathic effect (CPE) is determined against the Adames strain of PTV. Compounds exhibiting adequate CPE inhibition (Virus Rating [VR] ≥ 0.5) are retested and their effects on virus yield (Virus Titer Reduction [VTR]) at the maximum tolerated dose are also determined. Active compounds are further submitted to similar antiviral experiments using the Balliet strain of PTV, since a goal in our studies is to find compounds effective against both hepatotropic (Adames) PTV infections and neurotropic (Balliet) PTV infections.

This section describes our experiments with 295 AVS compounds evaluated during this report period.

Materials and Methods

Virus: A twice plaque isolated PTV, both Adames and Balliet strains, prepared in LLC-MK₂ cells as described in Section I of our First Annual Report was used.

Cells: LLC-MK₂ (Rhesus monkey kidney) cells were used. They were obtained initially from the ATCC. Various passages of the cells were used over the 1-year period of this study. Growth medium was medium essential medium (MEM, GIBCO Labs, Grand Island, NY) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan, UT) and 0.1% NaHCO₃ without antibiotics. All were determined to be mycoplasma-free.

Test Compounds: All materials were provided by Technassociates for these tests. Each was stored and handled according to instructions from Technassociates.

In Vitro Testing Procedures: Seven concentrations of test compound, these concentrations usually being 1000, 320, 100, 32, 10, 3.2 and 1 $\mu\text{g/ml}$, were added in 0.1 ml amounts to an 18-hr monolayer of cells in 96-well flat-bottom microplates. Adames strain PTV (320 CCID₅₀/ml) was added in 0.1 ml volume 15 minutes later. Three virus-containing cups in each microplate were used for each compound dosage level, with one cup used for toxicity controls (cells + sterile virus diluent + compound). Six cups in each panel were used for virus controls (cells + virus + drug diluent) and 6 cups in each panel were used for normal cell controls (cells + sterile virus diluent + drug diluent). Test medium in which virus and compound were suspended or dissolved was MEM with 2% FBS, 0.18% NaHCO₃ and 50 $\mu\text{g/ml}$ gentamicin. Viral CPE was graded from 0 (normal cells) to 4 (virtually complete destruction of the cell layer) 6-7 days post-virus exposure. The CPE was read by an individual who was well trained for CPE evaluation, then this reading was confirmed by a second, similarly trained individual.

Reduction in CPE was evaluated by VR as we have described previously (1, 2) and by 50% effective dose (ED₅₀). The VR is a numerical expression of antiviral activity, taking into account percent of CPE inhibition and partial cytotoxicity of the test compound. In our experience a VR of 1.0 is indicative of definite antiviral activity, a VR of 0.5 - 0.9 indicates moderate activity, and a VR of <0.5 suggests slight activity perhaps resulting from cytotoxicity only. The ED₅₀ was determined by plotting percent CPE inhibition vs test compound concentration, with the ED₅₀ level being that level causing an approximate 50% CPE inhibition. Also included in the test was an estimated minimum toxic concentration (MTC) of the test compound, this MTC being the lowest dosage causing visually discernible cytotoxic effects in the concurrently run toxicity controls. Cytotoxicity was determined by microscopic assessment of compound-induced cytopathic effects in treated cultures compared with those in control cells run in the same plate. A maximum tolerated dose (MTD) was also determined for use in evaluating effect on VTR.

Validation of apparent positive activity was done in early *in vitro* experiments by fixing the drained cells in 10% formalin and staining them with 1% crystal violet, which clearly demonstrated the complete cell monolayer. The stained plate was labeled and photographed. The experiment

was then repeated and, in addition to CPE inhibition being determined, virus yield was also determined by freezing the plate, thawing at room temperature, and the medium from each 10-fold dilution and from virus controls removed and virus quantified. The virus quantification was done by end-point dilution, determining CPE induced in triplicate cups containing LLC-MK₂ cells exposed to 0.1 ml of 10-fold dilutions of each sample collected.

As positive control, ribavirin (AVS01) was tested in parallel in each series of tests. This compound was shown by us (Section I, First Annual Report) and (3) to be highly active vs PTV *in vitro* and *in vivo*.

Compounds exhibiting confirmed positive activity in tests with the Adames PTV were retested in a similar manner using the Balliet strain of virus.

Results and Discussion

The results of all *in vitro* experiments run against the Adames PTV are summarized in Table V-1. A total of 295 compounds were tested, and 16 were considered positive against the virus, although some retesting for confirmation of results is still underway. The PTV-inhibitory materials were AVS111, 136, 139, 259, 1089, 1754, 1976, 1978, 2296, 2301, 2543, 2700, 2811, 2980 and 3038. We were not provided the names of the majority of these materials, so can identify them by AVS number only. Confirming tests were run to determine VTR at MTD on a portion of the positive compounds. Of the compounds tested, only AVS111, 2301 and 2700 were not effective by this parameter.

The Balliet PTV chemotherapy experiments are summarized in Table V-2. Twenty three AVS compounds which were positive vs the Adames PTV were evaluated. Of these, 11 had significant activity against the Balliet virus. The active compounds were AVS01, 52, 139, 149, 206, 215, 253, 257, 1089, 1159 and 1754. Some of the compounds shown in Table V-1 were reported active vs the Adames PTV in our First Annual Report.

We should point out that in mid-1987, it was decided to express our *in vitro* toxicity data as MTC instead of MTD as done in the first year of this project. We made this adjustment to allow our data to compare more closely with results reported by investigators at Southern Research Institute, who were already using the minimum toxic concentration reporting system, in *in vitro* experiments run for the U.S. Army Medical Research and Development Command.

It is interesting to note that AVS02, ribavirin triacetate, was only weakly active vs PTV *in vitro*, whereas as will be discussed in subsequent sections it was highly active *in vivo*. This is an excellent example of the occasional lack of good correlation between *in vitro* and *in vivo* results. In this case, the triacetyl groups are apparently cleaved enzymatically in the whole animal but this cleavage is only poorly done if at all in the cells. Many compounds defined as immunomodulators were not subjected *in vitro* assay since their activity is not usually as direct antiviral agents.

Conclusions

Compounds AVS111, 136, 139, 259, 1089, 1754, 1976, 1978, 2296, 2301, 2543, 2700, 2811, 2980 and 3038 were considered to have significant CPE-inhibitory effects against the Adames strain of PTV. A portion of these materials, as well as compounds found active in 1986 were also tested vs the Balliet PTV, with AVS01, 52, 139, 149, 206, 215, 253, 257, 1089, 1159 and 1754 exhibiting inhibition against this virus. The PTV-inhibitory compounds found in these *in vitro* tests should be considered for *in vivo* anti-PTV studies.

Literature Cited

1. Sidwell, R. W. and Huffman, J. H. 1971. Use of disposable micro tissue culture plates for antiviral and interferon studies. *Appl. Microbiol.* 22:797-801.
2. Sidwell, R. W. 1976. Virus disease: A review of chemotherapy systems. In, H. H. Gorbach (ed.) *Chemotherapy of Infectious Diseases*. CRC, Cleveland, pp. 31-53.
3. Sidwell, R.W., J.H. Huffman, B.B. Barnett and D.Y. Pifat. 1988. In vitro and in vivo phlebovirus inhibition by ribavirin. *Antimicrob. Ag. Chemother.* (in press).

Table V-1. Summary of *In Vitro* Anti-Punta Toro Virus (Adames Strain) Activity of AVS Compounds.

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	II ^e	Aqueous Solubility ^f
000001	0.9-1.2	4-10	3.2-100	nd	0.12-3.76	S
000002	0.4, 0.4	780, 220	>1000, >1000	-	1.3, >1.5	S
000033	0.0	<320	3.2	-	0.01	S
000065	0.3	61	10	-	0.16	S
000068	0.0	>1000	>1000	-	<1.0	INS
000071	0.4	150	<32	-	0.21	INS
000078	0.0, 1.0	21, >10	10, <3.2	-	0.48, 0.3	INS
000087	0.0	>1000	<1000	-	1.0	INS
000094	0.0	>1000	1000	-	<1.0	S
000095	0.0	>1000	320	-	0.3	S
000105	0.3	97	100	-	1.0	S
000111	0.8, 0.4, 0.8	21, 130, 18	3.2, 3.2, 3.2	0.0	0.15, 0.03, 0.18	S
000113	0.1	>1000	1000	-	<1.0	INS
000132	0.0	>1000	>1000	-	<1.0	INS
000136	0.5, 0.4	85, 270	100, 100	0.0, 0.5	1.2, 0.37	S
000139	0.8, 0.2, 0.8	24, 16, 22	3.2, 3.2, 3.2	-	0.1, 0.2, 0.2	S
000167	0.3	280	1000	-	3.6	INS
000217	0.2	750	1000	-	>0.4	S
000257	0.7, 1.0	31, 4	3.2, 3.2	-	<0.1, <0.8	S
000345	0.0	>1000	320	-	<0.3	INS
000701	0.0	>32	<10	-	<0.3	INS
001089	$\geq 1.4, 0.8$	<1.0, 0.16	3.2, 0.32	-	>3.2, 1.9	S
001160	0.6, 0.7	55, 24	3.2, 10	-	0.6, 0.4	S
001199	0.1	580	320	-	0.6	S
001754	1.7, 0.7	<1.0, 72	100, 32	0.5	<100, 0.4	S
001757	0.1	>1000	320	-	0.3	S
001767	0.0	>1000	>1000	-	<1.0	INS
001777	0.0	>0.1	0.1	-	<0.03	INS
001778	0.0	>1000	>1000	-	<1.0	INS
001846	0.2	1000	1000	-	1.0	S
001850	0.3	70	32	-	0.5	S
001915	0.4	220	100	-	0.005	S
001968	0.1	8.3	3.2	-	0.4	INS
001970	0.1	24	10	-	0.4	S
001976	0.6, 0.7	45, 25	10, 3.2	-	0.2, 0.12	S

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	TI ^e	Aqueous Solubility ^f
001977	0.0	>1000	32	-	<0.03	S
001978	0.4,0.5	1000,520	1000, 320	2.0	1.0, 0.6	S
001983	0.2	>1000	320	-	<0.3	S
001984	0.0	>1000	320	-	<0.3	INS
001985	0.0	>3.2	3.2	-	<1.0	S
001986	0.3	>1000	320	-	>0.3	S
001987	0.0	>1000	320	-	>0.3	S
001988	0.1	>1000	10	-	<0.01	S
001989	0.0	>1000	>1000	-	<1.0	INS
001990	0.0	>1000	>1000	-	<1.0	INS
001991	0.0	220	10	-	0.05	S
001992	0.0	>1000	>1000	-	<1.0	INS
001995	0.0	>1000	<1000	-	<1.0	INS
001996	0.1	>1000	<1000	-	<1.0	INS
001998	0.0	>1000	<1000	-	<1.0	INS
002006	0.0	>1000	>1000	-	<1.0	INS
002023	0.0	>1000	10	-	<0.01	S
002137	0.0	>1000	>1000	-	<1.0	INS
002138	0.0	>1000	<1000	-	<1.0	INS
002139	0.0	>1000	320	-	<0.3	INS
002140	0.0	>1000	1000	-	<1.0	S
002149	0.0, 0.2	>1000, 840	>1000, 1250	-	<1.0, 1.5	S
002159	0.1	>1000	1000	-	<1.0	S
002160	0.4	>1000	<320	-	<0.3	INS
002188	0.0	>320	<320	-	<1.0	INS
002191	0.0	>320	<100	-	<0.3	INS
002193	0.0	>1000	>1000	-	<1.0	INS
002217	0.1	>100	<320	-	<3.2	INS
002219	0.1	>100	<32	-	<0.3	INS
002220	0.2	>1000	>1000	-	<1.0	INS
002221	0.0	>1000	1000	-	<1.0	INS
002223	0.0	>1000	>1000	-	<1.0	INS
002224	0.0	>1000	320	-	<0.3	INS
002226	0.0	83	32	-	0.4	INS
002228	0.0	90	10	-	0.1	INS
002230	0.1	620	320	-	0.52	INS
002234	0.0	820	1000	-	1.2	INS
002235	0.0	>1000	>1000	-	<1	INS
002277	0.2	730	100	-	0.14	S

<u>Compd</u> <u>No. (AVS)</u>	<u>VR^a</u>	<u>ED50^b</u> <u>(μg/ml)</u>	<u>MTC^c</u> <u>(μg/ml)</u>	<u>VTR^d at</u> <u>MTD ($\log_{10}$)</u>	<u>TI^e</u>	<u>Aqueous</u> <u>Solubility^f</u>
002279	0.3	63	32		0.5	S
002282	0.3	760	320		0.42	S
002286	0.1	1000	100		0.1	S
002289	0.0	>320	1000	-	<3.1	S
002296	0.6	240	32	0.2	0.13	S
002301	0.8	9.5	<3.2	0.0	0.34	S
002336	0.0	>1000	320	-	<0.32	S
002340	0.0	>1000	>1000	-	<1.0	S
002344	0.0	<1.0	<3,2	-	<3.2	INS
002361	0.0	>1000	>1000	-	<1.0	S
002362	0.0	>1000	1000	-	<1.0	S
002364	0.1	>1000	1000	-	<1.0	INS
002365	0.0	>1000	>1000	-	<1.0	S
002366	0.0	>1000	<1000	-	<1.0	INS
002367	0.0	>320	320	-	<1.0	INS
002368	0.0	>1000	100	-	<0.01	INS
002369	0.0	>1000	320	-	<0.32	INS
002370	0.2	220	100	-	0.45	S
002371	0.0	>1000	32	-	<0.03	INS
002372	0.0	>1000	1000	-	<1.0	INS
002373	0.0	>320	<1000	-	<3.1	INS
002374	0.0	>100	100	-	<1.0	S
002375	0.1	32	10	-	0.31	S
002376	0.1	>100	100	-	<1.0	S
002377	0.0	>1000	<320	-	<1.0	INS
002405	0.1	>1000	<320	-	0.3	INS
002412	0.0	>1000	>1000	-	<1.0	INS
002413	0.0	>1000	>1000	-	<1.0	INS
002415	0.0	>1000	>1000	-	<1.0	INS
002417	0.3	450	<320	-	0.7	INS
002419	0.0	>1000	>1000	-	<1.0	INS
002421	0.0	>32	<10	-	<1.0	INS
002423	0.0	>1000	>1000	-	<1.0	INS
002425	0.0	>1000	<1000	-	<1.0	INS
002431	0.1	540	<100	-	0.2	INS
002442	0.0	>1000	>1000	-	<1.0	INS
002443	0.0	>320	<100	-	<1.0	INS
002444	0.0	>1000	>1000	-	<1.0	INS
002445	0.0	>1000	<320	-	<1.0	INS

Compd No. (AVS)	VR ^a	ED50 ^b (ug/ml)	MTC ^c (ug/ml)	VTR ^d at MTD (log ₁₀)	TI ^e	Aqueous Solubility ^f
002447	0.0	>1000	100	-	<1.0	S
002449	0.1	100	32	-	0.3	S
002450	0.2	83	<3.2	-	0.04	INS
002451	0.0	>1000	<1000	-	<1.0	INS
002452	0.0	>1000	<1000	-	<1.0	INS
002453	0.0	790	<100	-	<1.0	INS
002454	0.0	>1000	>1000	-	<1.0	INS
002456	0.2	830	<320	-	0.4	INS
002457	0.0	>1000	>1000	-	<1.0	INS
002458	0.0	810	<100	-	<1.0	INS
002464	0.1	975	<32	-	0.03	INS
002477	0.0	>1000	>1000	-	<1.0	INS
002479	0.0	>1000	>1000	-	<1.0	INS
002484	0.0	>1000	>1000	-	<1.0	INS
002507	0.2	>1000	>1000	-	<1.0	S
002538	0.2	>1000	1000	-	<1.0	S
002540	0.0	>1000	1000	-	<1.0	S
002541	0.2	>1000	100	-	0.1	S
002542	0.4	96	<3.2	-	0.03	INS
002543	0.4, 0.0, 0.7	20, 80, 30	10, 10, 3.2	0.2	0.5, 0.1, 0.1	S
002565	0.0	>320	320	-	<1.0	S
002566	0.2	790	10	-	0.01	S
002567	0.0	>1000	>1000	-	<1.0	INS
002568	0.0	>1000	32	-	0.03	S
002569	0.0	>1000	>1000	-	<1.0	INS
002685	0.2	>320	10	-	0.03	S
002686	0.1	>1000	100	-	0.1	S
002687	0.3	800	320	-	0.4	INS
002696	0.0	>100	10	-	<1.0	INS
002697	0.0	>100	32	-	<1.0	S
002698	0.1	>100	<1.0	-	<1.0	S
002699	0.0	>1000	320	-	<1.0	INS
002700	1.0, 0.7, 0.8	56, 10, 10	1.0, 3.2, <1.0	0.0	0.1, 0.1, 0.1	S
002701	0.1	>320	100	-	0.3	INS
002702	0.0	>1000	320	-	0.3	INS
002703	0.3	420	320	-	0.8	S
002704	0.0	>1000	1000	-	<1.0	S
002705	0.0	>1000	1000	-	<1.0	S
002707	0.0	>1000	320	-	<1.0	S

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	II ^e	Aqueous Solubility ^f
002709	0.0	>1000	320	-	<1.0	S
002710	0.1	>1000	320	-	0.3	S
002711	0.1	>1000	100	-	0.1	S
002714	0.3, 0.3, 0.6	<9, <9, 10	<1.0, <3.2, <1.0	0.0	0.1, 0.4, 0.1	INS
002716	0.2, 0.1, 0.9	<9, <46, 16	<3.2, <10, <1.0	0.0	0.3, 0.2, 0.06	INS
002717	0.4	850	1000	-	1.2	INS
002718	0.1	>1000	320	-	0.3	INS
002723	0.1	>1000	1000	-	<1.0	S
002739	0.0	>1000	>1000	-	<1.0	INS
002740	0.1	>1000	320	-	0.3	S
002741	0.0	>1000	1000	-	<1.0	S
002742	0.0	>1000	320	-	<1.0	S
002744	0.0	>1000	1000	-	<1.0	S
002745	0.1	>1000	1000	-	<1.0	S
002770	0.0, 0.0	>1000, >1000	<32, <1000	-	<0.03, <1.0	INS
002770	0.0	>320	320	-	<1.0	INS
002771	0.0	>1000	100	-	<1.0	INS
002772	0.1	>1000	320	-	0.3	INS
002773	0.0	>1000	>1000	-	<1.0	INS
002774	0.0	>1000	10	-	<1.0	INS
002775	0.2	450	32	-	0.07	S
002776	0.1	>1000	32	-	0.032	INS
002777	0.0	>1000	<1000	-	<1.0	INS
002778	0.1	>1000	100	-	0.1	INS
002880	0.1	850	320	-	0.38	S
002786	0.2	200	3.2	-	0.02	S
002787	0.3	420	<1.0	-	0.002	S
002788	0.1	>1000	320	-	0.3	S
002789	0.0	>1000	>1000	-	<1.0	S
002790	0.0	>1000	320	-	<1.0	S
002811	0.7, 0.7	<1.0, <3.0	<3.2, <1.0	-	<3.2, <0.3	INS
002869	0.0, 0.1, 0.5	220, 120, 150	100, 100, 320	0.3	0.5, 0.8, 2.1	S
002870	0.0	>1000	<1000	-	<1.0	INS
002871	0.0	>1000	<100	-	<0.1	INS
002872	0.0	>1000	>1000	-	<1.0	INS
002873	0.3	>1000	320	-	0.3	S
002874	0.0	>1000	>1000	-	<1.0	INS
002875	0.1	>1000	10	-	0.01	S
002876	0.2	740	1000	-	1.35	S

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	II ^e	Aqueous Solubility ^f
002877	0.0	>1000	>1000	-	<1.0	INS
002878	0.1	<220	<1000	-	<4.5	INS
002879	0.0	>1000	1000	-	<1.0	S
002883	0.1	>1000	>1000	-	<1.0	S
002884	0.1	>1000	>1000	-	<1.0	S
002885	0.0	>1000	>1000	-	<1.0	INS
002886	0.0	<820	<1000	-	<1.2	INS
002887	0.0	>1000	1000	-	<1.0	S
002888	0.0	>1000	>1000	-	<1.0	INS
002889	0.0	>1000	>1000	-	<1.0	S
002890	0.0	>1000	320	-	<0.3	S
002891	0.0	>1000	>1000	-	<1.0	INS
002893	0.2	870	320	-	0.37	S
002894	0.1	>1000	1000	-	<1.0	S
002895	0.1	>1000	32	-	<0.03	S
002896	0.1	>1000	10	-	<0.01	S
002898	0.0	<22	<3.2	-	0.15	INS
002899	0.0	22	<3.2	-	0.15	S
002900	0.0	22	<3.2	-	0.15	S
002901	0.0	22	32	-	1.5	S
002902	0.0	<83	<32	-	>0.4	INS
002903	0.0	<780	<320	-	>0.4	INS
002904	0.0	<840	<320	-	>0.4	INS
002905	0.0	<790	<1000	-	>1.3	INS
002906	0.3	<82	<100	-	>1.2	INS
002907	0.1	77	>1000	-	>12.99	S
002908	0.0	<83	<100	-	>1.2	INS
002909	0.0	>1000	100	-	<0.1	S
002910	0.0	94	100	-	1.1	S
002911	0.0	>1000	>1000	-	<1.0	S
002912	0.0	>1000	<1000	-	<1.0	INS
002913	0.0	>1000	<1000	-	<1.0	INS
002914	0.1	<190	<320	-	>1.7	INS
002915	0.0	>1000	>1000	-	>1.0	INS
002916	0.0	>1000	>1000	-	<1.0	INS
002917	0.2	>1000	320	-	<0.3	S
002918	0.0	82	100	-	1.2	S
002919	0.1	>1000	<1000	-	<1.0	INS
002926	0.0	>1000	320	-	<0.3	S

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	TI ^e	Aqueous Solubility ^f
002927	0.0	>1000	1000	-	<1.0	S
002928	0.3	830	1000	-	1.2	S
002929	0.0	>1000	1000	-	<1.0	S
002930	0.1	>1000	1000	-	<1.0	S
002931	0.2	>1000	320	-	<0.3	S
002932	0.0	>1000	320	-	<0.3	S
002934	0.0	<820	<10	-	0.01	INS
002936	0.0	820	1000	-	1.2	S
002939	0.0	220	100	-	0.5	S
002940	0.0	>1000	320	-	<0.3	S
002944	0.1	<820	<100	-	>0.1	INS
002946	0.2	<820	<100	-	>0.1	INS
002947	0.0	>1000	<320	-	<0.3	INS
002948	0.0	<820	<100	-	<0.1	INS
002949	0.0	<22	<10	-	<0.5	INS
002951	0.0	820	10	-	0.01	S
002956	0.1	<720	<320	-	<0.44	INS
002957	0.0	>1000	>1000	-	>1.0	INS
002958	0.0	>1000	<320	-	<0.3	INS
002959	0.0	>1000	<320	-	<0.3	INS
002960	0.0	220	100	-	0.5	S
002961	0.0	<820	<100	-	>0.12	INS
002962	0.1	>1000	>1000	-	<1.0	S
002963	0.0	780	1000	-	1.3	S
002964	0.0	>1000	<1000	-	<1.0	INS
002965	0.0	<220	<100	-	>0.5	INS
002966	0.0	>1000	<1000	-	<1.0	INS
002967	0.0	>1000	<1000	-	<1.0	INS
002968	0.0	82	100	-	1.2	S
002969	0.0	>1000	>1000	-	<1.0	INS
002970	0.0	>1000	>1000	-	<1.0	INS
002971	0.0	<770	<1000	-	>1.3	INS
002972	0.0	>1000	>1000	-	<1.0	INS
002973	0.0	<820	<320	-	>0.4	INS
002974	0.0	>1000	>1000	-	<1.0	S
002975	0.0	>1000	>1000	-	<1.0	INS
002978	0.0	>1000	>1000	-	<1.0	INS
002979	0.4	<96	<100	-	>1.0	INS
002980	0.8, 0.8	<8, <9	<3.2, <10	-	>0.4, >1.1	INS

Compd No. (AVS)	VR ^a	ED50 ^b ($\mu\text{g/ml}$)	MTC ^c ($\mu\text{g/ml}$)	VTR ^d at MTD ($\log_{10}$)	TI ^e	Aqueous Solubility ^f
002981	0.0	200	100	-	0.5	S
002982	0.0	21	3.2	-	0.15	S
002983	0.0	770	>1000	-	>1.3	S
002984	0.0	21	3.2	-	0.15	S
002985	0.0	770	1000	-	1.3	S
002986	0.0	<770	<320	-	>0.4	INS
002987	0.0	200	32	-	0.16	S
002988	0.1	<720	<320	-	>0.4	INS
002989	0.2	<40	<32	-	>0.8	INS
002990	0.1	<96	<320	-	>3.3	INS
002991	0.0	<780	<3.2	-	0.0	INS
002992	0.0	<810	<3.2	-	0.0	INS
002993	0.0	>1000	>1000	-	>1.0	S
002994	0.1	<650	<320	-	>0.5	INS
002995	0.1	>1000	1000	-	<1.0	S
002996	0.0	>1000	>1000	-	<1.0	INS
003035	0.0	<2	<3.2	-	>1.6	INS
003036	0.1	<82	<3.2	-	>0.04	INS
003037	0.0	>1000	>1000	-	<1.0	S
003038	≥ 1.0	1	3.2	-	>3.2	S
003039	0.0	>1000	320	-	<0.3	S
003585	0.4	820	>1000	-	>1.2	S
003587	0.5, 0.0	<94, <770	<100, <320	-	>1.1, >0.4	INS
003588	0.4	<88	<100	-	>1.1	INS
003589	0.2	>1000	>1000	-	<1.0	INS

^aVirus Rating.

^b50% Effective Dose.

^cMinimum toxic concentration.

^dVirus Titer Reduction as determined at the maximum tolerated dose (MTD).

^eTherapeutic Index (MTC + ED50)

^fS: Soluble; INS: Moderately Insoluble; VINS: Very Insoluble.

Table V-2. Summary of *In Vitro* Anti-Punta Toro Virus (Balliet Strain) Activity of AVS Compounds.

Compd No. (AVS)	VR ^a	ED50 ^b (μ g/ml)	MTC ^c (μ g/ml)	VTR ^d at MTD ($\log_{10}$)	TI ^e	Aqueous Solubility ^f
000001	1.1-1.4	5-4.9	32-100	1.0	6.4-20.4	S
000052	≥ 1.0 , 1.2	0.3, 1.4	<3.2, 0.1	0.0	10.7, 0.07	S
000111	0.4	130	3.2	-	0.02	S
000136	0.2	430	32	-	0.07	S
000139	0.6, 0.2	70, 82	3.2, 10	-	0.05, 0.1	S
000148	≥ 0.6 , ≥ 0.7	<1.0, .12	<3.2, <0.1	-	>3.2, 0.83	S
000195	0.3	62	<32	-	0.52	INS
000206	1.0, 0.8	27, 27	100, 10	2.8, 0.0	3.7, 0.37	S
000212	0.2	410	100	-	0.24	S
000215	0.8, 0.6	8.1, 8.6	<3.2, 10	0.8	0.4, 1.2	S
000233	0.2	>100	3.2	-	<0.032	S
000253	0.8, 0.6	>1.0, 17	<3.2, <3.2	-	<3.2, 0.19	S
000257	0.5, 0.6	75, 70	3.2, 3.2	-	0.04, 0.05	S
000360	0.0	>1.0	<3.2	-	<3.2	INS
001089	1.2, 1.4	0.45, 0.9	0.32, 0.1	-	0.7, 0.1	S
001159	0.5, 0.3	30, 135	<32, <32	0.0	1.1, 0.24	INS
001160	0.3	85	10	-	0.1	S
001199	0.0	>1000	320	-	<0.32	S
001754	0.3, 0.6	400, 130	320, 1000	-	0.8, 7.7	S
001976	0.4	85	32	-	0.38	S
002296	0.1	>1000	10	-	<0.01	S
002301	0.4	70	3.2	-	0.05	S
002700	0.2	850	10	-	0.01	S

^aVirus Rating.

^b50% Effective Dose.

^cMinimum toxic concentration.

^dVirus Titer Reduction as determined at the maximum tolerated dose (MTD).

^eTherapeutic Index (MTD + ED50)

^fS: Soluble; INS: Moderately Insoluble; VINS: Very Insoluble.

VI. INITIAL *IN VIVO* PUNTA TORO VIRUS EVALUATIONS OF COMPOUNDS.

Introduction

This report describes initial experiments run to determine if new AVS compounds submitted to us were active vs the hepatotropic PTV. The initial evaluation of potential anti-PTV compounds is performed using death only as endpoint. Compounds found positive in this initial evaluation are then retested using expanded evaluation parameters. If the compound is negative after the initial evaluation, further tests using other treatment regimens may be run in consultation with our Contracting Officer's Technical Representative. Figures VI-1 and VI-2 show flow charts for our *in vivo* evaluation process.

Materials and Methods

Virus: The Adames PTV was used. This was a twice-plaque isolated virus prepared in LLC-MK₂ cells as initially described in Section II of Report No. 1; the majority of the experiments run during this second year of the project used the new, more lethally potent PTV described in Section I of this Report.

Animals: C57BL/6 mice were obtained from Simonsen Laboratories. The mice were described further in Section II of this Report.

Compounds: All compounds were submitted to us by Technassociates, Inc. Compounds were usually prepared one day prior to being used for the first time in an experiment, using the vehicle considered most appropriate. Insoluble compounds were subjected to 15-30 min. treatment in a sonifying water bath, warmed to 45°C, vortexed, and used as a suspension if a full solution was not achieved. Each was distributed to sterile injection bottles, sealed and stored at 4°C until used. During use, each was stored at room temperature unless we were advised to the contrary. 1- β -D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, AVS01) was included in each series of experiments as a known positive control.

Experiment Design: A total of 10 s.c.-infected mice were treated with each drug dosage, and 20 infected mice were treated with placebo (drug vehicle) as virus controls. Five sham-infected mice were used in each drug dosage as toxicity controls, and 5 or 10 additional mice were used as normal controls. The toxicity and normal controls were held in a room separate from the infected area. Treatments were s.c., b.i.d. x 5 beginning 4 hr pre-virus inoculation unless another treatment schedule was recommended to us by the COTR or other individual acquainted with the material to be tested. Because of the pretreatments, the animals could not be randomized after virus infection, but the infection was given to each cage on a random, scattered basis in an attempt to randomize between cages. The animals were examined daily for death through day 21. Toxicity and normal controls were weighed on day 0 and again 18 hr after final drug treatment to ascertain weight loss or failure to gain weight. Dosages ranged in 2-fold dilutions, the number of dosages depending on the compound and what was initially known about it. A single dose of ribavirin was run in parallel as a positive control. This compound was described previously by us (Sections VIII-X of Report No. 1).

Statistical Analysis: Increases in mean survival time were evaluated using *t* test. Survivor increases were analyzed using chi square analysis with Yate's correction.

Results and Discussion

Thirty compounds were subjected to initial anti-PTV evaluations in mice. Overall, 138 experiments were run on these compounds and on compounds which we had begun to evaluate in 1986, altering treatment regimen in an effort to demonstrate or improve activity and also to determine therapeutic indices (TI) for active substances. An overview of the compounds evaluated is seen in Table VI-1. The new compounds evaluated included also compounds whose primary mechanism of action were considered to be directly antiviral and not immunomodulatory and 20 materials primarily characterized as immunomodulators.

For purposes of clarifying all data on individual compounds, each compound will be considered individually in Section IX of this Report.

Conclusions

Thirty compounds were evaluated for anti-PTV activity in mice using the hepatotropic (Adames) PTV. A total of 155 experiments were run in defining most appropriate treatment regimens or in further defining activity using expanded disease parameters for evaluation. See Section IX of this report for a detailed review of each active compound.

Figure VI-1. In Vivo Anti-Punta Toro Virus Evaluation of Non-Immunomodulating Compounds

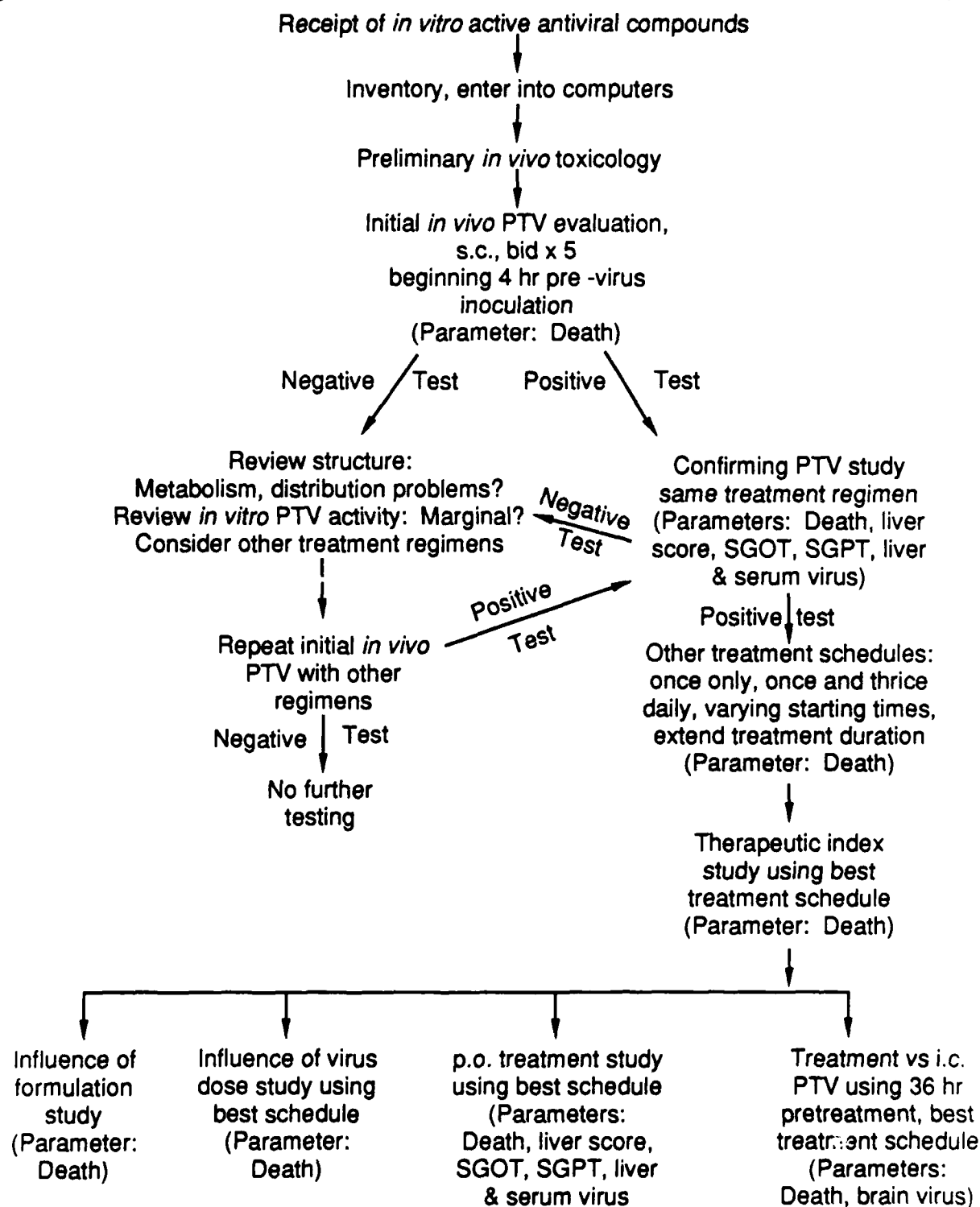


Figure VI-2. In Vivo Anti-Punta Toro Virus Evaluation of Immunomodulating Compounds

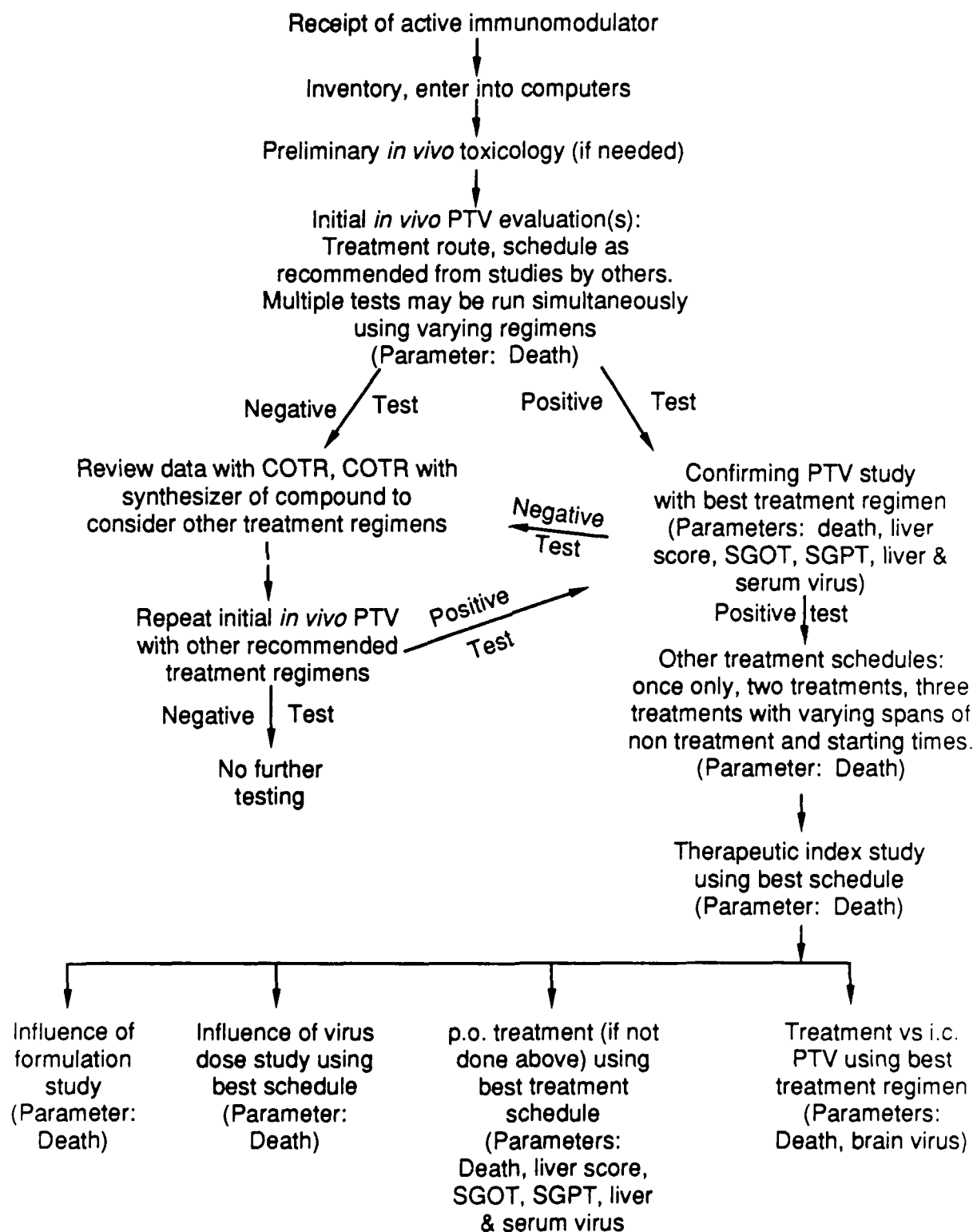


Table VI-1. Summary of In Vivo Anti-Punta Toro Virus (Adames Strain) Experiments with AVS Compounds^a.

AVS No.	Compound	Expt. No.	Dosage Range (mg/kg/day)	Treatment Schedule	Treatment Route	Toxic Dose	Results	Remarks
1	Ribavirin	1	9.4-75	bid x 5 beg 4 hr pre	s.c.	>75	+	TI = 16
		8	0.6-75	bid x 7 beg 4 hr pre	s.c.	>75	+	Active vs all parameters but liver virus.
		20	37.5-150	bid x 5 beg 24 hr post	s.c.	150	+	Active vs all parameters.
		21	37.5-150	bid x 5 beg 36 hr post	s.c.	150	+	Active vs all parameters but liver virus.
		43	3.2-100	bid x 5 beg 4 hr pre	p.o.	>100	+	Active vs all parameters.
		44	3.2-100	bid x 5 beg 4 hr post	p.o.	>100	+	Active vs all parameters but liver virus.
		45	3.2-100	bid x 5 beg 24 hr post	p.o.	>100	+	Active vs all parameters but liver virus.
		46	175-700	single, 4 hr post	s.c.	>700	+	TI > 4
		47	175-700	single, 8 hr post	s.c.	>700	+	TI > 4
		48	175-700	single, 24 hr post	s.c.	>700	+	TI > 4
		49	175-700	single, 48 hr post	s.c.	>700	+	TI > 4
2	Ribavirin triacetate	106	25-200	bid x 5 beg 4 hr pre	s.c.	>200	+	TI=2. Mean survival time increase only
		112	15.6-500	bid x 5 beg 4 hr pre	s.c.	>500	+	
		113	62.5-1000	single, 4 hr post	s.c.	>1000	+	TI > 16. Active vs all parameters.
		114	62.5-1000	single, 24 hr post	s.c.	>1000	+	TI ~ 16
		115	62.5-1000	single, 48 hr post	s.c.	>1000	+	TI > 16
		116	62.5-1000	single, 72 hr post	s.c.	>1000	-	TI > 16
		117	62.5-1000	single, 96 hr post	s.c.	>1000	-	
		134	9.4-600	bid x 5 beg 4 hr pre	p.o.	600	+	TI=8, Active vs all parameters
		185	31.3-1000	qd x 5 beg 4 hr pre	s.c.	>1000	+	TI=16 or >
52	Thioformycin B	2	62.5-250	bid x 5 beg 4 hr pre	s.c.	>250	-	
		22	300-1200	single, 4 hr post	s.c.	>1200	-	
		23	300-1200	single, 8 hr post	s.c.	>1200	-	
		24	300-1200	single, 24 hr post	s.c.	>1200	-	
		153	62.5-500	tid x 5 beg 4 hr pre	s.c.	>500	+	Survivor increase at 250 mpk only.
65	Formycin B	52	62.5-250	bid x 5 beg 4 hr pre	s.c.	>250	-	
79	9- β -D-Ribofuranosyl-purine-6-thio carboxamide	3	25-100	bid x 5 beg 4 hr pre	s.c.	~100	+	TI=2. Active vs all parameters but liver virus.
		12	6.25-50	bid x 5 beg 4 hr pre	s.c.	>50	+	Inhibited SGOT, SGPT only.
		102	25-200	bid x 5 beg 4 hr pre	p.o.	>200	±	
		107	18.8-150	bid x 5 beg 24 hr post	s.c.	>150	-	
		108	18.8-150	bid x 5 beg 36 hr post	s.c.	>150	-	
		109	18.8-150	bid x 5 beg 48 hr post	s.c.	>150	-	
		133	25-200	qd x 5 beg 4 hr pre	s.c.	200	±	Increased survivors @ 50 mpk only.
		154	87.5-700	single, 4 hr post	s.c.	~700	±	Increased mean survival time only.
		155	87.5-700	single, 24 hr post	s.c.	~700	±	Increased mean survival time only.
		156	87.5-700	single, 48 hr post	s.c.	~700	+	Increased survivors at all doses.

Table VI-1. Continued.

AVS No.	Compound	Expt. No.	Dosage Range (mg/kg/day)	Treatment Schedule	Treatment Route	Toxic Dose	Results	Remarks
79		157	87.5-700	single, 72 hr post	s.c.	~700	-	
		158	87.5-700	single, 96 hr post	s.c.	~700	-	
		187	6.25-200	bid x 5 beg 4 hr pre	i.p.	200	±	Increased mean survival time at 200 mpk only.
		188	6.25-200	tid x 5 beg 4 hr pre	i.p.	200	+	
111	Tiazofurin	53	31.3-250	bid x 5 beg 4 hr pre	s.c.	>250	+	
		68	31.3-500	bid x 5 beg 4 hr pre	s.c.	>500	+	Ti-16. Active vs all parameters but liver virus.
		110	15.7-2000	bid x 5 beg 4 hr pre	s.c.	2000	+	Ti = 8-16.
		135	125-1000	single, 4 hr post	s.c.	~1000	+	Increased survivors at 2 doses.
		136	125-1000	single, 24 hr post	s.c.	~1000	+	Increased survivors at 1 dose.
		137	125-1000	single, 48 hr post	s.c.	~1000	+	Increased survivors at 3 doses.
		138	125-1000	single, 72 hr post	s.c.	~1000	-	
		139	125-1000	single, 96 hr post	s.c.	~1000	±	Increased mean survival time at 1000 mpk only.
147	Enviroxime	15	25-100	bid x 5 beg 4 hr pre	s.c.	>100	-	
		34	250-1000	single, 4 hr post	s.c.	>1000	+	
		35	250-1000	single, 12 hr post	s.c.	>1000	±	
		36	250-1000	single, 24 hr post	s.c.	>1000	-	Increased mean survival time only.
		96	62.5-500	qd x 5 beg 4 hr pre	s.c.	>500	-	
167	Glycerhetic Acid	54	18.8-75	bid x 5 beg 4 hr pre	s.c.	>75	-	
		87	62.5-500	bid x 5 beg 4 hr pre	s.c.	>500	±	Increased mean survival time at 500 mpk only.
206	Ribavirin carboxamide	4	125-500	bid x 5 beg 4 hr pre	s.c.	>500	+	
		13	31.3-250	bid x 5 beg 4 hr pre	s.c.	>250	+	Active vs all parameters but liver virus.
		71	3.9-1000	bid x 5 beg 4 hr pre	s.c.	>1000	+	Ti > 32
		78	62.5-500	bid x 5 beg 24 hr post	s.c.	>500	+	Active vs all parameters.
		79	62.5-500	bid x 5 beg 36 hr post	s.c.	>500	+	Active vs all parameters.
		80	62.5-500	bid x 5 beg 48 hr post	s.c.	>500	+	Active vs all parameters.
		81	62.5-500	bid x 5 beg 72 hr post	s.c.	>500	+	Active vs all parameters.
		92	7.8-1000	bid x 5 beg 4 hr pre	p.o.	>1000	+	Ti = 64.
		169	15.7-1000	single, 4 hr post	s.c.	>1000	+	Ti > 4
		170	15.7-1000	single, 24 hr post	s.c.	>1000	+	Ti > 2
		171	15.7-1000	single, 48 hr post	s.c.	>1000	+	Ti > 4
		172	15.7-1000	single, 72 hr post	s.c.	>1000	-	
		173	15.7-1000	single, 96 hr post	s.c.	>1000	-	
212	Suramin	16	18.8-75	bid x 5 beg 4 hr pre	s.c.	>75	-	
		37	250-1000	single, 4 hr post	s.c.	>1000	-	
		38	250-1000	single, 12 hr post	s.c.	>1000	-	
		39	250-1000	single, 24 hr post	s.c.	>1000	-	
		103	75-200	bid x 5 beg 4 hr pre	p.o.	>200	-	
		159	18.8-150	tid x 5 beg 4 hr pre	s.c.	>150	-	

Table VI-1. Continued.

AVS No.	Compound	Expt. No.	Dosage Range (mg/kg/day)	Treatment Schedule	Treatment Route	Toxic Dose	Results	Remarks
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	55	31.3-250	bid x 5 beg 4 hr pre	s.c.	>250	-	Inhibition of liver score, SGOT, SGPT only.
		88	31.3-250	bid x 5 beg 4 hr pre	s.c.	>250	±	
233	Formycin	17	100-400	bid x 5 beg 4 hr pre	s.c.	~400	-	
		40	450-1800	single, 12 hr post	s.c.	~1800	±	Increase in mean survival time at 450 mpk only.
		41	450-1800	single, 24 hr post	s.c.	~1800	±	Increase in mean survival time at 450 mpk only.
253	Selenazofurin	5	80-320	bid x 5 beg 4 hr pre	s.c.	~320	+	
		14	20-160	bid x 5 beg 4 hr pre	s.c.	>160	+	Tl=2. Active vs all parameters but liver virus.
		97	40-320	qd x 5 beg 4 hr pre	s.c.	~320	±	To be repeated - Few deaths in controls.
		104	40-320	bid x 5 beg 4 hr pre	p.o.	~320	+	
272	3-Deazaguanine	186	25-200	bid x 5 beg 4 hr pre	s.c.	200	-	
360	7-Deoxynarciclasin	42	62.5-500	bid x 5 beg 4 hr pre	s.c.	>500	±	Increase in mean survival time at 250 mpk only.
1754	MVE-2	58	6.25-50	single, 24 hr pre	i.p.	>50	+	
		89	6.25-50	single, 24 hr pre	i.p.	>50	+	Active vs all parameters.
		98	6.25-100	single, 4 hr pre	i.p.	~100	+	
		99	6.25-100	single, 4 hr post	i.p.	~100	+	
		100	6.25-100	single, 24 hr post	i.p.	~100	+	
		101	6.25-100	single, 48 hr post	i.p.	~100	+	
		151	6.25-200	single, 24 hr pre	p.o.	~100-200	-	Inactive vs all parameters.
1757	Isoprinosine	76	250-1000	bid x 5 beg 4 hr pre	p.o.	>1000	-	
1767	AM-3	72	112.5-450	bid x 5 beg 4 hr pre	s.c.	>450	+	
		73	112.5-450	bid x 5 beg 4 hr pre	p.o.	>450	-	
		111	62.5-2000	bid x 5 beg 4 hr pre	s.c.	2000	+	Tl>2. Active vs all parameters.
1777	Streptonigrin	77	0.125-1	qd x 5 beg 4 hr pre	s.c.	~0.5-1	-	
1778	Mannozym	74	12.5-50	single, 4 hr pre	s.c.	~50	+	
		75	3.1-50	bid x 5 beg 4 hr pre	s.c.	>50	+	Tl > 16
		93	9.4-150	bid x 5 beg 4 hr pre	p.o.	>150	-	
		118	1.6-100	bid x 5 beg 4 hr pre	s.c.	>100	+	Tl=32. Active vs all parameters but liver virus.
		119	1.6-100	bid x 5 beg 4 hr pre	p.o.	>100	-	Inactive vs all parameters.
2149	Ampligen	56	0.6-5	qd x 8 beg 24 hr pre	s.c.	>5	+	Tl > 8
		57	0.6-5	eod x 8 beg 24 hr pre	s.c.	>5	+	Tl > 8
		69	0.3-5	qd x 8 beg 24 hr pre	s.c.	>5	+	Tl>16. Active vs all parameters but liver virus.
		128	0.6-5	single, 24 hr pre	i.p.	>5	+	Tl > 8

Table VI-1. Continued.

AVS No.	Compound	Expt. No.	Dosage Range (mg/kg/day)	Treatment Schedule	Treatment Route	Toxic Dose	Results	Remarks
2149		129	0.6-5	single, 4 hr pre	i.p.	>5	+	TI > 8
		130	0.6-5	single, 4 hr post	i.p.	>5	+	TI > 8
		131	0.6-5	single, 24 hr post	i.p.	>5	+	TI > 8
		132	0.6-5	single, 48 hr post	i.p.	>5	+	TI > 8
		142	0.04-5	qd x 5 beg 4 hr pre	p.o.	>5	±	Liver score, SGOT, SGPT reduction only.
2741	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-(1,4,5,6-tetrahydro-pyrimidin-2-yl)-HCl	149	31.3-500	bid x 5 beg 4 hr pre	s.c.	>500	-	
2742	1-(β-D-Ribofuranosyl)-1,2,4-triazole-3-(5-hydroxy-1,4,5,6-tetrahydropyrimidin-2-yl)-HCl	150	31.3-500	bid x 5 beg 4 hr pre	s.c.	>500	±	Survival increase at 500 mpk only.
2776	2-Amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP)	59	50-400	qd x 5 beg 24 hr pre	i.p.	400	+	TI = 4
		60	50-400	single, 24 hr pre	i.p.	400	+	TI = 4
		61	50-400	e3days x 3 beg 24 hr pre	i.p.	400	+	Survivor increase at 100 mpk only.
		90	100-400	single, 24 hr pre	i.p.	~400	+	Active vs all parameters
		143	100-400	single, 4 hr pre	i.p.	~400	+	TI > 4
		144	100-400	single, 4 hr post	i.p.	~400	+	TI = 2
		145	100-400	single, 24 hr post	i.p.	~400	+	TI = 2
		146	100-400	single, 48 hr post	i.p.	~400	+	TI = 1
		147	100-400	single, 72 hr post	i.p.	~400	-	
		148	100-400	single, 96 hr post	i.p.	~400	-	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	62	50-400	qd x 3 beg 24 hr pre	i.p.	400	+	Survivor increase at 200 mpk only.
		63	50-400	single, 24 hr pre	i.p.	~400	±	Only 35% virus controls died.
		64	50-400	e3days x 3 beg 24 hr pre	i.p.	>400	+	Only 35% virus controls died.
		91	100-400	single, 24 hr pre	i.p.	~400	+	Active at 400 mpk only.
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	65	50-400	qd x 3 beg 24 hr pre	i.p.	~400	+	Survivor increase at 200 mpk only.
		66	50-400	single, 24 hr pre	i.p.	>400	+	Survivor increase at 400, 50 mpk only.
		67	50-400	e3days x 3 beg 24 hr pre	i.p.	>400	+	Survivor increase at 400, 50 mpk only.
2880	Oxamisole	82	1.6-25	qd x 3 beg 24 hr pre	i.p.	>25	+	Survivor increase at 1.6 mpk only.
		83	1.6-25	qd x 3 beg 24 hr post	i.p.	>25	-	
		84	1.6-50	single, 24 hr post	i.p.	>50	+	Survivor increase at 25 mpk only.
		105	1.6-25	bid x 3 beg 24 hr pre	p.o.	>25	±	Mean survival time increase only at all doses.

Table VI-1. Continued.

AVS No.	Compound	Expt. No.	Dosage Range (mg/kg/day)	Treatment Schedule	Treatment Route	Toxic Dose	Results	Remarks
3585	Neurotropin	126 127 140 141	50-400 50-400 3-24 3-24	e3days x 2 beg 24 hr pre single, 24 hr pre qd x 3 beg 24 hr pre eodday x 3 beg 24 hr pre	i.p. i.p. s.c. s.c.	>400 >400 >24 >24	- - - -	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	120 121	50-400 50-400	qd x 3 beg 24 hr pre single, 24 hr pre	i.p. i.p.	400 400	- +	Survivor increase at 100 mpk only.
3588	Meta fluoro ABPP	122 123	50-400 50-400	qd x 3 beg 24 hr pre single, 24 hr pre	i.p. i.p.	>400 ~400	+ +	Tl > 4 Tl = 4
3589	5-Chloro-2,3-difluorophenyl ABPP	124 125	50-400 50-400	qd x 3 beg 24 hr pre single, 24 hr pre	i.p. i.p.	>400 ~400	+ -	Tl = 2
3925	du Pont A2222-1	189	25-200	single, 24 hr pre	i.p.	200	-	
3926	du Pont A2227-1	190	25-200	single, 24 hr pre	i.p.	100	-	
3927	du Pont A754-1	191	25-200	single, 24 hr pre	i.p.	200	-	
3934	Ge132	192	9.4-300	qd x 7 beg 36 hr pre	p.o.	>300	-	

^aFor purposes of comprehensive review of all compounds tested, data from 1986 Annual Report (Experiments 1-18) are also included.

VII. DETERMINATION OF INFLUENCE OF VIRAL CHALLENGE DOSE ON ANTIVIRAL ACTIVITY.

Introduction

Section IX of our first Annual Report described the influence of the concentration of viral challenge on the anti-PTV efficacy of ribavirin (AVS01). It was found that viral dose level affected this antiviral activity, but this was only seen at a low, MTD/8 dose of ribavirin, with antiviral effect lessening with higher PTV dose levels. Such data suggested the need to not use an overwhelming viral challenge in order to have an acceptable sensitive *in vivo* anti-PTV test.

The present experiment was run to determine if a similar effect would be seen using our new, more potent virus pool and using a compound closely related to ribavirin, 2',3',5'-triacetyl-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (AVS02, ribavirin triacetate), which has been shown by us (Sections VI, IX) to have a potent anti-PTV activity possibly exceeding that of ribavirin.

Materials and Methods

Virus: The newly prepared Adames strain PTV pool described in Section I, designated PTA/4LLC 6-19-87, was used. Virus dilutions were $10^{-1.5}$, $10^{-2.5}$, $10^{-3.5}$, and $10^{-4.5}$, which equated to 1000, 100, 10 and 1 LD50 of virus in 3 week-old mice.

Compound: Ribavirin triacetate (AVS02) was provided by Technassociates for this study. The compound was prepared in sterile physiological saline for this experiment. Five dosages: 15.6, 31.3, 62.5, 125 and 250 mg/kg/day were used.

Animals: C57BL/6 mice weighing 11-12 g were used. They were caged and maintained as described in Section I.

Antiviral Experiment: Ten PTV-infected mice were used with each drug dosage and at each virus level. Twenty PTV-infected mice were treated in parallel with saline at each virus dose. Five sham-infected mice were treated with each drug dose to serve as toxicity controls, and 5 normal control mice were used. The latter two groups were weighed immediately prior to initial drug treatment and 24 hr after final treatment. AVS02 was administered s.c., bid x 5 beginning 4 hr pre-virus inoculation. All animals were observed daily for death 21 days, with antiviral activity expressed as increased survivors and mean survival times.

Results and Discussion

The results of this study are summarized in Table VII-1. The virus control mice receiving 1000 and 100 LD50 of PTV all died with mean survival times of 4.4 and 4.1 days, respectively. Those control mice receiving 10 and 1LD50 of virus had 65% and 70% deaths, respectively, with a mean survival time of mice which died in each group of 4.6 days. Against all virus doses, 250 and 125 mg/kg/day of AVS02 was 100% protective; the 62.5 mg/kg/day dose was almost 100% protective, although one animal infected with this dose died. A definite difference was seen using the 31.3 mg/kg/day dosage. This drug dose protected 80% of the mice infected with 1LD50 of virus, but it was essentially inactive against all higher viral inocula. Similarly, the 15.6 mg/kg/day dosage of AVS02 was moderately effective only at the lowest viral inoculum, with 50% survivors seen. Figure VII-1 shows a bar graph of the data using the 31.3 mg/kg/day dosage of AVS02 vs all viral dosages.

These data suggest that, although viral challenge will affect the sensitivity of the *in vivo* antiviral test, the decreased sensitivity effect was not alarming since three of the five significantly active dosage levels were still antiviral at all virus concentrations. In all of our initial antiviral studies *in vivo*, multiple doses of each test substance are evaluated.

As described in Section IV, the LD50 of AVS02 was 850 mg/kg/day; no signs of toxicity were seen in the present study at any drug dosage, and it is apparent we could have used at least one 2-fold higher level of the compound without untoward effects in the mice. Thus, at least 4 dose

levels would be shown effective despite the concentrations used of the challenge virus. We are routinely using a $10^{-3.5}$ dilution (100 LD50) of this PTV pool in our antiviral studies.

Conclusions

Although virus dose effects *in vivo* anti-PTV effects of AVS02 to a degree, this effect was primarily seen at the lowest effective dose of AVS02, and was not manifested at higher doses. Based on data to date, we feel our current *in vivo* anti-PTV model to be acceptably sensitive for detection of compounds having antiviral potential.

Table VII-1. Expt. PtA178-181. Influence of Viral Inoculum Size on the Effect of AVS02 on Punta Toro Virus Infections in Mice.

Animals: 10.9-12.4 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected. Challenge dose: 1, 10, 100, 1000 LD50.

Treatment Route: s.c.

Drug Diluent: Saline.

Experiment Duration: 21 days.

Virus Dose (LD50)	Compound	Dosage (mg/kg/day)	Tox. Control		Infected. Treated	
			Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
1	AVS02	250	5/5	2.5	10/10**	>21.0**
		125	5/5	1.6	10/10**	>21.0**
		62.5	5/5	1.7	10/10**	>21.0**
		31.3	5/5	3.8	8/10**	6.0
		15.6	5/5	4.5	5/10	7.2**
	Saline	-	-	-	6/20	4.6
10	AVS02	250	5/5	2.5	10/10**	>21.0**
		125	5/5	1.6	10/10**	>21.0**
		62.5	5/5	1.7	10/10**	>21.0**
		31.3	5/5	3.8	0/10	6.7
		15.6	5/5	4.5	1/10	4.8
	Saline	-	-	-	7/20	4.6
100	AVS02	250	5/5	2.5	10/10**	>21.0**
		125	5/5	1.6	10/10**	>21.0**
		62.5	5/5	1.7	9/10**	7.0
		31.3	5/5	3.8	0/10	4.8
		15.6	5/5	4.5	0/10	4.8
	Saline	-	-	-	0/20	4.1
1000	AVS02	250	5/5	2.5	10/10**	>21.0**
		125	5/5	1.6	10/10**	>21.0**
		62.5	5/5	1.7	10/10**	>21.0**
		31.3	5/5	3.8	2/10	5.4
		15.6	5/5	4.5	2/10	4.1
	Saline	-	-	-	0/20	4.4
Normals		-	5/5	3.4	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

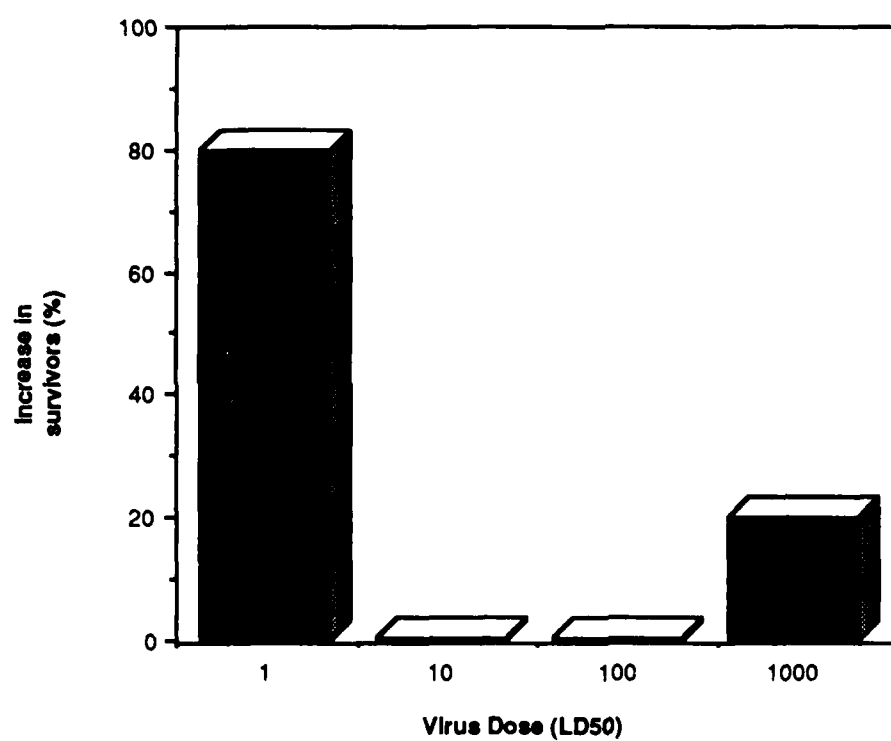
^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This series of experiments (PtA 178-181) was run to determine the influence of viral inoculum size on the AVS02 (ribavirin triacetate) anti-PTV activity. It appears that the compound's activity was affected little by viral inoculum size, since only at the 1LD50 viral challenge was anti-PTV effect seen at 31.3 mg/kg/day; the next higher dose, 62.5 mg/kg/day, was uniformly effective against all viral challenges.

Figure VII-1. Influence of PTV dose on in vivo antiviral activity of ribavirin triacetate (31.3 mg/kg/day)



VIII. EFFECT OF AVS COMPOUNDS ON INTRACEREBRAL INFECTIONS OF MICE INDUCED BY THE BALLIET STRAIN PUNTA TORO VIRUS.

Introduction

It has been stressed from the inception of this project that the infection in mice PTV is being used as a model for Rift Valley fever and sandfly fever infections in man. A late and often fatal form of Rift Valley fever involves encephalitis, and patients with sandfly fever also develop certain symptoms suggestive of central nervous system (CNS) infection. We therefore felt it was important to determine if AVS compounds active against the hepatotropic Adames PTV infection would also have an effect on an encephalitic disease induced in mice by the neurotropic (Balliet) strain of PTV. As described earlier, our protocol for *in vivo* evaluations of anti-PTV compounds includes follow-up testing of PTV-inhibitory compounds against the CNS disease in mice. The results of these follow-up investigations are described in this section.

Materials and Methods

Virus: The Balliet strain of PTV as described in Sections I and III of our Annual Report No. 1 was used. A mouse brain-prepared virus pool was used in the present studies. The virus, suspended in Pucks balanced salt solution (PBSS) was used at dilutions of 10^{-3} or 10^{-4} (10 and 1LD₅₀), coinciding with 10^4 and 10^3 Vero cell CCID₅₀ of virus. The latter dose was used in most studies in attempt to increase the sensitivity of the test.

Animals: Four week-old C57BL/6 mice were used. The source, caging and maintenance of these animals was described in Section I.

Compounds: The following 7 AVS compounds were evaluated during this contract period: AVS02, 79, 206, 253, 1754, 1778, and 2149. All were provided by Technassociates, Inc.

Experiment Design: Ether-anesthetized mice were infected by inoculating 0.05 ml of PTV i.c. into the right hemisphere of the brain. Twenty infected mice were used with each drug level, with 5 infected mice used as virus controls which received drug diluent only. Treatment and schedule varied depending upon the compound being evaluated, with those regimens considered highly effective against the hepatotropic virus infection selected for treatment of this CNS disease. Five toxicity control mice were used at each drug dose level, and 10 mice were used as normal controls. The latter two groups of controls were weighed before and after treatment as described in Section VI. On infection day 6, one-half (one or two pre-designated cages) of each group of infected animals were killed and their brains removed. Ten percent homogenates of each brain were diluted through a series of 10-fold dilutions and each was assayed for virus using CPE production in triplicate cups of LLC-MK₂ cells. The remaining animals were observed daily for death through infection day 21, which was the termination of the experiment.

Increases in survivor number were evaluated using chi square analysis with Yate's correction. Increases in mean survival time and decreases in mean brain virus titers were analyzed using *t* test.

Results and Discussion

The results with each AVS compound tested against this CNS infection are shown in Tables VIII-1 to 7. The following summarizes the activity of each. See also the conclusions indicated on each table.

AVS02 (Ribavirin triacetate) (Table VIII-1): Moderately effective as seen by increased mean survival times and decreased brain virus titers. We would expect the triacetate to have an increased lipid solubility and hence be a potentially better drug for penetrating the brain. These results suggest that to be the case, particularly since ribavirin has been shown to be totally inactive against this infection, as described in Section X of our Annual Report No. 1.

AVS79 (9-β-D-ribofuranosylpurine-6-thiocarboxamide) (Table VIII-2): No activity seen.

AVS206 (Ribavirin carboxamide) (Table VIII-3): Moderately effective as seen by increased survivors, increased survivor time, and particularly in significantly reduced brain virus titers. Of particular interest is that the highest dose used, 500 mg/kg/day, is 1/4 the MTD. Further CNS experiments are planned for this material.

AVS253 (Formycin) (Table VIII-4): No activity seen.

AVS1754 (MVE-2) (Table VIII-5): Moderately effective, as seen only by significantly increased mean survival times. It is important to note that more virus was seen in the brains of the treated animals than in the controls.

AVS1778 (Mannozym) (Table VIII-6): No activity seen.

AVS2149 (Ampligen) (Table VIII-7): Significantly active vs this CNS infection, as seen by increased survivors, mean survival prolongation, and decreased brain virus titers. This compound is considered to be the most active of those tested to date against this infection.

Conclusions

Compounds AVS02, 206, 1754 and 2149 were considered to have an effect on the CNS infection induced by the Balliet PTV.

Table VIII-1. Expt. PtA167. Effect of AVS02 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 13.3 - 14.4 g (4 wk) C57BL/6 Mice.

Virus: Balliet strain Punta Toro virus, i.c. injected.

Drug Diluent: Sterile saline.

Treatment Schedule: Twice daily X 5, beginning 24 hr pre-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Surv/ Total</u>	<u>Infected, Treated</u>	
		<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>		<u>MST^b (days)</u>	<u>Brain Virus Titers^d</u>
AVS02	1000	5/5	-0.9	0/10	10.4	5.7
	500	5/5	0.9	0/9 ^c	10.4	5.0**
	250	5/5	2.1	1/10	11.0*	5.6
	125	5/5	0.6	1/8 ^c	10.3	6.7
Saline	-	-	-	1/18 ^c	9.8	6.3
Normals	-	5/5	3.0	-	-	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cSome mice killed during inoculation.

^dLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: AVS02 (ribavirin triacetate) was moderately effective against the intracerebral Balliet strain PTV infection, as seen by increased mean survival times and lowered brain virus titers. The LD₅₀ of this compound in younger (3 week-old) mice was previously determined to be 850 mg/kg/day, so the 1000 mg/kg/day maximum dose used in this study is considered the MTD in these older mice. The virus challenge was quite high in this study; such a high challenge is somewhat unrealistic compared to a naturally occurring human infection.

Table VIII-2. Expt. PtA18. Effect of AVS79 on Intracerebral Punta Toro Virus Infections in Mice.

Animals: 14-19 g (5 wk) C57BL/6 Mice

Treatment Schedule: Twice daily X 5,
beginning 30 hr pre-virus inoculation.

Virus: Balliet strain Punta Toro virus,
i.c. injected.

Treatment Route: s.c.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Controls</u>	<u>LD50 Virus Dose</u>		
		<u>Surv/ Total</u>	<u>Surv/ Total</u>	<u>MST^a (days)</u>	<u>Brain Virus Titers^b</u>
000079	75	5/5	7/10	9.0	2.2
	37.5	5/5	7/10	11.3	3.8
	18.8	2/2	8/10	3.0	2.8
	9.4	2/2	5/10	14.0	1.5
Saline	-	-	13/20	12.2	1.1
Normals	-	3/3	-	-	0.0

^aMean survival time of mice dying on or before day 21.

^bLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: Twice daily treatment with AVS79 (9-β-D-ribofuranosylpurine-6-thiocarboxamide) beginning 30 hr pre-virus inoculation was not effective against intracerebral Balliet strain PTV infections. This compound was highly active against the subcutaneously administered Adames strain of this virus, so we presume the lack of efficacy in this experiment was due to an inability of the compound to pass the blood-brain barrier in the mouse. We deliberately used a low virus challenge (1 LD₅₀) in order to allow the compound more opportunity to be efficacious. The LD₅₀ of this compound is approximately 150 mg/kg/day, so the MTD was used in this study.

Table VIII-3. Expt. PtA86. Effect of AVS206 on Intracerebrally Injected Punta Toro Virus Infections in Mice (Initial Test).

Animals: 15.2-15.8 g (4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 24 hr pre-virus inoculation.

Virus: Balliet strain Punta Toro virus, i.c. injected. Treatment Route: s.c.

Drug Diluent: Sterile saline. Experiment Duration: 19 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected Treated</u>		
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>	<u>Brain</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>	<u>Virus Titers^c</u>
AVS000206	500	5/5	2.0	8/10	11.0	1.5*
	250	5/5	2.1	3/8	12.6	2.5
	125	5/5	2.8	5/8	10.0	0.0**
Saline	-	-	-	10/20	10.7	3.2
Normals	-	5/5	3.2	-	-	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: AVS 206, the carboxamidine derivative of ribavirin, was moderately active against the i.c. Balliet PTV infection in this study, as seen particularly by decreased brain virus titers and in a moderate increase in survivor number and mean survival time. The maximum tolerated dose (~2000 mg/kg/day) was not used in this study.

Table VIII-4. Expt. PtA19. Effect of AVS253 on Intracerebral Punta Toro Virus Infections in Mice.

Animals: 14-19 g (5 wk) C57BL/6 Mice

Treatment Schedule: Twice daily X 5,
beginning 30 hr pre-virus inoculation.
Treatment Route: s.c.

Virus: Balliet strain Punta Toro virus,
i.c. injected.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Controls</u>		<u>LD50 Virus Dose</u>		<u>Brain</u> <u>Virus</u> <u>Titers^b</u>
		<u>Surv/</u> <u>Total</u>	<u>Surv/</u> <u>Total</u>	<u>MST^a</u> <u>(days)</u>	<u>MST^a</u> <u>(days)</u>	
AVS000253	150	5/5	6/10	12.8	12.8	1.4
	75	5/5	9/10	9.0	9.0	2.8
	37.5	2/2	7/10	13.0	13.0	2.8
	18.8	2/2	6/10	11.5	11.5	1.3
Saline	-	-	13/20	12.2	12.2	1.1
Normals	-	3/3	-	-	-	0.0

^aMean survival time of mice dying on or before day 21.

^bLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: Twice daily treatment with AVS253 (Formycin) beginning 30 hr pre-virus inoculation was not effective against intracerebral Balliet strain Punta Toro virus infections. This compound was highly active against the s.c. administered Adames strain of this virus, so we presume the lack of efficacy in this experiment was due to an inability of the compound to pass the blood-brain barrier in the mouse. We deliberately used a low virus challenge (1 LD50) in order to allow the compound more opportunity to be efficacious. The LD50 for this compound is approximately 500 mg/kg/day, so the 150 mg/kg/day maximum dose used was approaching the MTD.

Table VIII-5. Expt. PtA161. Effect of AVS1754 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.4 - 13.4 g (4 wk) C57BL/6 Mice.

Virus: Balliet strain Punta Toro virus, i.c. injected.

Drug Diluent: Sterile H₂O then saline.

Treatment Schedule: Once only, beginning 4 hr pre-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Surv/ Total</u>	<u>Infected, Treated</u>	
		<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>		<u>MST^b (days)</u>	<u>Brain Virus Titters^d</u>
AVS1754	100	5/5	-0.5 ^c	2/10	14.2**	5.3
	50	5/5	-0.1 ^c	2/10	11.8**	6.1
	25	5/5	0.3 ^c	4/10	8.8	5.9
	12.5	5/5	0.2 ^c	3/10	9.0	6.4
Saline	-	-	-	3/20	9.1	5.2
Normals	-	5/5	1.0	-	-	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cAnimals weighed one day late.

^dLog₁₀ infectious virus recovered from animals killed on infection day 6. Titters determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: AVS1754 (MVE-2) has been previously shown to be highly active vs the hepatotropic PTV when administered once only from 24 hr pre to 48 hr post-virus inoculation. The present experiment used a single treatment 4 hr pre-virus inoculation to treat the neurotropic PTV infection. Moderate activity was seen, expressed as significant increases in mean survival time only.

Table VIII-6. Expt. PtA152. Effect of AVS1778 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 13.3 - 14.4 g (4 wk) C57BL/6 Mice.

Virus: Balliet strain Punta Toro virus, i.c. injected.

Drug Diluent: Sterile saline.

Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.

Treatment Route: s.c.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>		
		<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>	<u>Surv/ Total</u>	<u>MST^b (days)</u>	<u>Brain Virus Titers^d</u>
AVS1778	100	5/5	0.7	4/10	8.8	2.5
	50	5/5	0.9	5/10	9.2	3.9
	25	5/5	0.4	2/10	10.6	3.4
	12.5	5/5	0.9	4/9 ^c	8.4	1.6
	6.25	5/5	1.2	1/7 ^c	9.3	3.2
Saline	-	-	-	9/20	9.4	2.3
Normals	-	5/5	1.4	-	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cSome mice killed during inoculation.

^dLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: AVS1778 (mannozym) has been highly active against the hepatotropic PTV when given by this treatment regimen. Essentially no activity was seen when used against the neurotropic PTV in this experiment, however. The LD₅₀ of mannozym is 200 mg/kg/day using this treatment regimen, so essentially the MTD was used in this experiment.

Table VIII-7. Expt. PtA160. Effect of AVS2149 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.4 - 13.4 g (4 wk) C57BL/6 Mice.

Virus: Balliet strain Punta Toro virus, i.c. injected.

Drug Diluent: Sterile H₂O then saline.

Treatment Schedule: Once daily X 5, beginning 4 hr pre-virus inoculation.

Treatment Route: i.p.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>		
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>	<u>Brain</u> <u>Virus Titers^c</u>
AVS2149	5	5/5	1.2	5/10*	14.2**	1.9**
	2.5	5/5	1.8	5/10*	11.8**	3.4**
	1.25	5/5	1.9	5/10*	8.8	4.1
	0.63	5/5	0.7	4/10	9.0	3.7*
Saline	-	-	-	3/20	9.1	5.2
Normals	-	5/5	1.0	-	-	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cLog₁₀ infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK₂ cells.

*P<0.05

**P<0.01

Conclusions: AVS2149 (ampligen) was considered to have significant activity against i.c. injected PTV. This AVS material is considered to have the greatest activity of all the AVS compounds evaluated to date.

IX. OVERVIEW OF *IN VIVO* ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS COMPOUNDS.

Introduction

This section includes the expanded data on all *in vivo* experiments run against the Adames strain of PTV except those studying effect of virus dose described in Section VII and effects on the CNS infection described in Section VIII. Commentary is provided on each compound tested to date regarding our overall conclusions regarding each.

Materials and Methods

All were as described in Section VI.

Results and Discussion

AVS01 (Ribavirin) (Tables IX-1-3): This compound has been used as our positive control, being run in parallel at a single dose in the majority of the *in vivo* Adames PTV chemotherapy experiments. The initial data concerning this compound were described in our Annual Report No. 1, wherein it was considered highly active against Adames hepatotropic PTV infections, with a TI of 16. Ribavirin was inactive vs the i.c.-inoculated Balliet PTV. Subcutaneous treatment of Adames PTV infections, beginning 24 or 36 hr post-virus inoculation, were highly effective, although liver virus was reduced in only a single incidence (Table IX-1). Orally administered ribavirin was similarly effective, with treatments beginning 4 hr pre-, 4 hr post- or 24 hr post-virus inoculation active, although by the latest post-treatment therapy, activity began to decline at lower dosage levels (Table IX-2). Single s.c. treatments of the Adames infection prevented death when begun prior to or up to 48 hr after virus inoculation (Table IX-3). Despite the positive anti-PTV activity exhibited by ribavirin, we feel there are other materials which may have even greater promise for treatment of PTV. These include AVS02 (ribavirin triacetate), AVS206 (ribavirin carboxamidine), and certain immunomodulators, most notably AVS2149 (ampligen).

AVS02 (Ribavirin triacetate) (Tables IX-4-8): This compound exhibited strong activity against PTV infections, with a TI at least equivalent to ribavirin. The compound is about 8 times more tolerated than ribavirin but the minimum effective dose is higher. Like ribavirin, single s.c. treatment given up to 48 hr after virus inoculation was highly effective (Table IX-6). Of particular significance was the observation that orally administered AVS02 was active to the extent of totally eliminating all detectable virus from livers and spleens of infected mice at 3 dosage levels (Table IX-7). Liver virus reduction has been a formidable obstacle in our anti-PTV studies with most compounds studied. Single treatments daily (Table IX-8) appeared equally as effective as twice daily treatments. A meaningful observation was the moderate activity this compound exhibited against i.c. Balliet strain PTV infections (Table VIII-1).

AVS52 (Thioformycin B) (Tables IX-9-10): In our first Annual Report, Section IV, we reported AVS52 to have strong *in vitro* anti-PTV activity. In Section VI of that Report, however, the compound was considered inactive against the Adames PTV infection *in vivo*. During this report period we studied two other treatment regimens, single s.c. treatment and three times daily treatment, in an attempt to demonstrate *in vivo* efficacy with this material. The single treatment, given 4, 12 or 24 hr post-virus inoculation, had no apparent effect on the infection (Table IX-9). However, the three times daily treatment yielded positive results (Table IX-10), indicating this compound is apparently relatively quickly metabolized to an inactive form by the host. We are currently examining the effects of four times daily therapy, which we anticipate should further improve this compound's *in vivo* efficacy.

AVS65 (Formycin B) (Table IX-11): AVS65 has exhibited only a slight *in vitro* anti-PTV activity (VR=0.3, Table V-1). It was recommended to us for *in vivo* evaluation, presumably because of its relationship to AVS52 and 233. A single experiment was run using our standard treatment regimen (Table IX-11), but no activity was seen. It also was nontoxic at the dosages used, suggesting the need for further evaluation at higher dosage levels. This compound may behave metabolically in a similar manner to AVS52, indicating a treatment schedule using more treatments per day may be appropriate.

AVS79 (9- β -D-Ribofuranosylpurine-6-thiocarboxamide) (Tables IX-12-17): In our First Annual Report, we reported s.c.-administered AVS79 to be active vs PTV infections, but with a relatively low TI of 2. Further experiments were run to explore more fully the anti-PTV effects of this compound. Treatment by oral gavage (Table IX-12) was essentially ineffective. Delaying the bid x 5 s.c. treatments (Table IX-13) yielded less activity than treatment beginning 4 hr pre-virus inoculation. Once daily s.c. treatments for 5 days (Table IX-14) was considered less effective than bid x 5 therapy. Single s.c. treatments given at varying times relative to virus inoculation were studied (Table IX-15), with surprisingly strong activity seen when those treatments were given only 48 hr post-virus inoculation. This result may suggest the compound has a mechanism of action best manifested when the infection has progressed through several replications of the virus and when the virus has reached near maximal levels in serum and liver (the latter as shown in our First Annual Report, Section II). Finally, i.p. treatments bid x 5 or tid x 5 were less active than s.c. treatments (Tables IX-16, 17). AVS79, when administered s.c. bid x 5 beginning 30 hr pre-i.c. Balliet PTV inoculation, was ineffective. We conclude at this stage that this material is less promising than ribavirin.

AVS111 (Tiazofurin) (Tables IX-18-21): AVS111 was slightly less active *in vitro* vs PTV than ribavirin. It also was somewhat less effective than ribavirin when administered by similar treatment schedules to PTV-infected mice (Tables IX-18-20). Using expanded parameters to study AVS111 anti-PTV effects (Table IX-19), efficacy was not seen using reduction of liver virus as parameter, although in this experiment the usual dose of ribavirin was similarly not effective in reducing those liver virus titers. Single treatment given at varying times relative to virus inoculation was, like AVS79, most effective at 48 hr post-virus inoculation (Table IX-21). We consider AVS111 to be slightly less effective than ribavirin.

AVS147 (Enviroxime) (Tables IX-22, 23): Enviroxime was shown by us in the last Annual Report to be inactive vs PTV infections when administered s.c. bid x 5 beginning 4 hr pre-PTV inoculation. During this report period we found the material to be moderately active when administered in a single s.c. treatment 4 hr post-virus inoculation (Table IX-22). Single daily treatment x 5 were not effective (Table IX-23). At this point, AVS147 is not considered to have potential as an anti-PTV compound.

AVS167 (Glycerrhetic Acid) (Tables IX-24, 25): This compound was only weakly active (VR=0.3) against PTV *in vitro*. Two *in vivo* experiments were run vs PTV, both using our standard s.c. bid x 5 treatment schedule. No activity was seen, although the MTD may not yet have been reached in the studies completed to date.

AVS206 (Ribavirin carboxamidine) (Tables IX-26-29): We reported this ribavirin derivative to be highly active *in vivo* vs PTV in the First Annual Report. Experiments have been run since then to further define this activity. Until recently, we have been limited in our studies because of inadequate available quantities of the compound. We have determined that the LD50 of AVS206 is approximately 3000 mg/kg/day when administered s.c. bid x 5. Assuming the MTD to then be 2000 mg/kg/day, and the minimum effective dose to be 62.5 (Table IX-26), we determine this compound's TI to be 32, or at least twice as large as ribavirin. This s.c. treatment was shown to be effective when begun as late as 72 hr post-virus inoculation (Table IX-27), with anti-PTV activity seen using every disease parameter. Oral therapy appeared equal in activity to s.c. treatment (Table IX-28), with a TI of 32 again seen. Single s.c. treatments, like ribavirin, could be given effectively as late as 48 hr post-virus inoculation (Table IX-29). It is interesting that in the single experiment run to date against the i.c. PTV infection using AVS206 (Table VIII-3), activity was seen at doses well below MTD levels, an observation not seen with ribavirin. We consider AVS206 to be among the most promising compounds seen to date for treatment of PTV infections.

AVS212 (Suramin) (Tables IX-30-32): Suramin was initially found inactive vs PTV as reported in our First Annual Report. Experiments were run since that time to determine if single s.c. treatments, s.c. treatments given tid x 5, or oral treatments bid x 5 would be efficacious. In no case was antiviral activity seen. We consider suramin to be inactive vs PTV.

AVS222 (3-Bromo-4-chloropyrazolo-[3,4-d]-pyrimidine) (Tables IX-33, 34): This compound was not considered active vs PTV *in vitro*. It was submitted also for *in vivo* testing, however, but only weak activity was seen (Table IX-34). A further study will be run to determine an LD50 to ascertain if an MTD was used in these experiments.

AVS233 (Formycin) (Table IX-35): Formycin was moderately active *in vitro* vs PTV (VR=0.7-0.8). An initial experiment run during 1986 using our standard s.c. bid x 5 treatment regimen indicated the material was inactive in mice against the PTV infection. We have run 2 additional experiments using the compound in single s.c. injections 12 hr or 24 hr post-PTV inoculation. Moderate activity was seen, implying AVS233 may be metabolized to an inactive form at a relatively high rate, preventing it from attaining antiviral levels in the blood when administered on a more chronic basis, with lower dose levels.

AVS253 (Selenazofurin) (Tables IX-36, 37): Selenazofurin has exhibited a positive *in vitro* activity vs PTV (VR=0.7-0.9). We previously reported the material to be active against *in vivo* PTV infections (First Annual Report) using our s.c. bid x 5 treatment regimen, but the TI was only 2, and liver virus was not inhibited. A single daily treatment study was inconclusive (Table IX-36) during this current report period. Oral therapy was effective, but the TI was considered to only be 4-8 depending on the parameter used (Table IX-37). We presently consider selenazofurin to be inferior to ribavirin for PTV infections. We have previously found selenazofurin to be moderately effective against *in vivo* influenza (1) and murine hepatitis (2) virus infections.

AVS272 (3-Deazaguanine) (Table IX-38): 3-Deazaguanine has previously been found to be moderately effective against a number of RNA viruses, including influenza, parainfluenza, rhino, vesicular stomatitis, bluetongue, reo and rota viruses (3-6). The compound was only slightly effective vs PTV *in vitro* (VR=0.3), and it was essentially inactive in the single *in vivo* PTV study run to date (Table IX-38). We feel this lack of effect vs PTV to be quite surprising, and question if the right material has been sent to us.

AVS360 (7-Deoxynarciclasin) (Table IX-39): *In vitro* activity vs PTV has ranged from a VR of 0.4 to 0.7 with AVS360. In a single experiment run to date (Table IX-39), moderate activity was seen as increased mean survival time only. The MTD was not attained in our study, however, and further studies have been curtailed because of a shortage of the compound.

AVS1754 (MVE-2) (Tables IX-40-43): AVS1754 is a known immunomodulating substance. Single i.p. treatments from 24 hr pre- to 48 hr post-virus inoculation were markedly effective against Adames PTV infections (Tables IX-40-42). All disease parameters were significantly inhibited by the compound. Oral treatment with AVS1754 was ineffective (Table IX-43). A single i.p. treatment 4 hr pre-i.c. inoculation of Balliet PTV significantly prolonged mean survival times of infected mice (Table VIII-5). We feel this compound is of considerable significance as a potential anti-PTV compound.

AVS1757 (Isoprinosine) (Table IX-44): Isoprinosine has had mixed reviews as an antiviral agent. An extensive review of literature published on this material indicated the ideal treatment regimen to be p.o., bid x 5 beginning 4 hr pre-virus inoculation. Using this regimen, however, no anti-PTV effect was seen.

AVS1767 (AM-3) (Tables IX-45-47): AM-3 is an immune modulator of which little has been published. In experiments run with this material, it was considered highly active when administered s.c. bid x 5 beginning 4 hr pre-virus inoculation. All disease parameters were inhibited by the treatment. Activity was seen at doses nearly 100-fold below the LD50 level, yet higher dose levels were not effective -- a not unusual observation for an immune modulating substance. Oral therapy was ineffective. We feel this material is of considerable interest as a potential PTV drug.

AVS1777 (Streptonigrin) (Table IX-48): In a single experiment run with this material, with treatment regimen determined after review of published data concerning this compound, no anti-PTV activity was seen.

AVS1778 (Mannozym) (Tables IX-49-53): Mannozym is an immune modulating material. It was initially evaluated vs PTV in parallel using two treatment regimens: s.c. once only 4 hr pre-PTV inoculation and s.c. bid x 5 beginning 4 hr pre-PTV inoculation. Both methods of treatment yielded positive results (Tables IX-49, 50). Using our expanded parameters to follow-up the bid x 5 treatment results, positive activity was again seen, but although treatment prevented death in the infected mice, liver and serum virus titers were not affected except at a single, relatively low dose level (Table IX-51). Oral therapy bid x 5 was not effective (Tables IX-52, 53). No activity was seen using AVS1778 against the Balliet PTV infection (Table VIII-6).

AVS2149 (Ampligen) (Tables IX-54-57): This compound was used by three treatment regimens against *in vivo* PTV infections: s.c. qd x 8 beginning 24 hr pre-virus inoculation, s.c. every other day x 8 beginning 24 hr pre-virus inoculation, and i.p. once only at varying times relative to virus inoculation (Tables IX-54-56). Marked activity was seen by every treatment schedule, and using our expanded disease parameters, all were significantly reduced except liver virus titers (Table IX-57). Oral therapy (Table IX-57A) yielded weakly positive results, with activity seen only as reductions in liver score, SGOT and SGPT. AVS2149 has been the most active compound used to date against the Balliet PTV infection (Table VIII-7). We have selected this compound to be run in parallel with ribavirin in a combination chemotherapy study described in Section X of this Report.

AVS2741 (1-[β -D-Ribofuranosyl]-1,2,4-triazole-3-[1,4,5,6-tetrahydropyrimidine]-HCl) (Table IX-58): A single test run with AVS2741 showed no anti-PTV activity. The compound was well tolerated at all doses used, however, so the experiment will be repeated using higher dosages.

AVS2742 (1-[β -D-Ribofuranosyl]-1,2,4-triazole-3-[5-hydrox-1,4,5,6-tetrahydropyrimidine]-HCl) (Table IX-59): A single test run with AVS2742 showed moderate anti-PTV activity at the highest dose used. This dose, 500 mg/kg/day, was well tolerated, so the experiment will be repeated using higher dosages.

AVS2776 (2-Amino-5-bromo-6-phenyl-4(3H)-pyrimidinone [ABPP]) (Tables IX-60-64): ABPP has been described as an immunomodulating agent. In initial tests, 3 different treatment schedules were used, qd x 3, once only, and every 3 days x 3, all i.p. beginning 24 hr pre-virus inoculation. The doses used were as recommended to USAMRIID by UpJohn, the producer of ABPP. The qd x 3 and once only treatments were most effective against the PTV infection. Activity was also seen using all expanded parameters when the once only treatment study was repeated (Table IX-63). A last study using single treatment revealed that the therapy could be given as late as 48 hr post-virus inoculation with positive effects seen (Table IX-64). We consider this ABPP to be among the best anti-PTV compounds to be evaluated to date.

AVS2777 (2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone [AIPP]) (Tables 65-68): AIPP was evaluated in parallel with ABPP (AVS2776) described above. The same 3 treatment schedules were used in initial evaluations. Positive activity was seen using all schedules, but the efficacy was less than that of ABPP. A last study run, using the expanded disease parameters, showed the single i.p. treatment given 24 hr pre-virus inoculation to have only a moderate anti-PTV effect, with no significant effects on serum or liver virus. At present, we consider AIPP to be inferior to ABPP as an anti-PTV compound.

AVS2778 (2-Amino-5-bromomethyl-4(3H)-pyrimidinone [ABMP]) (Tables IX-69-71): ABMP was used initially in parallel with ABPP and AIPP (AVS2776, 2777), described above. The same 3 treatment regimens were also used with this compound. Positive, but erratic (non-dose-responsive) activity was seen with each treatment schedule, a result often expected from an immune modulating substance. Further studies are planned for ABMP, but at this point the activity seen has been inferior to that of ABPP.

AV2880 (Oxamisole) (2,3,5,6,7,8-Hexahydro-2-phenyl-8,8-dimethoxyimidazo[1,2a]pyridine) (Tables IX-72-75): We have run extensive studies with oxamisole used against other viruses in work sponsored by Pennwalt Corporation, the producer of this material. We have found the material significantly effective against murine hepatitis (7) and influenza (8) virus infections of mice, and combinations of oxamisole and ribavirin were considered synergistically effective against influenza virus infections in mice. Our predominant observation in these earlier studies was that oxamisole, which is an immune modulator, exerted positive, but highly erratic activity, so that different dosage levels were active in different experiments, and initial activity often could not be exactly repeated. We also found activity to be highly dependent on treatment route and schedule. This material was evaluated in 4 experiments to date vs PTV, using 4 different treatment regimens, qd x 3 beginning 24 hr pre- or 24 hr post-virus inoculation (Tables IX-72, 73), once only 24 hr post-virus inoculation (Table IX-74), all administered i.p., and p.o. bid x 3 beginning 24 hr pre-virus inoculation (Table IX-75). All of these regimens were active in our earlier studies with the other viruses. Against PTV the qd x 3 treatment beginning 24 hr pre-virus inoculation was active at the lowest dosage level used (1.6 mg/kg/day) only; the same treatment begun 24 hr post-virus inoculation was not significantly effective. The once only treatment was efficacious at the 25 mg/kg dose only. Used orally, activity was seen primarily as significant

increases in mean survival time. Further experiments are planned for oxamisole, but at this point we feel this material is inferior to some of the other PTV-inhibitory compounds. It may prove efficacious in use as a combination therapy with some other compound, however.

AVS3585 (Neurotropin) (Tables IX-76-79): AVS3585 was used via 2 treatment schedules: every 3 days x 2, beginning 24 hr pre-virus inoculation, and once only 24 hr pre-virus inoculation. All treatments were i.p. No activity was seen in any experiment, although the material was well tolerated at every dose used. The doses and schedules used were as recommended by the manufacturer of the material to USAMRIID.

AVS3587 (2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone) (Tables IX-80, 81): This compound, another UpJohn material related to AVS2776 (ABPP), was evaluated in two experiments. Treatment regimens were i.p., qd x 3 beginning 24 hr pre-virus inoculation and once only 24 hr pre-virus inoculation. Using the latter schedule (Table IX-81), a single dose in the mid-range of those used was efficacious, an expected finding for an immune modulator. The qd x 3 treatment was not active (Table IX-80). At this point, we consider this material to be inferior to AVS2776 (ABPP) vs PTV.

AVS3588 (Meta fluoro derivative of ABPP) (Tables IX-82, 83): This is another UpJohn immunomodulating substance, a derivative of AVS2776 (ABPP). It was evaluated in parallel with AVS3587, using the same qd x 3 and once only treatment schedules. The material was quite active, essentially equivalent to ABPP, in both studies (Tables IX-82, 83), and more work is planned for it.

AVS3589 (5-Chloro-2,3-difluoro ABPP) (Tables IX-84, 85): This UpJohn immunomodulator derivative of AVS2776 (ABPP) was also run in parallel with AVS3587 and 3588 described above. The same qd x 3 and single shot treatment schedules were used. The qd x 3 treatment was efficacious (Table IX-84), but the activity was slightly weaker than ABPP. Further work will be done with this material.

AVS3925 (duPont A2222-1) (Table IX-86): A single experiment was run with this material using duPont's recommended regimen, but no PTV-inhibitory activity was seen. It had been recommended to us that the compound be initially prepared in 5 ml of 100% DMSO to which 0.01 ml Tween 80 had been added. We suspect this vehicle may have been toxic to the mice, since, as seen in Table IX-86, PTV-infected mice treated with that vehicle only all died, some after the first treatment, whereas virus control animals treated with the 0.25% methocel diluent used for the lower concentrations had a 70% death rate. Further studies are underway to investigate this possible vehicle toxicity.

AVS3926 (duPont A2227-1) (Table IX-87): This compound, like AVS3925, was inactive when used according to duPont recommendations. The same comments regarding toxicity described for AVS3925 also apply here.

AVS3927 (duPont A754-1) (Table IX-88): AVS3927 was run in parallel with AVS3925 and 3826, with the same negative and possibly toxic vehicle results.

AVS3934 (Ge132) (Table IX-89): This compound has been run in a single experiment to date using a treatment regimen described in literature sent by the (unidentified) manufacturer. No activity was seen.

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Table IX-1. Expt. PIA20, 21. Effect of Late Ribavirin (AVS01) Therapy on Punta Toro Virus Infections in Mice.
Animals: 10.5-11.8 g (4 wk) C57BL/6 Mice

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: Twice daily X 5, beginning 24 or 36 hr post-virus inoculation.

Treatment Route: s.c.

Experiment Duration: 21 days.

Time		Tox. Controls			Infected Treated					
		Dosage	Surv/	Host Wt.	Surv/	MST ^b	Mean	SGOT	SGPT	Mean Liver
Compound	Treatment Began	(mg/kg/day)	Total	Change ^a (g)	Total	(days)	Liver Score ^c	Neg/Total ^d	(Mean)	Virus Titer ^f
Ribavirin	24 hr post	150	1/5	-1.5	10/10*	>21.0**	1.7**	9/10*(259)**	10/10**(81)**	3.5
Ribavirin		75	5/5	2.0	10/10*	>21.0**	1.7**	10/10**(211)**	10/10**(46)**	3.5
Ribavirin		37.5	5/5	2.3	10/10*	>21.0**	2.3**	7/8(304)**	8/8**(211)**	3.5
Ribavirin	36 hr post	150	1/5	-1.5	10/10*	>21.0**	2.4**	9/9**(80)**	9/9**(44)**	3.0
Ribavirin		75	5/5	2.0	10/10*	>21.0**	1.5**	10/10**(102)**	10/10**(53)**	1.9**
Ribavirin		37.5	5/5	2.3	9/9**	>21.0**	2.8**	6/9(976)	6/9*(1015)	2.5
Saline		-	-	-	12/20	6.4	3.8	10/20(1736)	5/20(1301)	3.2
Normals		-	5/5	2.7	-	-	0.3	-	-	3.4

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: Ribavirin has previously been shown to be highly active against the Punta Toro virus by this same treatment schedule, with treatments beginning at 4 hr pre-virus inoculation. These experiments indicate this drug is similarly effective when treatment was not begun until 24 or 36 hr post-virus inoculation, although virus titers in the liver were reduced only in the single instance shown above.

*P<0.05 **P<0.01

Table IX-2. Expt. PTA43-45. Effect of Oral Ribavirin (AVS01) Therapy on Punta Toro Virus Infections in Mice.
 Animals: 10.0-10.8 g (4 wk) C57BL/6 Mice.
 Inoculation: Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile saline.

Treatment Route: p.o.
 Experiment Duration: 21 days.

Serum	Toxicity Controls				Infected/Treated								
	Time				Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	Neg/Total ^d (Mean)	SGPT (Mean)	Mean Liver	Virus Titer ^f (log ₁₀)	Virus Titer (log ₁₀)
Compound	4 hr pre	Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.8	5/5	10/10**	>21.0**	0.2**	10/10**(219)**	10/10**(49)**	2.8	1.4**
Ribavirin	4 hr post	Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.6	5/5	10/10**	>21.0**	0.3**	10/10**(149)**	10/10**(68)**	2.6	0.7**
H ₂ O	24 hr post	Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	10/10**	>21.0**	1.2**	8/10**(985)**	7/10*(847)**	0.7	2.4*
Normals		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.3	5/5	6/10	6.3	1.2**	2/10(6323)	1/10(5706)	0.4*	3.9
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	9/10**	7.0	2.7	2/10(4930)	1/10(4812)	0.3*	4.0
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.2	5/5	4/10	5.7	3.9	1/9(13428)	1/9(9270)	1.2	5.2
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.8	5/5	10/10**	>21.0**	0.4**	10/10**(289)**	10/10**(54)**	0.4*	0.0**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.6	5/5	10/10**	>21.0**	0.3**	9/9**(192)**	9/9**(61)**	0.3*	0.2**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	10/10**	>21.0**	1.2**	9/10**(1017)*	9/10**(798)**	0.0**	0.4**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.3	5/5	6/10	6.3	1.2**	2/8(5140)	1/8(4848)	0.8	1.7*
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	9/10**	7.0	2.7	2/8(5960)	1/8(4807)	0.8	3.3
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.2	5/5	4/10	5.7	3.9	0/9(6965)	0/9(5127)	1.8	4.2
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.8	5/5	10/10**	>21.0**	0.8**	8/8**(347)**	8/8(124)	0.0**	0.2**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.6	5/5	10/10**	>21.0**	3.0	9/9**(263)**	9/9(224)	0.0**	0.0**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	7/10*	6.0	0.8**	3/10(3053)	1/10(3301)	1.2	0.4**
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.3	5/5	5/10	6.6	3.1	3/9(5910)	2/9(5221)	1.2	3.4
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	2.8	5/5	7/10*	6.7	3.3	2/10(8160)	2/10(6588)	0.8	4.7
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	1.2	5/5	5/10	7.8	3.2	0/9(13109)	1/9(8496)	3.0	5.4
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	-	-	5/20	6.4	3.2	5/19(6954)	5/19(5642)	1.0	3.6
		Dosage (mg/kg/day)	Surv/Total	Surv/Total	3.4	5/5	-	-	0.0	5/5(236)	5/5(39)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Titers determined by production of viral cytopathogenic effect in LLC/MK₂ cells exposed to 10-fold dilutions of individual liver homogenates. Geometric means.

*P<0.05 **P<0.01

Conclusions: These experiments, PTA 43, 44, 45, indicate that orally administered ribavirin is highly effective against PTV infections even when therapy was delayed to 24 hr post-virus inoculation. The compound appeared well tolerated at all doses administered. One disturbing observation was rather markedly increased liver and serum virus titers at some dosage levels of ribavirin. We can offer no explanation for this apparent anomaly.

Table IX-3. Expts. PtA46-51. Effect of Single Treatments with AVS01 (Ribavirin) on Punta Toro Virus Infections in Mice.

Animals: 10.4-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, beginning 4, 8, 24, 48, 72, or 96 hr post-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected. Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS01	700	5/5	-0.4	10/10**	>21.0**
4 hr post	350	5/5	0.5	10/10**	>21.0**
	175	5/5	0.7	10/10**	>21.0**
AVS01	700	-	-	10/10**	>21.0**
8 hr post	350	-	-	10/10**	>21.0**
	175	-	-	10/10**	>21.0**
AVS01	700	-	-	10/10**	>21.0**
24 hr post	350	-	-	10/10**	>21.0**
	175	-	-	10/10**	>21.0**
AVS01	700	-	-	4/10	6.8**
48 hr post	350	-	-	10/10**	>21.0**
	175	-	-	8/10*	6.0
AVS01	700	-	-	1/10	6.6**
72 hr post	350	-	-	4/10	6.3**
	175	-	-	4/10	5.8
AVS01	700	-	-	2/10	5.1
96 hr post	350	-	-	2/10	5.9
	175	-	-	7/10	5.0
Saline	-	-	-	8/20	5.3
Normals	-	10/10	1.1	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: Ribavirin was highly effective against PTV infections when administered in a single s.c. injection as late as 48 hr post-virus inoculation. The highest dosage level used, 700 mg/kg, while not lethally toxic, caused some weight loss, indicating it was the approximate maximum tolerated dose.

Table IX-4. Expt. PtA106. Effect of AVS02 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.8-14.0 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS02	200	5/5	1.8	10/10**	>21.0**
	100	5/5	0.8	10/10**	>21.0**
	50	5/5	2.0	7/10**	8.0**
	25	5/5	1.0	2/10*	5.6
Ribavirin	75	5/5	1.7	10/10**	>21.0**
Saline	-	-	-	0/20	5.3
Normals	-	5/5	3.1	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS02 is the triacetate derivative of ribavirin. In this initial test, the material was considered highly active against PTV. This experiment has been confirmed (Expt. PtA 112), with significant activity seen, as evidenced by increased survivors and decreased liver scores, at dosages ranging from 15.6 mg/kg/day through 500 mg/kg/day. The latter dose was well tolerated in the mice.

Table IX-5. Expt. P1A112. Effect of AVS02 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).
 Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile saline.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: s.c.
 Experiment Duration: 21 days.

Toxicity controls				Infected/Treated						
Compound	Dosage (mg/kg/day)	Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
AVS02	500	5/5	2.5	10/10**	>21.0**	0.0**	10/10** (132**)	10/10** (28**)	1.8**	0.0**
	250	5/5	2.7	10/10**	>21.0**	0.0**	10/10** (100**)	10/10** (27**)	0.9**	0.5**
	125	5/5	2.6	10/10**	>21.0**	0.0**	10/10 (98**)	10/10** (49**)	0.7**	3.3**
	62.5	5/5	2.8	10/10**	>21.0**	0.6**	9/9** (69**)	9/9** (79**)	2.7**	4.8
	31.3	5/5	3.6	8/10	5.5	0.9**	6/9 (1566)	3/9 (1556)	3.2*	5.5
Ribavirin	15.6	5/5	3.5	7/10	4.0	2.4	1/10 (5109)	1/10 (5290)	3.7	5.7
	75	5/5	2.5	10/10**	>21.0**	0.0**	10/10** (306**)	10/10** (71**)	1.9**	2.1**
Saline	-	-	-	5/20	5.3	2.5	4/19 (10583)	4/19 (8354)	4.4	6.1
Normals	-	5/5	5.0	-	-	0.0	5/5 (106)	5/5 (41)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Livers dark purple color, they were scored on this color.

Conclusions: This is ribavirin triacetate, shown in PTA 106 to be active vs PTV. The activity was confirmed in the present study. Note the toxicity (>500 mg/kg/day; LD50 shown in separate toxicity study to be 850 mg/kg/day) is considerably less than that of ribavirin (~100 mg/kg/day). The therapeutic index is considered 16 or > based on this study.

*P<0.05 **P<0.01

Table IX-6. Expt. PtA113-117. Effect of Single Treatment with AVS02 Administered at Varying Times Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.2-14.3 g (3-4 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Sterile saline.

Treatment Schedule: Once only, varying times post-virus inoculation.

Treatment Route: s.c.

Experiment Duration: 21 days.

Compound	Treatment Initiation	Dosage (mg/kg)	Tox. Control		Infected, Treated	
			Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS02	4 hr post	1000	5/5	0.3	10/10**	>21.0**
		500	5/5	0.9	10/10**	>21.0**
		250	5/5	0.6	8/10**	6.0
		125	5/5	0.8	6/10	6.8
		62.5	5/5	0.4	9/10**	7.0
	24 hr post	1000	5/5	0.3	10/10**	>21.0**
		500	5/5	0.9	10/10**	>21.0**
		250	5/5	0.6	9/10**	11.0
		125	5/5	0.8	9/10**	9.0
		62.5	5/5	0.4	9/10**	7.0
	48 hr post	1000	5/5	0.3	9/10**	5.0
		500	5/5	0.9	10/10**	>21.0**
		250	5/5	0.6	10/10**	>21.0**
		125	5/5	0.8	10/10**	>21.0**
		62.5	5/5	0.4	7/10*	5.7
	72 hr post	1000	5/5	0.3	3/10	6.3
		500	5/5	0.9	2/10	5.5
		250	5/5	0.6	1/10	5.8
		125	5/5	0.8	4/10	6.3
		62.5	5/5	0.4	4/10	5.2
	96 hr post	1000	5/5	0.3	3/10	5.9
		500	5/5	0.9	2/10	6.4
		250	5/5	0.6	4/10	5.8
		125	5/5	0.8	5/10	7.0
		62.5	5/5	0.4	6/10	8.3
Ribavirin		75	5/5	2.5 ^c	10/10**	>21.0**
Saline		-	-	-	5/20	6.3
Normals		-	5/5	1.0	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cWeight 4 days later than the other doses.

*P<0.05

**P<0.01

Conclusions: AVS02, the triacetate derivative of ribavirin, has exhibited marked activity vs PTV in previous experiments (PtA 106, 112). This series of experiments was run to determine if a single treatment administered at varying times relative to virus inoculation would also be effective. The data indicate treatment as late as 48 hr post-virus inoculation was highly efficacious in preventing death of the PTV-infected animals. Treatments 72 or 96 hr post-virus inoculation were not effective. It should also be noted that the highest dosage used, 1000 mg/kg, was well tolerated in this study, indicating higher dosages could have been used.

Table IX-7. Expt. P1A134. Effect of Oral AVS02 Therapy on Punta Toro Virus Infections in Mice.
Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H₂O.
Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Compound	Toxicity controls				Infected Treated					
	Dosage	Surv/	Host Wt.	Surv/	MST ^b	Mean	SGOT	SGPT	Mean Liver	Mean Serum
	(mg/kg/day)	Total	Change ^a (g)	Total	(days)	Liver Score ^c	Neg/Total ^d (Mean)	(Mean)	Virus Titer ^f (log ₁₀)	Virus Titer ^f (log ₁₀)
AVS02	600	0/5	-1.6	0/10	8.9	0.5*	10/10** (138**)	10/10** (35**)	0.3**	0.0**
	300	5/5	1.7	10/10**	>21.0**	0.4**	10/10** (96**)	10/10** (30**)	0.0**	0.0**
	150	4/5	0.8	10/10**	>21.0**	0.3**	10/10** (69**)	10/10 (28**)	0.0**	0.0**
	75	5/5	1.9	10/10**	>21.0**	0.5*	10/10** (75**)	10/10** (29**)	0.0**	0.6**
	37.5	5/5	2.6	10/10**	>21.0**	0.4**	10/10** (127**)	10/10** (89**)	4.1**	5.1
	18.8	5/5	3.1	0/10	5.0	0.6	5/9 (1154)	5/9 (1082)	6.2	6.4
	9.4	5/5	2.7	1/10	4.3	2.0	2/10 (3472)	0/10 (5091)	5.4	5.9
Ribavirin	75	5/5	2.5	10/10**	>21.0**	0.3**	10/10** (142**)	10/10** (33**)	3.0**	2.5**
H ₂ O	-	-	-	0/20	4.3	1.1	11/20 (3226)	7/20 (3637)	5.8	4.9
Normals	-	5/5	3.8	-	-	0.3	5/5 (160)	5/5 (43)	3.4	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This experiment indicates that oral treatment with AVS02 (ribavirin triacetate) to be highly effective against PTV infections. A striking observation is the elimination of all detectable virus from livers and sera of mice treated with the 3 highest nontoxic dose levels. The p.o. therapeutic index is 8 in this study.

*P<0.05

**P<0.01

Table IX-8. Expt. PtA185. Effect of AVS02 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.5 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS02	1000	5/5	1.2	10/10**	>21.0**
	500	5/5	2.1	10/10**	>21.0**
	250	5/5	2.3	10/10**	>21.0**
	125	5/5	2.8	10/10**	>21.0**
	62.5	5/5	3.6	10/10**	>21.0**
	31.3	5/5	3.4	3/10	5.9
Ribavirin	75	5/5	2.7	10/10**	>21.0**
Saline	-	-	-	0/20	5.5
Normals	-	5/5	3.0	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This experiment was run to determine if treating once daily instead of twice daily with AVS02 (ribavirin triacetate) would affect the compound's anti-PTV efficacy. The data indicate the efficacy was identical using either treatment schedule, with a TI of 16 or > seen.

Table IX-9. Expts. PtA22-24. Effect of Single Treatments with AVS52 on Punta Toro Virus Infections in Mice.

Animals: 11-12 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, beginning 4, 12, or 24 hr post-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>	<u>Surv/ Total</u>	<u>MST^b (days)</u>
AVS052	1200	5/5	0.1	2/10	5.9
4 hr post	600	5/5	0.4	3/10	6.9
	300	5/5	0.4	6/10	6.5
AVS052	1200	-	-	1/10	5.7
12 hr post	600	-	-	4/10	7.3
	300	-	-	3/10	6.0
AVS052	1200	-	-	5/10	6.4
24 hr post	600	-	-	3/10	5.6
	300	-	-	2/10	5.9
Ribavirin ^c	700	5/5	0.1	10/10	>21.0**
Saline	-	-	-	15/20	5.4
Normals	-	10/10	0.5	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin administered in a single treatment 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS52 (thioformycin B) was apparently ineffective against PTV infections when administered in a single treatment. The compound was relatively well tolerated at the maximum dose (1200 mg/kg) used, but the lesser weight gain seen in the toxicity controls suggests that dose was approaching the maximum tolerated. In this experiment, the virus control mice did not die as expected, so even ribavirin was lacking in striking activity.

Table IX-10. Expt. PtA153. Effect of AVS52 on Punta Toro Virus Infections in Mice.

Animals: 10.7-12.3 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Three x daily x 5, 4 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Saline.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS52	500	5/5	0.2	0/10	6.8
	250	5/5	1.9	10/10**	>21.0**
	125	5/5	2.2	1/10	5.2
	62.5	5/5	2.7	1/10	4.2
Ribavirin	75	5/5	2.1	10/10**	>21.0**
Saline	-	-	-	8/20	5.4
Normals	-	5/5	3.9	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS52 (thioformycin B) has exhibited strong activity in vitro against PTV, but in earlier in vivo experiments no activity was seen. In the present study, the daily treatments were increased to 3 per day, and highly significant activity was seen at the 250 mg/kg/day dosage, which we note was the highest dosage that seemed well tolerated by the toxicity controls. These data suggest this compound may be relatively rapidly metabolized, requiring multiple treatments per day.

Table IX-11. Expt. PtA52. Effect of AVS65 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.8-14.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS65	250	5/5	4.0	1/10	5.2
	125	5/5	4.0	2/10	6.9
	62.5	5/5	3.8	0/10	6.4
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS65 (Formycin B), was not considered significantly active against PTV in this study. Ribavirin exerted the positive activity expected. This compound was well tolerated at all doses used, so the experiment will be repeated using higher dosage levels.

Table IX-12. Expt. PtA102. Effect of Oral AVS79 Therapy on Punta Toro Virus Infections in Mice.

Animals: 11.9-14.0 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile H₂O.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: p.o.
 Experiment Duration: 21 days.

Toxicity controls				Infected Treated				
Dosage	Surv/	Host Wt.		Mean	SGPT	Mean Liver	Mean Serum	
Compound (mg/kg/day)	Total	Change ^a (g)	Surv/	Liver Score ^c	Neg/Total ^d	Virus Titer ^f	Virus Titer ^f	
			Total	(days)	(Mean)	(log ₁₀)	(log ₁₀)	
AVS079	200	5/5	0/10	6.0	8/10*(535**)	8/10**(479**)	5.7	6.4
	100	5/5	0/10	5.1	1/10(9450)	1/10(7485)	6.1	6.5
	50	5/5	0/10	5.4	5/9(6174)	5/9(4165)	6.2	6.1
	25	5/5	0/10	5.3	2/10(9252)	2/10(8982)	5.7	6.5
Ribavirin	75	5/5	10/10**	>21.0**	10/10**(120**)	10/10**(89**)	2.7**	4.4**
H ₂ O	-	-	1/20	5.5	7/20(7301)	3/20(6627)	5.1	6.2
Normals	-	2.7	-	-	5/5(73)	5/5(28)	1.7	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Some livers were darker than normal, suggesting possible hemorrhage in them. Appeared somewhat dose-responsive, with most at 100, least at 25 mg/kg/day.

Conclusions: This compound is 9-β-D-ribofuranosylpurine-6-thiocarboxamide, which was shown previously (Expts. PtA 3, 12) to be active vs PTV using this treatment schedule with the compound administered s.c. Only weak activity was seen in the present study, however, when it was administered orally.

*P<0.05 **P<0.01

Table IX-13. Expt. PtA107-109. Effect of Varying Treatment Initiation with AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.7-14.0 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, beginning varying times post-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Treatment Initiation</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
			<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>	<u>Surv/ Total</u>	<u>MST^b (days)</u>
AVS79	24 hr post	150	5/5	0.0	0/10	6.9**
		75	5/5	1.1	0/10	6.2**
		37.5	5/5	1.2	0/10	6.1*
		18.8	5/5	1.9	1/10	6.1*
	36 hr post	150	5/5	0.0	0/10	7.9**
		75	5/5	1.1	0/10	6.3**
		37.5	5/5	1.2	0/10	6.7**
		18.8	5/5	1.9	1/10	5.6
	48 hr post	150	5/5	0.0	3/10	5.9
		75	5/5	1.1	1/10	5.2
		37.5	5/5	1.2	1/10	5.3
		18.8	5/5	1.9	2/10	4.4
Ribavirin		75	5/5	1.7	10/10**	>21.0**
Saline		-	-	-	0/20	5.3
Normals		-	5/5	3.1	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS79, 9-β-D-ribofuranosyl-purine-6-thiocarboxamide, was shown previously (Expts. PtA 3, 12) to be active vs PTV when administered bid x 5 beginning 4 hr pre-virus inoculation. The present experiments were run to determine if treatment initiation could be delayed with efficacy still seen. The material was much less active when administered on these therapeutic treatment schedules.

Table IX-14. Expt. PtA133. Effect of AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.4 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS79	200	4/5	-0.2	2/10	8.5
	100	5/5	2.3	4/10	7.8
	50	5/5	3.9	7/10*	7.7
	25	5/5	2.3	6/10	7.5
Ribavirin	75	5/5	2.7	10/10**	>21.0**
Saline	-	-	-	5/20	7.7
Normals	-	5/5	3.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS79 (9-β-D-ribofuranosylpurine-6-thiocarboxamide) has been found effective vs PTV when administered on a twice daily treatment schedule. Treatment by this once daily treatment schedule yielded a moderate survival increase at a single dosage level only.

Table IX-15. Expt. PtA154-158. Effect of Time of Single Treatment of AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.0-12.2 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Saline.

Treatment Schedule: Once only, varying times relative to virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

Compound	Time of Treatment ^a	Dosage (mg/kg)	Tox. Control		Infected, Treated	
			Surv/ Total	Host Wt. Change (g) ^b	Surv/ Total	MST ^c (days)
AVS79	4 hr post	700	5/5	0.1	0/10	5.4
		350	5/5	0.5	1/10	5.6**
		175	5/5	0.9	1/10	4.9
		87.5	5/5	0.3	0/9	4.7
	24 hr post	700	5/5	0.1	0/10	6.0**
		350	5/5	0.5	0/8	5.8**
		175	5/5	0.9	0/10	5.7**
		87.5	5/5	0.3	0/6	5.0
	48 hr post	700	5/5	0.1	3/10*	4.7
		350	5/5	0.5	6/10**	6.3
		175	5/5	0.9	8/10**	6.5
		87.5	5/5	0.3	9/10**	7.0
	72 hr post	700	5/5	0.1	0/10	4.4
		350	5/5	0.5	1/10	4.7
		175	5/5	0.9	2/10	4.8
		87.5	5/5	0.3	1/10	4.4
	96 hr post	700	5/5	0.1	0/10	4.3
		350	5/5	0.5	0/10	4.4
		175	5/5	0.9	0/10	4.2
		87.5	5/5	0.3	0/10	4.1
Ribavirin	4 hr post	700	5/5	0.3	9/10**	3.0
Saline	4 hr post	-	-	-	0/20	4.5
Normals	-	-	5/5	0.4	-	-

^aRelative to virus inoculation.

^bDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^cMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS79 (1-β-D-ribofuranosylpurine-6-thiocarboxamide) in previous studies has been active vs PTV when administered s.c., bid x 5. The present series of experiments (PtA 154-158) was run to determine if a single s.c. treatment given at varying times relative to virus inoculation would be effective against the infection. Surprisingly, the maximal effect was seen when the compound was administered 48 hr post-virus inoculation.

Table IX-16. Expt. PtA187. Effect of AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS79	200	0/5	-2.7	1/10	6.7**
	100	5/5	0.9	8/10	7.5
	50	5/5	1.9	7/10	6.7
	25	5/5	2.6	6/10	6.5
	12.5	5/5	2.7	2/10	5.6
	6.25	5/5	2.2	5/10	4.8
Ribavirin	75	5/5	2.7	10/10**	>21.0**
Saline	-	-	-	10/20	5.0
Normals	-	5/5	3.0	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS079 (1-β-D-ribofuranosylpurine-6-thiocarboxamide) was less active in this experiment where the compound was administered i.p., than in previous experiments where a s.c. treatment route was used.

Table IX-17. Expt. PtA188. Effect of AVS79 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Three times daily x 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS79	200	0/5	-2.2	0/10	6.5**
	100	5/5	1.1	1/10	6.7**
	50	5/5	3.3	7/10**	7.7**
	25	5/5	2.0	6/10**	6.3**
	12.5	5/5	2.6	0/10	5.3**
	6.25	5/5	2.5	2/10*	5.3**
Ribavirin	75	5/5	2.1	10/10**	>21.0**
Saline	-	-	-	0/20	3.7
Normals	-	5/5	3.0	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS079 (1-β-D-ribofuranosylpurine-6-thiocarboxamide) was less active vs PTV administered i.p. three times daily than given s.c. twice daily.

Table IX-18. Expt. PtA53. Effect of AVS111 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.6-12.8 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS111	250	5/5	3.5	10/10**	>21.0**
	125	5/5	3.7	6/10	8.8
	62.5	5/5	4.4	7/10*	7.7
	31.3	5/5	4.1	6/10	6.5
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS111, (thiazofurin), was considered active against PTV in this initial experiment, and will be retested to confirm the activity seen.

Table IX-19. Expt. PIA68. Effect of AVS111 Therapy on Punta Toro Virus Infections in Mice.

Animals: 10 5 11 8 g (4 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile saline.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: s.c.
 Experiment Duration: 21 days.

Toxicity controls				Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/	Host Wt.	MST ^b (days)	Mean Liver Score ^c	SGOT		SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀ l)	Mean Serum Virus Titer ^f (log ₁₀ l)
		Total	Change ^a (g)			Neg/Total ^d (Mean)				
AVS0111	500	5/5	2.4	>21.0**	0.4**	10/10** (140**)		10/10** (38**)	3.9	4.1**
	250	5/5	3.3	>21.0**	0.6**	9/9** (136**)		9/9** (50**)	3.9	5.0*
	125	5/5	3.4	7.0**	0.9**	6/10** (1276**)		6/10** (1287**)	3.7	6.0
	62.5	5/5	3.8	6.9**	0.7**	3/10* (3330**)		2/10 (2399**)	4.5	6.2
Ribavirin	31.3	5/5	3.6	5.6	1.5**	3/10* (4095*)		2/10 (3840*)	4.0	6.1
	75	5/5	2.3	>21.0**	0.5**	9/9** (95**)		9/9** (38**)	3.6	2.4**
Saline	-	-	-	5.3	5.3	0/18 (7874)		0/18 (7681)	3.8	6.0
Normals	-	5/5	3.1	-	0.1	5/5 (136)		5/5 (29)	<2.5	<2.5

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This compound, tiazofurin, was previously shown in Expt. PTA 53 to have significant activity vs PTV. This experiment confirms those earlier findings. The compound was well tolerated at the highest dosage used, and significant activity was seen using some parameters at dosages as low as 31.3 mg/kg/day, indicating a therapeutic index of at least 16, or equivalent to ribavirin. Further experiments will be run to more fully determine the therapeutic index and most acceptable treatment regimen.

*P<0.05

**P<0.01

Table IX-20. Expt. PtA110. Effect of AVS111 on Punta Toro Virus Infections in Mice (Therapeutic Index Determination).

Animals: 11.2-14.3 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS111	2000	5/5	-0.6	-	-
	1000	5/5	0.6	10/10**	>21.0**
	500	5/5	1.4	10/10**	>21.0**
	250	5/5	2.7	8/10**	6.5*
	125	5/5	1.8	3/10	6.7**
	62.5	5/5	3.1	0/10	5.7
	31.3	5/5	2.8	0/10	5.3
	5.7	5/5	2.3	0/10	5.5
Ribavirin	75	5/5	1.7	10/10**	>21.0**
Saline	-	-	-	0/20	5.3
Normals	-	5/5	3.1	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS111 (tiazofurin) was found previously to be active vs PTV (Expts. PtA 53, 68). The present study was run to determine the therapeutic index (TI) of the material. Considering 2000 mg/kg/day to be the MTD, the minimum effective dose was 125 to 250 mg/kg/day, for a TI of 8 to 16.

Table IX-21. Expt. PtA135-139. Effect of Time of Single Treatment of AVS111 on Punta Toro Virus Infections in Mice.

Animals: 11.0-12.3 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, varying times relative to virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Treatment Time^a</u>	<u>Dosage (mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
			<u>Surv/ Total</u>	<u>Host Wt. Change (g)^b</u>	<u>Surv/ Total</u>	<u>MST^c (days)</u>
AVS111	4 hr post	1000	5/5	-0.2	4/10**	5.2
		500	5/5	-0.1	4/10**	6.5
		250	5/5	-0.1	2/10*	4.5
		125	5/5	0.3	0/10	4.5
	24 hr post	1000	5/5	-0.2	4/10**	5.8
		500	5/5	-0.1	0/10	5.8
		250	5/5	-0.1	1/10	4.7
		125	5/5	0.3	0/10	5.3
	48 hr post	1000	5/5	-0.2	9/10**	3.0
		500	5/5	-0.1	10/10**	>21.0**
		250	5/5	-0.1	8/10**	5.5*
		125	5/5	0.3	0/10	5.1
	72 hr post	1000	5/5	-0.2	0/10	4.8
		500	5/5	-0.1	1/10	4.4
		250	5/5	-0.1	0/10	4.4
		125	5/5	0.3	0/10	4.7
	96 hr post	1000	5/5	-0.2	1/10	5.6**
		500	5/5	-0.1	2/10	4.6
		250	5/5	-0.1	1/10	4.4
		125	5/5	0.3	0/10	4.3
Ribavirin	4 hr post	700	5/5	0.1	10/10**	>21.0**
Saline	4 hr post	-	-	-	0/20	4.3
Normals	-	-	5/5	3.7	-	-

^aRelative to virus inoculation.

^bDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^cMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: Single treatment with AVS111 (tiazofurin) 48 hr post-virus inoculation had the greatest effect in preventing death of PTV infected mice.

Table IX-22. Expts. PtA34-36. Effect of Single Treatments with AVS147 on Punta Toro Virus Infections in Mice.

Animals: 10.4-11.5 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, beginning 4, 12, or 24 hr post-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS147	1000	5/5	0.2	10/10**	>21.0**
4 hr post	500	5/5	0.6	7/10	6.3
	250	5/5	0.7	8/10	5.5
AVS147	1000	-	-	0/10	6.8
12 hr post	500	-	-	5/10	7.8
	250	-	-	6/10	5.3
AVS147	1000	-	-	3/10	6.1
24 hr post	500	-	-	4/10	6.5
	250	-	-	3/10	6.3
Ribavirin ^c	700	5/5	0.1	10/10**	>21.0**
Saline	-	-	-	10/20	5.4
Normals	-	5/5	0.4	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin administered s.c. bid x 5 beginning 4 hr pre-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS147 (Enviroxime), when administered in a single treatment, was effective at the approximate maximum tolerated dose against the PTV infection when administered 4 hr post-virus inoculation; later treatments were not effective. This compound was not active against the virus *in vitro*. It was run against this virus *in vivo* because it had a moderate activity against Venezuelan equine encephalitis and Rift Valley fever infections in mice.

Table IX-23. Expt. PtA96. Effect of Single Daily Treatments with AVS147 on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.8 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, Treatment Route: s.c.
 s.c. injected.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS147	500	3/3	2.7	3/10	6.6
	250	3/3	2.1	3/10	7.0
	125	5/5	1.9	7/10	7.7
	62.5	5/5	2.0	5/10	7.4
Ribavirin	75	5/5	0.3	10/10	>21.0**
Saline	-	-	-	18/20	7.5
Normals	-	5/5	2.2	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS147 (enviroxime) was inactive in the initial test run with it (Expt. PtA 15). The present experiment utilized a once daily treatment schedule instead of the initial twice daily schedule. Once again, it was inactive. It should be pointed out that the compound was quite insoluble and a moderate degree of gas formed when it was mixed with saline.

Table IX-24. Expt. PtA54. Effect of AVS167 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.8-13.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS167	75	5/5	4.4	5/10	4.2
	37.5	5/5	4.6	5/10	6.0
	18.8	5/5	4.7	1/10	5.6
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS167, designated as glycyrrhetic acid, was not considered significantly active against PTV in this study. The compound did not exhibit any toxicity at the doses used, however, so will be retested at higher dosage levels.

Table IX-25. Expt. PtA87. Effect of AVS167 on Punta Toro Virus Infections in Mice (repeat of initial test using higher doses).

Animals: 11.8-13.6 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile Saline. Experiment Duration: 18 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS167	500	5/5	3.3	5/10	8.0**
	250	5/5	2.9	5/10	6.2
	125	5/5	3.2	6/10	7.5
	62.5	5/5	3.9	4/10	5.5
Ribavirin	75	5/5	2.0	10/10**	>21.0**
Normals	-	5/5	4.6	-	-
Saline	-	-	-	11/20	5.6

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This experiment repeats Expt. PTA 54, with higher dosages of AVS167 used in an attempt to reach a maximum tolerated dose (MTD). The material was again considered inactive, although the MTD was apparently not yet reached.

Table IX-26. Expt. PtA71. Effect of AVS206 on Punta Toro Virus Infections in Mice (Therapeutic Index Experiment).

Animals: 11.5-12.9 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/	Host Wt.	Surv/	MST ^b
		Total	Change (g) ^a	Total	(days)
AVS206	1000	5/5	1.9	-	-
	250	5/5	3.0	10/10**	>21.0**
	125	5/5	3.2	10/10**	>21.0**
	62.5	5/5	3.5	10/10**	>21.0**
	31.3	5/5	3.2	8/10**	8.5**
	15.7	5/5	3.6	4/10	6.8
	7.8	5/5	4.1	5/10	6.6
	3.9	5/5	4.1	1/10	6.2
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Normals	-	5/5	4.8	-	-
Saline	-	-	-	5/20	6.1

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS 206 (carboxamidine derivative of ribavirin) was active down to a dosage of 31.3 mg/kg/day in this therapeutic index determination study. The compound was tolerated up to 1000 mg/kg/day, but the toxicity controls at that level gained much less weight than those in the lower dosages, implying we were approaching the maximum tolerated dose at 1000 mg/kg/day. The therapeutic index was determined to be 32 or greater, or approximately twice as much as ribavirin.

Table IX-27. Expt. P1A78-81. Effect of AVS206 Therapy Beginning at Varying Time Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.1-13.6 g (4 wk) C57BL/6 Mice.

Treatment Schedule: Twice daily X 5, beginning 24, 36, 48, or 72 hr post-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Route: s.c.
Experiment Duration: 21 days.

Time Treatment Began	Toxicity controls		Infected Treated								
	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)	
AVS206	24 hr post	500	5/5	2.5	10/10**	>21.0**	0.8	10/10** (149**)	10/10** (31**)	2.3	0.0**
		250	5/5	3.5	10/10**	>21.0**	0.6**	10/10** (134**)	10/10** (37**)	1.0**	1.1**
		125	5/5	3.6	10/10**	>21.0**	0.5**	10/10** (238**)	9/10 (259**)	0.0**	3.5**
		62.5	5/5	3.6	10/10**	>21.0**	0.7	9/10 (493**)	7/10 (391**)	1.0**	4.9
	36 hr post	500	5/5	2.5	10/10**	>21.0**	0.8	10/10** (145**)	10/10** (45**)	3.6	0.4**
		250	5/5	3.5	10/10**	>21.0**	0.7	10/10** (181**)	10/10** (75**)	3.0	2.4**
		125	5/5	3.6	10/10**	>21.0**	0.6**	8/10 (693)	8/10 (402*)	0.9**	4.8
		62.5	5/5	3.6	10/10**	>21.0**	1.3	4/9 (2026)	3/9 (1643)	2.8	5.2
	48 hr post	500	5/5	2.5	10/10**	>21.0**	0.5**	10/10** (150**)	10/10** (48**)	0.3**	0.5**
		250	5/5	3.5	10/10**	>21.0**	0.4**	10/10** (197**)	10/10** (87**)	6.3**	3.4**
		125	5/5	3.6	10/10**	>21.0**	0.2**	10/10** (306**)	9/10 (198**)	0.0**	5.3
		62.5	5/5	3.6	5/10	6.6**	0.8	4/10 (3129)	2/10 (2857)	2.9	6.0
	72 hr post	500	5/5	2.4	10/10**	>21.0**	2.2	6/10 (2568)	6/10 (2093)	1.3**	4.5**
		250	5/5	2.8	10/10**	>21.0**	1.1**	9/10 (392**)	9/10 (275**)	1.8**	4.9**
		125	5/5	3.3	10/10**	>21.0**	1.8	5/10 (2641)	5/5 (2695)	0.5**	5.4*
Ribavirin	75	5/5	2.4	10/10**	>21.0**	nr ^g	nr	nr	nr	nr	nr
Saline	-	-	-	8/20	4.9	1.8	12/19 (1189)	9/19 (1952)	3.1	5.6	
Normals	-	5/5	3.9	-	-	0.3	5/5 (107)	5/5 (25)	0.0	0.0	

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Not run

Conclusions: In this series of experiments (PTA 78-81), AVS206 (ribavirin carboxanidine) was studied to determine its effects when administered at varying times after virus inoculation. The compound was still highly active when s.c. treatment began as late as 72 hr post-virus inoculation, as seen by all parameters.

*P<0.05

**P<0.01

Table IX-28. Expt. PtA92. Effect of Oral Treatment with AVS206 on Punta Toro Virus Infections in Mice.

Animals: 14.5-17.8 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: p.o.
 Drug Diluent: H₂O. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS206	1000	5/5	0.6	10/10**	>21.0**
	500	5/5	1.1	10/10**	>21.0**
	250	5/5	0.5	10/10**	>21.0**
	125	5/5	0.6	10/10**	>21.0**
	62.5	5/5	1.4	9/10*	7.0**
	31.3	5/5	2.2	8/10	7.5**
	15.7	5/5	1.7	5/10	6.0
	7.8	5/5	1.9	4/10	6.3
Ribavirin	75	5/5	1.8	9/10*	17.0**
H ₂ O	-	-	-	10/19	6.4
Normals	-	5/5	3.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This experiment was run to determine the oral efficacy of AVS206 (ribavirin carboxamidine) on PTV; it also was run to determine the approximate therapeutic index of the material when used orally. The compound was considered markedly active, with a therapeutic index of approximately 32, assuming 2000 mg/kg/day is the MTD. We could not use the latter dose in this study because of a shortage of the compound.

Table IX-29. Expt. PtA169-173. Effect of Time of Single Treatment of AVS206 on Punta Toro Virus Infections in Mice.

Animals: 11.0-12.2 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Saline.

Treatment Schedule: Once only, varying times of post-virus inoculation.

Treatment Route: s.c.

Experiment Duration: 21 days.

Compound	Time of Treatment ^a	Dosage (mg/kg)	Tox. Control		Infected, Treated	
			Surv/ Total	Host Wt. Change (g) ^b	Surv/ Total	MST ^c (days)
AVS206	4 hr post	1000	5/5	0.3	8/10**	9.0
		500	5/5	0.3	10/10**	>21.0**
		250	5/5	0.4	7/10**	5.7
		125	5/5	1.1	1/10	5.6
		62.5	5/5	0.5	3/10	6.1**
		31.3	5/5	0.8	2/10	5.4
		15.7	5/5	0.6	1/10	4.6
	24 hr post	1000	5/5	0.3	10/10**	>21.0**
		500	5/5	0.3	10/10**	>21.0**
		250	5/5	0.4	5/10	6.6
		125	5/5	1.1	3/10	6.4
		62.5	5/5	0.5	0/10	5.6
		31.3	5/5	0.8	0/10	4.3
		15.7	5/5	0.6	0/10	4.2
	48 hr post	1000	5/5	0.3	10/10**	>21.0**
		500	5/5	0.3	8/10**	8.0
		250	5/5	0.4	9/10**	4.0
		125	5/5	1.1	6/10	5.8
		62.5	5/5	0.5	4/10	4.3
		31.3	5/5	0.8	1/10	4.6
		15.7	5/5	0.6	0/10	4.5
	72 hr post	1000	5/5	0.3	2/10	4.3
		500	5/5	0.3	2/10	4.3
		250	5/5	0.4	0/10	4.6
		125	5/5	1.1	1/10	4.6
		62.5	5/5	0.5	0/10	4.1
		31.3	5/5	0.8	1/10	4.2
		15.7	5/5	0.6	0/10	4.0
	96 hr post	1000	5/5	0.3	0/10	4.1
		500	5/5	0.3	0/10	4.0
		250	5/5	0.4	0/10	4.2
		125	5/5	1.1	0/10	4.0
		62.5	5/5	0.5	3/11	5.5
		31.3	5/5	0.8	1/10	4.2
		15.7	5/5	0.6	0/10	4.2
Ribavirin	4 hr post	350	5/5	0.2	4/10*	7.2**
Saline	4 hr post	-	-	-	0/10	4.2
Normals	-	-	5/5	0.7	-	-

^aRelative to virus inoculation.

^bDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^cMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS206, ribavirin carboxamide, was run in a series of experiments (PtA 169-173) to determine how late in the PTV infection a single treatment would still be efficacious. It appears the treatment could be given as late as 48 hr post-virus inoculation. The compound was well tolerated at 1000 mg/kg, indicating the single treatment therapeutic index may be considerably higher than seen here.

Table IX-30. Expts. PtA37-39. Effect of Single Treatments with AVS212 on Punta Toro Virus Infections in Mice.

Animals: 10.8-11.8 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, beginning 4, 12, or 24 hr post-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS212	1000	5/5	0.4	0/10	4.7
4 hr post	500	5/5	0.8	2/10	5.3
	250	5/5	0.9	6/10	6.8
AVS212	1000	-	-	0/10	4.8
12 hr post	500	-	-	1/10	5.3
	250	-	-	7/10	6.0
AVS212	1000	-	-	0/10	5.1
24 hr post	500	-	-	3/10	5.9
	250	-	-	5/10	7.0
Ribavirin ^c	700	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	10/20	5.4
Normals	-	5/5	0.4	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin administered s.c. bid x 5 beginning 4 hr pre-virus inoculation.

*P<0.05

**P<0.01

Conclusions: Single s.c. treatments with AVS212 (Suramin) were not effective against PTV infections, regardless of time of administration relative to virus inoculation. This compound was mildly active against this virus *in vitro*, but also failed to exert a positive antiviral effect when administered twice daily for 5 days beginning 4 hr pre-virus inoculation.

Table IX-31. Expt. PtA103. Effect of Oral AVS212 Therapy on Punta Toro Virus Infections in Mice.

Animals: 12.5-14.1 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile H₂O.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: p.o.
 Experiment Duration: 21 days.

Compound	Toxicity controls			Infected/Treated					Mean Serum Virus Titer ^f (log ₁₀)
	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)
AVS212	200	5/5	1.5	0/10	5.6	2.9	3/10(5418)	1/10(6241)	4.3
	100	5/5	3.6	1/10	5.8	3.0	1/10(7731)	1/10(8654)	4.7
	50	5/5	3.3	0/10	5.2	2.8	4/10(3621)	3/10(3432)	5.6
	25	5/5	2.1	0/10	5.2	2.5	3/10(7370)	3/10(6138)	5.4
Ribavirin	75	5/5	1.5	10/10**	>21.0**	2.0	10/10**(120**)	10/10**(89**)	2.7**
H ₂ O	-	-	-	1/20	5.5	2.9	7/20(7301)	3/20(6627)	5.1
Normals	-	5/5	2.7	-	-	0.0	5/5(73)	5/5(28)	1.7

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This compound, suramin, had weak activity against PTV in previous experiments where the compound was administered s.c. The present study investigated the effects of this material administered orally against PTV infections. No activity was seen.

*P<0.05

**P<0.01

Table IX-32. Expt. PtA159. Effect of AVS212 on Punta Toro Virus Infections in Mice.

Animals: 10.7-12.3 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Three x daily x 5, 4 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Saline.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS212	150	5/5	0.3	0/10	3.9
	75	5/5	2.0	0/10	4.5
	37.5	5/5	2.4	0/10	4.3
	18.8	5/5	2.8	0/10	4.3
Ribavirin	75	5/5	2.1	10/10**	>21.0**
Saline	-	-	-	8/20	5.4
Normals	-	5/5	3.9	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS212 (suramin) was inactive vs PTV when given once only or bid x 5. The present study indicates the material is also ineffective when given three times daily. The relatively low host weight change at 150 mpk suggests the MTD was essentially reached in this experiment.

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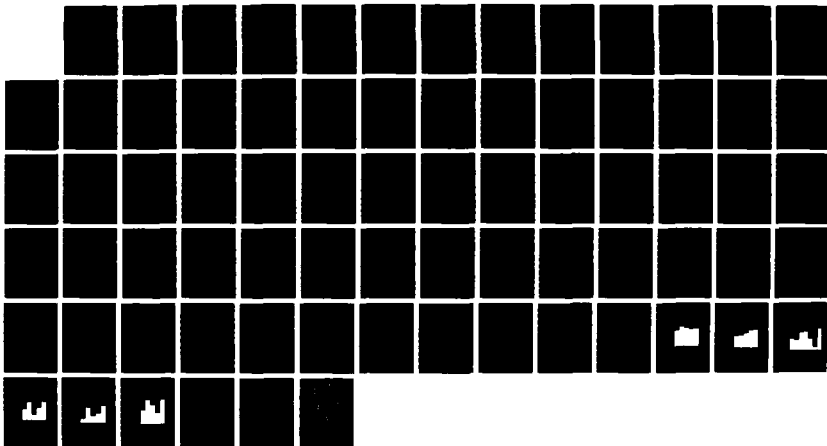
DETERMINATION OF THE IN VITRO AND IN VIVO ACTIVITY OF
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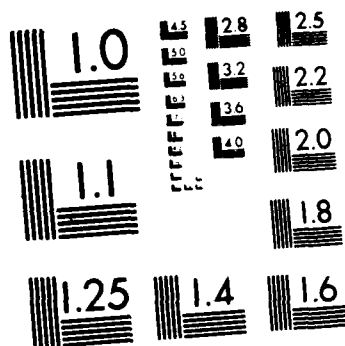
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Table IX-33. Expt. PtA55. Effect of AVS222 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.2-13.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS222	250	5/5	4.3	1/10	6.3
	125	5/5	4.1	1/10	6.2
	62.5	5/5	5.0	4/10	6.2
	31.3	5/5	4.4	5/10	6.2
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS222, (3-bromo-4-chloropyrazolo-[3,4-d]-pyrimidine), was not considered significantly active against PTV in this study.

Table IX-34. Expt. PtA88. Effect of AVS222 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).

Animals: 10.9-12.2 g (4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: Twice daily, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 20 days.

Toxicity controls				Infected Treated					
Dosage	Surv/	Host Wt.	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)	
Compound	(mg/kg/day)	Total							
AVS222	250	5/5	4.1	2.3*	3/10(3368**)	3/10(4225**)	2.5	5.8	
	125	5/5	3.6	2.8	1/10(6542)	1/10(6492)	3.7	6.8	
	62.5	5/5	4.4	2.5	2/10(3582**)	2/10(3667**)	2.9	5.8	
	31.3	5/5	3.8	2.5	1/10(5911*)	1/10(5672*)	2.6	6.7	
Ribavirin	75	5/5	2.0	0.4**	9/10**(447**)	10/10**(76**)	0.0**	1.6**	
Saline	-	-	-	3.4	2/20(9747)	2/20(8662)	2.7	6.3	
Normals	-	5/5	4.6	0.5	5/5(393)	5/5(71)	0.0	0.0	

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cScores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^dSerum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^eSerum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^fGeometric mean.

Conclusions: This experiment was run to further determine the effects of AVS222 (3-bromo-4-chloropyrazolo-[3,4-d]pyrimidine) on PTV in mice. Initially (Expt. PTA 55) the compound was not considered active using survivors only as parameter for evaluation. In this experiment, the expanded parameters were used, and moderate activity was seen, primarily at the highest dosage used (250 mg/kg/day) and only as moderate decreased liver scores, SGPT and SGOT. We will run an LD50 determination to be sure sufficiently high dosages were used before discontinuing studies with this compound.

*P<0.05 **P<0.01

Table IX-35. Expts. PtA40-41. Effect of Single Treatments with AVS233 on Punta Toro Virus Infections in Mice.

Animals: 11.0-11.6 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, beginning 12 or 24 hr post-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Sterile saline.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS233	1800	5/5	0.1	8/10	5.5
12 hr post	900	4/5	-0.1	6/10	6.0
	450	5/5	0.6	8/10	7.0**
AVS233	1800	-	-	0/10	6.4
24 hr post	900	-	-	3/10	5.3
	450	-	-	2/10	7.3**
Ribavirin ^c	700	5/5	0.1	10/10**	>21.0**
Saline	-	-	-	10/20	5.4
Normals	-	5/5	0.4	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin administered s.c. bid x 5 beginning 4 hr pre-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS233 (Formycin), when administered in single treatment, was slightly effective against PTV infections if the compound was administered as early as 12 hr post-virus inoculation. Later treatment, administered 24 hr post-virus inoculation, was much less effective, although mean survival time was extended. The compound was not well tolerated in these animals at the 1800 and 900 mg/kg dosages, but the 450 mg/kg dose level appeared to not adversely affect the animals. Insufficient compound was available to evaluate this compound at other time periods. This compound was considered moderately effective against this virus *in vitro*, but was not active when administered twice daily for 5 days beginning 4 hr pre-virus inoculation (Expt. PTA17). The present data suggest that AVS233 may be metabolized to an inactive form at a relatively high rate, preventing it from attaining antiviral levels in the blood when administered in lower doses.

Table IX-36. Expt. PtA97. Effect of Single Daily Treatments with AVS253 on Punta Toro Virus Infections in Mice.

Animals: 11.4-13.4 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS253	320	5/5	-0.5	10/10	>21.0**
	160	5/5	1.0	10/10	>21.0**
	80	5/5	2.1	9/10	7.0
	40	5/5	2.0	10/10	>21.0**
Ribavirin	75	5/5	0.3	10/10	>21.0**
Saline	-	-	-	18/20	7.5
Normals	-	5/5	2.2	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This compound is selenazofurin, a ribavirin derivative. Previous experiments with it (Expt. PtA 5, 14) indicated it to be moderately active vs PTV in mice. The present study was run to determine if once daily treatments would enhance its activity. The virus used, however, appeared to be defective, since only 10% of the virus control mice died, thus compromising the experiment. This experiment will be repeated.

Table IX-37. Expt. PtA104. Effect of Oral AVS253 Therapy on Punta Toro Virus Infections in Mice.

Animals: 12.0-14.7 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile H₂O.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: p.o.
 Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Toxicity controls		Infected, Treated					Mean Serum Virus Titer ^f	
		Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
AVS253	320	5/5	-1.7	8/10**	9.5**	0.0**	10/10** (129**)	10/10** (48**)	0.8**	3.2**
	160	5/5	2.5	7/10**	7.7**	0.1**	10/10** (151**)	10/10** (50**)	2.6**	4.6*
	80	5/5	2.7	4/10*	6.3**	1.1**	10/10** (119**)	10/10** (51**)	4.4	5.3
	40	5/5	2.5	0/10	6.1	1.6**	2/10 (3412)	3/10 (2693)	5.2	6.3
Ribavirin	75	5/5	1.5	10/10**	>21.0**	2.0*	10/10** (120**)	10/10** (89**)	2.7**	4.4**
H ₂ O	-	-	-	1/20	5.5	2.9	7/20 (7301)	3/20 (6627)	5.1	6.2
Normals	-	5/5	2.7	-	-	0.0	5/5 (73)	5/5 (28)	1.7	0.0

98

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: AVS253 (selenazofurin) has proven moderately active against PTV in previous experiments (Expts. PTA 5, 14). The present study indicates this compound is also active against PTV when administered orally, although the activity was considered less than that exerted by ribavirin.

*P<0.05 **P<0.01

Table IX-38. Expt. PtA186. Effect of AVS272 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.5 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/	Host Wt.	Surv/	MST ^b
		Total	Change (g) ^a	Total	(days)
AVS272	200	5/5	-0.5	0/10	5.8
	100	5/5	-0.3	3/10	5.1
	50	5/5	1.0	5/10 ^a	5.2
	25	5/5	2.3	3/10	4.6
Ribavirin	75	5/5	2.7	10/10 ^{**}	>21.0 ^{**}
Saline or CMC	-	-	-	10/20	5.0
Normals	-	5/5	3.0	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS272 (3-Deazaguanine) was not considered active vs PTV when used by this treatment schedule. Other treatment regimens will be considered, although the compound was only slightly active against PTV *in vitro*. We have found this compound to have significant antiviral activity against a variety of other RNA viruses.

Table IX-39. Expt. PtA42. Effect of AVS360 on Punta Toro Virus Infections in Mice.

Animals: 10.3-11.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS360	500	5/5	2.2	6/10	6.0
	250	4/5	2.8	8/10	7.5**
	125	5/5	2.5	7/10	6.3
	62.5	5/5	2.1	6/10	5.3
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	11/20	5.5
Normals	-	5/5	2.7	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS360 (7-deoxynarciclasin) was slightly active against the PTV infection in this experiment. Ribavirin exerted the positive activity expected. These data were somewhat compromised by the death of only 45% of the saline-treated virus controls. Also, AVS360 was well tolerated at all dosages used, indicating the maximum tolerated dose may not have been used. Further experiments will be run with this compound using other treatment schedules to further define its antiviral potential.

Table IX-40. Expt. PtA58. Effect of AVS1754 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS1754	50	5/5	0.7	10/10**	>21.0**
	25	5/5	0.7	10/10**	>21.0**
	12.5	5/5	0.9	10/10**	>21.0**
	6.25	5/5	1.1	9/10	7.0
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS 1754, (MVE-2), was considered markedly inhibitory to PTV infections in this experiment. Ribavirin was also considered active, but because of a low death rate (35%) in the virus controls, the ribavirin survivors/total increase was not statistically significant. The dose range, route of inoculation, and treatment schedule were as recommended by Dr. M. Kende of USAMRIID, based on studies with this material run in other systems. The experiment will be repeated to confirm the positive activity seen.

Table IX-41. Expt. PtA89. Effect of AVS1754 Therapy on Punta Toro Virus Infections in Mice (Confirming Experiment).
Animals: 10.9-11.5 g (4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

Toxicity controls				Infected Treated								
Compound	Dosage (mg/kg/day)	Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Liver Score ^c		SGOT		SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
						Mean	Liver Score ^c	Neg/Total ^d (Mean)	Neg/Total ^e (Mean)			
AVS1754	50	5/5	nr ^g	10/10**	>21.0**	0.1**	10/10** (149**)	10/10** (22**)	0.0**	0.3**		
	25	5/5	0.6	9/10*	7.0**	0.2**	9/10** (418**)	9/10** (284**)	0.9**	3.2*		
	12.5	5/5	0.8	9/10*	9.0**	0.6**	8/10** (1003**)	8/10** (1562**)	1.7*	4.8*		
	6.25	5/5	0.8	10/10**	>21.0**	1.0**	6/10** (1312**)	6/10** (766**)	2.5	4.6*		
Ribavirin	75	5/5	nr	10/10**	>21.0**	0.4**	9/10** (447**)	10/10** (76**)	0.0**	1.6**		
Saline	-	-	-	11/20	5.6	3.3	2/17 (8485)	2/17 (7984)	2.4	6.1		
Normals	-	5/5	nr	-	-	0.5	5/5 (393)	5/5 (71)	0.0	0.0		

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Not run.

Conclusions: This experiment confirms and extends our previous findings (Expt. PtA 58) in which AVS1754, designated as MVE-2 was found markedly active against PTV infections.

*P<0.05

**P<0.01

Table IX-42. Expt. PtA98-101. Effect of Time of Single Treatment with AVS1754 on Punta Toro Virus Infections in Mice.

Animals: 11.7-13.4 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, varying times relative to virus inoculation.
Virus: Adames strain Punta Toro virus, Treatment Route: i.p.
s.c. injected.
Drug Diluent: Sterile saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Time of Treatment</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
			<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>	<u>Surv/ Total</u>	<u>MST^b (days)</u>
AVS1754	4 hr pre	100	5/5	-0.1	10/10**	>21.0**
		50	5/5	-0.2	10/10**	>21.0**
		25	5/5	-0.1	10/10**	>21.0**
		12.5	5/5	0.7	10/10**	>21.0**
		6.3	5/5	0.4	10/10**	>21.0**
	4 hr post	100	5/5	-0.1	10/10**	>21.0**
		50	5/5	-0.2	10/10**	>21.0**
		25	5/5	-0.1	10/10**	>21.0**
		12.5	5/5	0.7	10/10**	>21.0**
		6.3	5/5	0.4	10/10**	>21.0**
	24 hr post	100	5/5	-0.1	9/10	8.0**
		50	5/5	-0.2	10/10**	>21.0**
		25	5/5	-0.1	10/10**	>21.0**
		12.5	5/5	0.7	10/10**	>21.0**
		6.3	5/5	0.4	10/10**	>21.0**
	48 hr post	100	5/5	-0.1	10/10**	>21.0**
		50	5/5	-0.2	9/10	8.0**
		25	5/5	-0.1	8/10	7.0
		12.5	5/5	0.7	10/10**	>21.0**
		6.3	5/5	0.4	9/10	9.0**
Ribavirin		75	5/5	0.3	10/10**	>21.0**
Saline		-	-	-	14/20	6.7
Normals		-	5/5	2.2	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This compound is designated as MVE-2, an immunomodulating agent. It was found active vs PTV (Expt. PtA 58) when given i.p. once 24 hr pre-virus inoculation. The present series of experiments (PtA 98-101) were designed to determine how long relative to virus inoculation the material could be given and still be active. These data indicate it can be administered as late as 48 hr post-virus inoculation and still exert a significant antiviral effect.

Table IX-43. Expt. PtA151. Effect of Oral AVS1754 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).

Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: Sterile H₂O.

Treatment Schedule: Once only, 24 hr pre-virus inoculation.

Treatment Route: p.o.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Toxicity controls		Infected, Treated					Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
		Surv/Total	Host Wt. Change ^a (g)	Surv/Total	I/ST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)		
AVS1754	200	5/5	-0.4	2/10	5.5	1.5	4/10(1814)	4/10(1871)	5.5	6.5
	100	5/5	-0.3	4/10	5.8	0.9	8/9(378)	8/9(231)	5.1	4.8
	50	5/5	0.1	1/10	6.0	0.8	6/10(771)	6/10(647)	5.0	4.6
	25	5/5	0.4	3/10	6.0	0.7	6/10(748)	6/10(631)	5.1	5.7
	12.5	5/5	-0.1	2/10	5.8	1.7	5/10(2719)	5/10(1949)	4.4	4.2
	6.25	5/5	-0.2	1/10	5.7	1.1	6/9(780)	5/9(625)	5.2	4.6
Ribavirin	700	5/5	-0.1	1/10	6.8**	0.7	10/10**(149**)	9/10**(150**)	4.3	5.6
H ₂ O	-	-	-	2/20	5.7	1.2	17/20(1183)	14/20(1104)	4.5	5.2
Normals	-	5/5	0.1	-	-	0.2	5/5(47)	5/5(13)	2.0	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cScores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^dSerum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^eSerum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^fGeometric mean.

Conclusions: AVS1754 (MVE-2) has previously exhibited anti-PTV activity when administered i.p. No activity was seen in the present study using this orally administered material.

*P<0.05

**P<0.01

Table IX-44. Expt. PtA76. Effect of AVS1757 on Punta Toro Virus Infections in Mice (initial test).

Animals: 12.8-13.3 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: p.o.
Drug Diluent: Sterile H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS1757	1000	5/5	3.5	1/10	6.7
	500	5/5	3.6	1/10	5.6
	250	5/5	3.1	4/10	5.8
Ribavirin	100	5/5	3.4	10/10**	>21.0**
Normals	-	5/5	4.4	-	-
Saline	-	-	-	8/20	6.7

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS1757, (isoprinosine), was inactive against PTV infections in this experiment. The treatment regimen used was derived from extensive literature search concerning this compound. Ribavirin exerted the positive activity expected. We do not propose to run further experiments with AVS1757.

Table IX-45. Expt. PtA72. Effect of AVS1767 on Punta Toro Virus Infections in Mice (initial test).

Animals: 11.5-12.8 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS1767	450	5/5	1.7	10/10**	>21.0**
	225	5/5	2.3	10/10**	>21.0**
	112.5	5/5	1.7	10/10**	>21.0**
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Normals	-	5/5	4.8	-	-
Saline	-	-	-	5/20	6.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This immunomodulating substance (AM-3), was used s.c. and p.o. in separate experiments (see also PTA 73), since preliminary information supplied to us indicated the material was active orally. The s.c. treatment, shown in this table, was highly active against the PTV infection, with all dosages used exhibiting similar activity. All dosages, while not lethally toxic, caused moderate failure to gain weight. Treatment p.o. was ineffective.

Table IX-46. Expt. PIA111. Effect of AVS1767 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).

Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.

Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.

Treatment Route: s.c.

Experiment Duration: 21 days.

Toxicity controls				Infected, Treated				Mean Serum	
Dosage	Surv/	Host Wt.	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)	
Compound	(mg/kg/day)	Total Change ^a (g)							
AVS1767	2000	4/5	-	-	-	-	-	-	-
	1000	5/5	-	-	-	-	-	-	-
	250	5/5	0/10	1.89	3/10(5244)	3/10(4363)	6.6	6.4	
	125	5/5	8/10**	1.2	7/10**(959**)	7/10**(705**)	4.3*	5.1**	
	62.5	5/5	10/10**	>21.0**	8/10**(1728**)	6/10**(1811**)	2.5**	4.3**	
Ribavirin	75	5/5	10/10**	0.0**	10/10**(85**)	10/10**(24**)	0.8**	3.7**	
Saline	-	-	0/20	2.8	2/20(6270)	1/20(7029)	5.3	6.1	
Normals	-	5/5	-	0.0	5/5(77)	5/5(27)	0.6	0.0	

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Livers dark purple color, they were scored on this color.

Conclusions: This compound is AM-3, an immune modulating agent. Two high dosages were run to determine the approximate MTD, which was observed to be between 1000 and 2000 mg/kg/day. The compound, found previously to be active vs PTV, was confirmed to be active in this experiment. Note the 250 mg/kg/day dose was apparently ineffective, a not unusual observation for such agents.

*P<0.05

**P<0.01

Table IX-47. Expt. PtA73. Effect of AVS1767 on Punta Toro Virus Infections in Mice (initial test, oral).

Animals: 11.7-12.9 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: p.o.
 Drug Diluent: Sterile H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS1767	450	5/5	2.6	6/10	5.5
	225	5/5	3.2	3/10	6.7
	112.5	5/5	4.7	5/10	6.4
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Normals	-	5/5	4.8	-	-
H ₂ O	-	-	-	5/20	6.5

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See comments under PTA 72 (Table IX-45).

Table IX-48. Expt. PtA77. Effect of AVS1777 on Punta Toro Virus Infections in Mice (initial test).

Animals: 12.8-13.3 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS1777	1	0/5	-0.6	0/10	4.9
	.5	4/5	-0.7	0/10	4.9
	.25	5/5	0.7	0/10	5.4
	.125	5/5	0.3	0/10	6.0
Ribavirin	75	5/5	2.4	10/10**	>21.0**
Normals	-	5/5	3.6	-	-
Saline	-	-	-	14/20	8.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This immunomodulating compound, streptonigrin, was considered inactive against PTV infections in this experiment. The treatment schedule used was derived from published reports by others (Cancer Res. 26:727-732, 1966). The maximum tolerated dose was approximately 0.25 mg/kg/day. We do not propose to run further experiments with AVS1777.

Table IX-50. Expt. PtA75. Effect of AVS1778 on Punta Toro Virus Infections in Mice (initial test, b.i.d. x 5).

Animals: 11.1-12.7 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: CMC.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS1778	50	5/5	2.2	8/10	14.0**
	25	5/5	2.7	10/10**	>21.0**
	12.5	5/5	2.3	10/10**	>21.0**
	6.25	5/5	2.4	9/10*	8.0**
	3.13	5/5	2.9	10/10**	>21.0**
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Normals	-	5/5	4.8	-	-
CMC	-	-	-	9/20	6.3

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See comments under PTA 74 (Table IX-49).

Table IX-51. Expt. P1A118. Effect of AVS1778 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).
Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

Toxicity controls				Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT		SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
							Neg/Total ^d (Mean)				
AVS1778	100	5/5	1.6	9/10**	8.0	0.0**	10/10** (156**)		10/10** (173**)	4.2	5.7
	50	5/5	1.9	9/10**	5.0	0.5**	10/10** (351**)		8/10** (384**)	4.2	4.9
	25	5/5	3.1	7/10**	8.7	0.7**	9/10** (440**)		8/10** (384**)	4.3	5.3
	12.5	5/5	2.7	10/10**	>21.0**	1.3	8/9** (487**)		8/9** (462**)	3.8	4.8
	6.3	5/5	2.8	8/10**	4.0	0.8**	4/10 (4345)		3/10 (4210)	4.1	5.2
Ribavirin	3.1	5/5	2.7	7/10**	5.7	1.6	7/9** (671**)		6/9** (694**)	3.8	3.8**
	1.6	5/5	3.4	6/10	7.3	1.5	4/7 (4375)		2/7 (3226)	4.1	4.7
	75	5/5	2.6	10/10**	>21.0**	0.0**	10/10** (148**)		10/10** (39**)	0.9**	1.9**
	-	-	-	2/20	5.4	2.0	7/20 (4695)		4/20 (4415)	4.4	5.6
	Normals	-	5/5	4.5	-	-	0.0	3/3 (196)		3/3 (43)	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: AVS1778 (mannozym) was highly active when used initially by this treatment schedule (PTA 75). That activity was confirmed in the present study, although it should be noted that liver and serum virus titers were not reduced.

*P<0.05

**P<0.01

Table IX-52. Expt. PtA93. Effect of Oral Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 14.5-17.8 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Twice daily X 5, 4 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: p.o.
 Drug Diluent: H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS1778	150	4/4	0.6	5/10	6.8
	75	5/5	0.6	4/10	6.2
	37.5	5/5	1.0	3/10	6.4
	18.8	5/5	2.0	4/10	6.0
	9.4	5/5	1.6	5/10	6.8
Ribavirin	75	5/5	1.8	9/10*	17.0**
H ₂ O	-	-	-	10/19	6.4
Normals	-	5/5	3.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This immunomodulating substance, mannozym, was considered markedly active against PTV when used s.c. by this treatment schedule (Expt. PtA 75). The present experiment was run to determine if this activity would also be exerted when the compound was administered orally. No activity was seen, however, and we conclude mannozym is not effective when administered orally.

Table IX-53. Expt. P1A119. Effect of Oral AVS1778 Therapy on Punta Toro Virus Infections in Mice (Confirming Study).
 Animals: 12.6-14.0 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: CMC.
 Treatment Schedule: Twice daily X 5, beginning 4 hr pre-virus inoculation.
 Treatment Route: p.o.
 Experiment Duration: 21 days.

Toxicity controls				Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	SGOT		SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
							Neg/Total ^d (Mean)				
AVS1778	100	5/5	2.7	1/9	6.1	2.0	0/9(5546)		0/9(8033)	5.4	6.3
	50	5/5	4.0	0/10	6.1	2.7	0/10(5556)		0/10(6375)	4.3	6.1
	25	5/5	3.0	1/10	6.0	2.0	0/8(7400)		0/8(6650)	5.1	6.0
	12.5	5/5	3.6	2/10	5.3	2.0	2/10(5727)		2/10(5038)	3.9	6.1
	6.3	5/5	3.2	1/10	5.7	1.5	0/10(2775)		0/10(2669)	4.7	6.2
Ribavirin	3.1	5/5	3.0	1/10	5.1	3.2	0/9(11,822)		0/9(10,561)	5.2	5.9
	1.6	5/5	2.4	2/10	5.9	3.0	0/10(6805)		0/10(9035)	4.3	6.1
	75	5/5	2.6	10/10**	>21.0**	0.0**	10/10**(148**)		10/10**(39**)	0.9**	1.9**
CMC	-	-	-	1/20	5.1	2.3	1/18(5215)		1/18(6353)	4.9	6.3
Normals	-	5/5	4.5	-	-	0.0	3/3(196)		3/3(43)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: AVS1778 (mannozym) was shown in other studies to be active vs PTV when administered s.c. We previously found this material to be inactive vs PTV when administered p.o.; the present study was run to determine if efficacy could be seen by other, more sensitive, parameters. No activity was seen.

*P<0.05

**P<0.01

Table IX-54. Expt. PtA56. Effect of AVS2149 on Punta Toro Virus Infections in Mice (Initial Test, q.d. x 8).

Animals: 12.8-13.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 8, beginning 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Sterile pyrogen free H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2149	5	5/5	4.4	10/10**	>21.0**
	2.5	5/5	4.4	10/10**	>21.0**
	1.25	5/5	4.9	10/10**	>21.0**
	0.625	5/5	4.2	10/10**	>21.0**
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2149 (ampligen), was considered highly active against PTV infections at every dose used in this study in which the compound was administered once daily for 8 days beginning prior to infection. The dosages used were all non-toxic, but were as recommended to us. This compound will be retested to confirm the positive activity seen.

Table IX-55. Expt. PtA57. Effect of AVS2149 on Punta Toro Virus Infections in Mice (Initial Test, e other day x 8).

Animals: 12.2-13.1 g (3 wk) C57BL/6 Mice. Treatment Schedule: Every other day X 8, beginning 24 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.

Drug Diluent: Sterile pyrogen free H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2149	5	5/5	7.0	10/10**	>21.0**
	2.5	5/5	6.4	10/10**	>21.0**
	1.25	5/5	8.0	10/10**	>21.0**
	0.625	5/5	7.0	10/10**	>21.0**
Ribavirin	75	5/5	4.2	10/10**	>21.0**
Saline	-	-	-	5/20	6.5
Normals	-	5/5	5.5	-	-

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2149 (ampligen) was considered highly active vs PTV at every dose used in this study in which the compound was administered on an every other day treatment schedule. All doses were nontoxic, and were used as recommended.

Table IX-56. Expt. PtA128-132. Effect of Time of Treatment Initiation of AVS2149 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.4 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 5, beginning at varying times relative to virus inoculation.
 Virus: Adames strain Punta Toro virus, Treatment Route: i.p.
 s.c. injected.
 Drug Diluent: H₂O. Experiment Duration: 21 days.

Compound	Time of Treatment ^a	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
			Surv/ Total	Host Wt. Change (g) ^b	Surv/ Total	MST ^c (days)
AVS2149	24 hr pre	5	5/5	2.9	10/10**	>21.0**
		2.5	5/5	3.5	10/10**	>21.0**
		1.25	5/5	2.4	10/10**	>21.0**
		0.625	5/5	2.6	10/10**	>21.0**
	4 hr pre	5	5/5	2.9	10/10**	>21.0**
		2.5	5/5	3.5	10/10**	>21.0**
		1.25	5/5	2.4	10/10**	>21.0**
		0.625	5/5	2.6	10/10**	>21.0**
	4 hr post	5	5/5	2.9	10/10**	>21.0**
		2.5	5/5	3.5	10/10**	>21.0**
		1.25	5/5	2.4	10/10**	>21.0**
		0.625	5/5	2.6	10/10**	>21.0**
	24 hr post	5	5/5	2.9	10/10**	>21.0**
		2.5	5/5	3.5	9/10**	21.0**
		1.25	5/5	2.4	10/10**	>21.0**
		0.625	5/5	2.6	10/10**	>21.0**
	48 hr post	5	5/5	2.9	10/10**	>21.0**
		2.5	5/5	3.5	10/10**	>21.0**
		1.25	5/5	2.4	10/10**	>21.0**
		0.625	5/5	2.6	10/10**	>21.0**
Ribavirin ^d	4 hr pre	75	5/5	2.7	10/10**	>21.0**
Saline	24 hr pre	-	-	-	5/20	7.7
Normals	-	-	5/5	3.6	-	-

^aRelative to virus inoculation.

^bDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^cMean survival time of mice dying on or before day 21.

^dRibavirin administered s.c. bid x 5 beginning 4 hr pre-virus inoculation.

*P<0.05

**P<0.01

Conclusions: This series of experiments was run to determine how late treatment with AVS2149 (ampligen) could begin relative to virus inoculation and still provide a significant effect against PTV infections. In this study, treatments begun as early as 24 hr pre- or as late as 48 hr post-virus inoculation were considered equally effective.

Table IX-57. Expt. PtA69. Effect of AVS2149 Therapy on Punta Toro Virus Infections in Mice.
 Animals: 11.3-11.9 g (4 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile saline.
 Treatment Schedule: Once daily X 8, beginning 24 hr pre-virus inoculation.
 Treatment Route: s.c.
 Experiment Duration: 21 days.

Toxicity controls				Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
AVS2149	5.0	5/5	5.5	10/10**	>21.0**	0.1**	10/10** (117**)	10/10** (27**)	3.6	2.5**
	2.50	5/5	6.0	10/10**	>21.0**	0.2**	10/10** (117**)	10/10** (29**)	3.6	0.3**
	1.25	5/5	5.7	10/10**	>21.0**	0.2**	10/10** (142**)	10/10** (37**)	3.5	0.4**
	0.625	5/5	5.9	10/10**	>21.0**	0.2**	9/9** (120**)	9/9** (33**)	3.6	1.1**
	0.313	5/5	4.0	10/10**	>21.0**	0.6**	10/10** (117*)	10/10** (48**)	3.5	1.8**
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.5**	9/9** (95**)	9/9** (38**)	3.6	2.4**
Saline	-	-	-	1/20	5.3	3.0	0/18 (7874)	0/18 (7681)	3.8	6.0
Normals	-	5/5	3.1	-	-	0.1	5/5 (136)	5/5 (29)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This experiment confirms and extends the results seen in Expt. PTA 56 using AVS2149 (ampligen) against PTV. This immune modulating compound has a potent effect against the infection, seen at all dosages used. No toxicity was demonstrated. Surprisingly, mean liver virus titers were not reduced in this experiment by AVS2149 or by ribavirin, the latter run as positive control.

*P<0.05

**P<0.01

Table IX-57A. Expt. PtA142. Effect of Oral AVS2149 Therapy on Punta Toro Virus Infections in Mice.
Animals: 10.3-12.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile pyrogen-free H₂O.
Treatment Schedule: Once daily X 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Toxicity controls				Infected, Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT		SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
							Neg/Total ^d (Mean)				
AVS2149	5	5/5	3.5	2/10	4.6	1.8	6/10(586**)		5/10(714**)	5.7	5.3
	2.5	5/5	4.3	1/10	4.9	1.3**	4/9(1079**)		3/9(1265**)	5.4	5.8
	1.25	5/5	3.3	2/10	4.1	2.7	0/10(7713)		0/10(9765)	5.8	6.5
	0.63	5/5	3.1	2/10	4.8	2.5	0/9(10,894)		0/9(12,228)	6.4	6.3
	0.31	5/5	3.2	0/10	4.0	2.2	3/10(2514**)		3/10(4944)	5.4	5.5
Ribavirin	0.16	5/5	3.3	1/10	4.4	2.5	2/8(5928)		2/8(6559)	5.2	5.8
	0.078	5/5	2.1	0/10	4.6	2.3	2/10(2737*)		1/10(4160)	6.0	6.5
	0.039	5/5	3.1	2/10	4.1	1.6*	4/10(2897)		4/10(2816)	5.5	6.5
	75	5/5	1.4	10/10**	>21.0**	1.4**	10/10**(142**)		10/10**(42**)	4.9**	6.1
	H ₂ O	-	-	1/20	4.1	2.4	4/20(5693)		3/20(6060)	6.1	6.3
Normals	-	5/5	3.5	-	-	0.5	5/5(68)		5/5(14)	0.6	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: AVS2149 (ampligen) was moderately inhibitory to PTV infections when the material was administered by oral gavage. This activity was expressed as inhibition of liver score and associated reductions in SGOT and SGPT.

*P<0.05

**P<0.01

Table IX-58. Expt. PtA149. Effect of AVS2741 on Punta Toro Virus Infections in Mice (initial test).

Animals: 10.6-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
Drug Diluent: Saline. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS2741	500	5/5	1.5	0/10	4.8
	250	5/5	2.5	0/10	4.3
	125	5/5	1.8	0/10	4.4
	62.5	5/5	2.1	0/10	4.5
	31.3	5/5	2.5	0/10	4.3
Ribavirin	75	5/5	0.9	10/10**	>21.0**
Saline	-	-	-	0/20	4.6
Normals	-	5/5	1.9	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2741 [1-(β-D-ribofuranosyl)-1,2,4-triazole-3-(1,4,5,6-tetrahydropyrimidine)•HCl] was inactive against PTV in this experiment. The compound was well tolerated at the highest dose given, however, so further studies will be run to determine if higher dosages or different treatment regimens will be effective.

Table IX-59. Expt. PtA150. Effect of AVS2742 on Punta Toro Virus Infections in Mice (initial test).

Animals: 10.6-12.2 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: s.c.

Drug Diluent: Saline.

Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS2742	500	5/5	1.6	3/10*	4.6
	250	5/5	1.7	0/10	4.4
	125	5/5	2.3	0/10	5.0
	62.5	5/5	2.4	0/10	4.2
	31.3	5/5	2.1	0/10	4.4
Ribavirin	75	5/5	0.9	10/10**	>21.0**
Saline	-	-	-	0/20	4.6
Normals	-	5/5	1.9	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2742 [1-(β-D-ribofuranosyl)-1,2,4-triazole-3-(5-hydroxy-1,4,5,6-tetrahydropyrimidine)•HCl] was slightly active vs PTV at the highest dose used in this experiment. since that dose was apparently well tolerated, the experiment will be repeated to determine the LD50 and to see if higher doses will be more effective.

Table IX-60. Expt. PtA59. Effect of AVS2776 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.5-11.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: 1% carboxymethylcellulose. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2776	400	4/5	1.2	10/10**	>21.0**
	200	5/5	1.4	10/10**	>21.0**
	100	5/5	2.4	9/9*	>21.0**
	50	5/5	1.6	8/10	8.5
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2776 (2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone) was considered markedly inhibitory to PTV infections in this experiment, wherein the compound was administered once daily for 3 consecutive days beginning 24 hr prior to virus inoculation. Ribavirin was also considered active, but because of a low death rate (35%) in the virus controls, the ribavirin survivors/total increase was not statistically significant. The dose range, route of inoculation, and treatment schedule were as recommended by Dr. M. Kende of USAMRIID, based on studies with this material run in other systems. The experiment will be repeated to confirm the positive activity seen.

Table IX-61. Expt. PtA60. Effect of AVS2776 on Punta Toro Virus Infections in Mice (Initial Test, Single Treatment).

Animals: 10.5-11.2 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, 24 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: i.p.

Drug Diluent: 1% carboxymethylcellulose. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2776	400	4/5	0.0	10/10**	>21.0**
	200	5/5	0.6	10/10**	>21.0**
	100	5/5	0.4	10/10**	>21.0**
	50	5/5	0.8	7/10	8.3
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2776 (2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone) was considered markedly inhibitory to PTV infections in this experiment, wherein the compound was administered in a single injection 24 hr before virus inoculation. This experiment was run in parallel with PTA59, with virtually identical activity seen in that experiment using the compound for 3 consecutive days.

Table IX-62. Expt. PtA61. Effect of AVS2776 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.5-11.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Every 3 days X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2776	400	4/5	3.6	9/10	8.0
	200	5/5	5.2	9/10	8.0
	100	5/5	5.9	10/10**	>21.0**
	50	5/5	5.9	5/10	8.4
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2776 (2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone) was considered moderately inhibitory to PTV infections in this experiment, wherein the compound was administered once every 3 days for 3 treatments, beginning 24 hr before virus inoculation. This experiment was run in parallel with PTA59 and PTA60, with this every 3 day treatment schedule slightly less efficacious than the regimens used in those experiments.

Table IX-63. Expt. P1A90. Effect of AVS2776 Therapy on Punta Toro Virus Infections in Mice (Confirming Experiment).
 Animals: 11.2-11.8 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile saline.
 Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Treatment Route: i.p.
 Experiment Duration: 21 days.

Toxicity controls				Infected, Treated						
Dosage	Surv/	Host Wt.	Surv/	MST ^b	Mean	SGOT	SGPT	Mean Liver	Mean Serum	
Compound (mg/kg/day)	Total	Change ^a (g)	Total	(days)	Liver Score ^c	Neg/Total ^d	(Mean)	Virus Titer ^f	Virus Titer ^f	
								(log ₁₀)	(log ₁₀)	
AVS2776	400	5/5	5/5	10/10**	>21.0**	0.2**	10/10** (199**)	10/10** (38**)	0.8**	2.8**
	200	5/5	5/5	10/10**	>21.0**	0.6**	10/10** (270**)	9/10** (135**)	1.2**	3.0**
	100	5/5	5/5	8/10	7.0	0.7**	10/10** (349**)	10/10** (163**)	2.3*	4.9
Ribavirin	75	5/5	5/5	10/10**	>21.0**	0.4**	9/10** (447**)	10/10** (76**)	0.0**	1.6**
CMC	-	-	-	6/10	6.8	2.8	2/10 (4016)	1/10 (3183)	3.5	5.0
Normals	-	5/5	5/5	-	-	0.5	5/5 (393)	5/5 (71)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

9 Weight 4 days latter.

Conclusions: This experiment confirms and extends our previous observations (Expt. PTA 60) that AVS2776, 2-amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP), was markedly active against PTV infections. Three treatment schedules were used initially (every 3 days x 3, once daily x 3 beginning 24 hr pre, once only 24 hr pre), but chose the latter because it appeared to provide the most positive results.

*P<0.05

**P<0.01

Table IX-64. Expt. PtA143-148. Effect of Time of Single Treatment of AVS2776 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.2 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, varying times relative to virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Time of Treatment^a</u>	<u>Dosage (mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
			<u>Surv/ Total</u>	<u>Host Wt. Change (g)^b</u>	<u>Surv/ Total</u>	<u>MST^c (days)</u>
AVS2776	4 hr pre	400	5/5	0.1	7/10**	6.3
		200	5/5	0.2	8/10**	8.5**
		100	5/5	0.7	7/10**	6.0
	4 hr post	400	5/5	0.1	8/10**	8.0**
		200	5/5	0.2	8/10**	5.0
		100	5/5	0.7	3/10	5.0
	24 hr post	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.2	9/10**	2.0
		100	5/5	0.7	2/10	4.9
	48 hr post	400	5/5	0.1	7/10**	5.3
		200	5/5	0.2	2/10	4.4
		100	5/5	0.7	0/10	5.0
	72 hr post	400	5/5	0.1	3/10	4.1
		200	5/5	0.2	0/10	4.3
		100	5/5	0.7	0/10	4.4
	96 hr post	400	5/5	0.1	1/10	4.1
		200	5/5	0.2	2/10	4.3
		100	5/5	0.7	2/10	4.1
Ribavirin ^d	4 hr pre	75	5/5	-0.6	10/10**	>21.0**
CMC	4 hr pre	-	-	-	2/20	5.1
Normals	-	-	5/5	1.6	-	-

^aRelative to virus inoculation.

^bDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^cMean survival time of mice dying on or before day 21.

^dRibavirin administered bid x 7 beginning 4 hr pre-virus inoculation.

*P<0.05

**P<0.01

Conclusions: We have shown previously (PtA 60, 90) that a single i.p. treatment with AVS2776 (ABPP) given 24 hr pre-virus inoculation, was highly active against PTV infections. This series of experiments (PtA 143-148) were run to determine if ABPP would be active if administered in a single i.p. injection later in the infection. Treatment as late as 48 hr post-virus inoculation was still considered effective.

Table IX-65. Expt. PtA62. Effect of AVS2777 on Punta Toro Virus Infections in Mice (Initial Test, q.d. x 3).

Animals: 10.5-11.2 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS2777	400	4/5	0.0	9/10	6.0
	200	5/5	0.6	10/10**	>21.0**
	100	5/5	0.4	8/9	9.0
	50	5/5	0.8	5/10	8.2
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2777 (2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone) was considered moderately inhibitory to PTV infections in this experiment, wherein the compound was administered once daily for 3 consecutive days beginning 24 hr prior to virus inoculation. Ribavirin was also considered active, but because of a low death rate (35%) in the virus controls, the ribavirin survivors/total increase was not statistically significant. The dose range, route of inoculation, and treatment schedule were as recommended by Dr. M. Kende of USAMRIID, based on studies with this material run in other systems. The experiment will be repeated to confirm the positive activity seen. (Compare with Expts. PTA 63 and 64).

Table IX-66. Expt. PtA63. Effect of AVS2777 on Punta Toro Virus Infections in Mice (Initial Test, single shot).

Animals: 10.6-11.4 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2777	400	5/5	-0.3	9/10	7.0
	200	5/5	-0.2	9/10	9.0
	100	5/5	0.5	8/10	9.0
	50	5/5	0.3	9/10	6.0
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2777 (2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone) was considered moderately inhibitory to PTV infections in this experiment, wherein the compound was administered once only beginning 24 hr prior to virus inoculation. The experiment will be repeated to confirm the positive activity seen. (Compare with Expts. PTA 62 and 64).

Table IX-67. Expt. PtA64. Effect of AVS2777 on Punta Toro Virus Infections in Mice (Initial Test, e 3 days x 3).

Animals: 10.6-11.4 g (3 wk) C57BL/6 Mice. Treatment Schedule: Every 3 days X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> (mg/kg/day)	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2777	400	5/5	5.1	10/10**	>21.0**
	200	5/5	4.9	9/10	6.0
	100	5/5	6.7	9/10	9.0
	50	5/5	6.2	9/10	9.0
Ribavirin	75	5/5	0.6	8/10	9.0
Saline	-	-	-	13/20	7.1

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2777 (2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone) was considered moderately inhibitory to PTV infections in this experiment, wherein the compound was administered once every 3 days for 3 consecutive days beginning 24 hr prior to virus inoculation. The experiment will be repeated to confirm the positive activity seen. (Compare with Expts. PTA 62 and 63).

Table IX-68. Expt. P1A91. Effect of AVS2777 Therapy on Punta Toro Virus Infections in Mice (Confirming Experiment).
Animals: 11.5-11.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

Toxicity controls				Infected Treated			
Dosage	Surv/	Host Wt.	Surv/	SGOT	SGPT	Mean Liver	Mean Serum
Compound (mg/kg/day)	Total	Change ^a (g)	Total	Neg/Total ^d	(Mean)	Virus Titer ^f	Virus Titer ^f
				(Mean)		(log ₁₀)	(log ₁₀)
AVS2777	400	5/5	8/10	8/10** (890**)	7/10** (750**)	2.3	4.4
	200	5/5	6/10	5/10 (3142)	5/10* (2628)	3.2	5.8
	100	5/5	7/10	3/10 (2535)	3/10 (2420)	3.5	4.2
Ribavirin	75	5/5	10/10**	9/10** (447**)	10/10** (76**)	0.0**	1.6**
CMC	-	-	6/10	2/10 (4016)	1/10 (3183)	3.5	5.0
Normals	5/5	4.69	-	5/5 (393)	5/5 (71)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

9Weight 4 days latter.

Conclusions: The initial tests (Expts. PTA 62-64) with AVS 2777, 2-amino-5-iodo-6-phenyl-4(3H)-pyrimidinone, (AIPP), indicated this material was moderately active against PTV when used by each of three treatment procedures. In those studies, a relatively high virus control survival rate occurred, however, which somewhat compromised the data. The present experiment was run to confirm the single treatment study run previously. Again, a slight to moderate effect was seen. We conclude this compound is less active than AVS2776 (ABPP), a closely related analog.

*P<0.05

**P<0.01

Table IX-69. Expt. PtA65. Effect of AVS2778 on Punta Toro Virus Infections in Mice (Initial Test, q.d. x 3).

Animals: 11.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS2778	400	5/5	0.1	3/10	6.4**
	200	5/5	0.2	4/10*	6.8**
	100	5/5	1.6	0/10	5.9**
	50	5/5	1.3	1/10	6.4**
Ribavirin	75	5/5	2.3	10/10**	>21.0**
Saline	-	-	-	1/20	5.3

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS2778 (2-amino-5-bromo-6-methyl-4(3H)pyrimidinone) was used via three treatment schedules in the initial experiments in order to not miss any potential effect. The treatments, i.p. qd x 3 beginning 24 hr pre-virus inoculation, once only 24 hr pre-virus inoculation (Expt. PTA 66), and once every 3 days x 3 (Expt. PTA 67), were all moderately effective against the PTV infection. The activity seen was somewhat erratic, as might be expected from an immune modulating substance. The once only experiment will be repeated to confirm the activity seen.

Table IX-70. Expt. PtA66. Effect of AVS2778 on Punta Toro Virus Infections in Mice (Initial Test, single shot).

Animals: 10.4-12.3 g (3 wk) C57BL/6 Mice.

Treatment Schedule: Once only, 24 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: i.p.

Drug Diluent: CMC.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> (mg/kg/day)	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2778	400	5/5	4.4	9/10**	7.0**
	200	5/5	4.9	3/10	7.6**
	100	5/5	5.5	3/10	8.9**
	50	5/5	5.4	4/10*	8.2**
Ribavirin	75	5/5	2.3	10/10**	>21.0**
Saline	-	-	-	1/20	5.3

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See conclusions under PTA 65.

Table IX-71. Expt. PtA67. Effect of AVS2778 on Punta Toro Virus Infections in Mice (Initial Test, e 3 days x 3).

Animals: 10.4-12.3 g (3 wk) C57BL/6 Mice. Treatment Schedule: Every 3 days X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2778	400	5/5	4.4	5/10**	8.4**
	200	5/5	4.9	1/10	7.6**
	100	5/5	5.5	3/10	7.3**
	50	5/5	5.4	4/10*	7.3**
Ribavirin	75	5/5	2.3	10/10**	>21.0**
Saline	-	-	-	1/20	5.3

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See conclusions under PTA 65.

Table IX-72. Expt. PtA82. Effect of AVS2880 on Punta Toro Virus Infections in Mice (initial test, q.d. x 3, 24 hr pre).

Animals: 10.9-12.3 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS2880	25	5/5	2.4	4/10	7.0
	12.5	5/5	2.3	5/10	7.0
	6.25	5/5	1.7	2/10	8.0
	3.13	5/5	1.6	3/10	8.3
	1.6	5/5	1.8	8/10**	7.0
Ribavirin	75	5/5	0.7	10/10**	>21.0**
Normals	-	5/5	1.4	-	-
Saline	-	-	-	5/20	8.5

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This immunomodulating compound (Pennwalt Corporation's oxamisole) has been previously tested by us under another project for efficacy against murine hepatitis and influenza virus infections in mice. The treatment schedules used in these PTV experiments (q.d. x 3 beginning 24 hr pre-virus inoculation, q.d. x 3 beginning 24 hr post-virus inoculation, once only 24 hr post-virus inoculation) were all found effective against those other virus infections. The results seen here were quite similar to those seen by us previously, i.e., significant activity at one or two dosage levels, with little consistency between experiments. It appeared that the pretreatment initiation and single treatment were most effective. The most efficacious schedules will be retested to confirm the activity seen. Ribavirin exerted the positive activity expected.

Table IX-73. Expt. PtA83. Effect of AVS2880 on Punta Toro Virus Infections in Mice (initial test, q.d. x 3, 24 hr post).

Animals: 10.9-12.3 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr post-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2880	25	5/5	2.4	5/10	7.2
	12.5	5/5	2.3	5/10	5.8
	6.25	5/5	1.7	2/10	6.3
	3.13	5/5	1.6	4/10	7.0
	1.6	5/5	1.8	2/10	6.8
Ribavirin	75	5/5	0.7	10/10**	>21.0**
Normals	-	5/5	1.4	-	-
Saline	-	-	-	5/20	8.5

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See comments under PTA 82 (Table IX-72).

Table IX-74. Expt. PtA84. Effect of AVS2880 on Punta Toro Virus Infections in Mice (initial test, single shot).

Animals: 12.0-13.1 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr post-virus inoculation.
Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
Drug Diluent: Sterile Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS2880	50	5/5	0.6	4/10	6.0
	25	5/5	0.8	8/10**	6.0
	12.5	5/5	0.7	4/10	6.0
	6.25	5/5	0.3	4/10	7.7
	3.13	5/5	0.2	5/10	6.2
	1.6	5/5	0.7	3/10	7.7
Ribavirin	75	5/5	0.7	10/10**	>21.0**
Normals	-	5/5	1.4	-	-
Saline	-	-	-	5/20	8.5

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: See comments under PTA 82 (Table IX-72).

Table IX-75. Expt. PtA105. Effect of Oral AVS2880 Therapy on Punta Toro Virus Infections in Mice.

Animals: 11.7-13.2 g (3 wk) C57BL/6 Mice.
 Virus: Adames strain Punta Toro virus, s.c. injected.
 Drug Diluent: Sterile H₂O.
 Treatment Schedule: Twice daily X 3, beginning 24 hr pre-virus inoculation.
 Treatment Route: p.o.
 Experiment Duration: 21 days.

Compound	Toxicity controls				Infected, Treated					Mean Serum	
	Dosage	Surv/	Host Wt.	Change ^a	Surv/	MST ^b	Mean	SGOT	SGPT	Liver Virus Titer ^f	Virus Titer ^f
	(mg/kg/day)	Total	(g)	(g)	Total	(days)	Score ^c	Neg/Total ^d	(Mean)	(log ₁₀)	(log ₁₀)
AVS2880	25	5/5	0.4	0.4	2/10	7.6**	2.9	0/10(8638)	0/10(9620)	5.1	6.4
	12.5	5/5	0.4	0.4	1/10	7.1**	2.8	0/10(6470)	0/10(6990)	5.2	6.4
	6.25	5/5	0.9	0.9	4/10*	7.5**	2.8	0/9(6659)	0/9(6428)	5.1	6.3
	3.13	5/5	0.3	0.3	1/10	6.3**	1.7	1/10(6259)	1/10(6269)	5.6	6.4
	1.56	5/5	0.8	0.8	0/10	7.5**	1.3	2/10(4751)	2/10(5846)	5.4	5.7
Ribavirin	75	5/5	1.5	1.5	10/10**	>21.0**	2.0	10/10**(120**)	10/10**(89**)	2.7**	4.4
H ₂ O	-	-	-	-	1/20	5.5	2.9	7/20(7301)	3/20(6627)	5.1	6.2
Normals	-	5/5	2.7	2.7	-	-	0.0	5/5(73)	5/5(28)	1.7	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: AVS2880 (oxamisole) had moderate activity against PTV when administered i.p. Treatment by oral gavage was also moderately effective, as evidenced primarily by increases in mean survival time.

*P<0.05

**P<0.01

Table IX-76. Expt. PtA126. Effect of AVS3585 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.1-13.6 g (3-4 wk) C57BL/6 Mice.

Treatment Schedule: Every 3 days x 2, beg. 24 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: i.p.

Drug Diluent: CMC.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u>	<u>Host Wt.</u>	<u>Surv/</u>	<u>MST^b</u>
		<u>Total</u>	<u>Change (g)^a</u>	<u>Total</u>	<u>(days)</u>
AVS3585	400	5/5	2.0	0/10	6.0
	200	5/5	2.2	0/10	6.2
	100	5/5	1.2	0/10	6.5
	50	5/5	1.7	0/10	6.7
Ribavirin	300 ^c	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS3585(neurotropin) exhibited no anti-PTV activity in the present study. No toxicity was seen, suggesting higher dosages could be used. The doses used were as recommended to us.

Table IX-77. Expt. PtA127. Effect of AVS3585 on Punta Toro Virus Infections in Mice (Initial Test, Single Shot).

Animals: 10.1-13.6 g (3-4 wk) C57BL/6 Mice.

Treatment Schedule: Once only, 24 hr pre-virus inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Treatment Route: i.p.

Drug Diluent: CMC.

Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3585	400	5/5	2.0	1/10	6.2
	200	5/5	2.2	1/10	6.6
	100	5/5	1.2	0/10	6.1
	50	5/5	1.7	0/10	6.0
Ribavirin	300 ^c	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS3583 (neurotropin) exhibited no anti-PTV activity in the present study. No toxicity was seen, suggesting higher dosages could be used. The doses used were as recommended to us.

Table IX-78. Expt. PtA140. Effect of AVS3585 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.2 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3585	24	5/5	1.4	1/10	4.8
	12	5/5	0.4	2/10	5.0
	6	5/5	0.4	0/10	4.9
	3	5/5	1.6	1/10	5.8
Ribavirin	75	5/5	-0.6	10/10**	>21.0**
Saline	-	-	-	2/20	5.1
Normals	-	5/5	1.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS3585 (neurotropin) exhibited no anti-PTV activity in the present study. We note the dosage levels are relatively low, and may have been erroneously selected.

Table IX-79. Expt. PtA141. Effect of AVS3585 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.2 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Every other day x 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: s.c.
 Drug Diluent: Saline. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3585	24	5/5	1.9	0/10	5.0
	12	5/5	1.7	1/10	5.0
	6	5/5	3.1	1/10	5.2
	3	5/5	3.0	1/10	5.2
Ribavirin	75	5/5	-0.6	10/10**	>21.0**
Saline	-	-	-	2/20	5.1
Normals	-	5/5	1.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS3585 (neurotropin) exhibited no anti-PTV activity in the present study. We note the dosage levels are relatively low, and may have been erroneously selected.

Table IX-80. Expt. PtA120. Effect of AVS3587 on Punta Toro Virus Infections in Mice.

Animals: 10.8-12.2 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily X 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected. Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3587	400	4/5	-0.7	0/10	6.5
	200	5/5	1.5	3/10	7.1
	100	5/5	1.5	3/10	6.4
	50	5/5	0.5	1/10	6.0
Ribavirin ^c	300	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	2.1,2.7	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS3587 is the 5-chloro analog of ABPP (AVS2776). The latter compound was highly active vs PTV using this treatment schedule, but AVS3587 was not considered effective.

Table IX-81. Expt. PtA121. Effect of AVS3587 on Punta Toro Virus Infections in Mice.

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3587	400	4/5	-0.1	2/10	7.4
	200	5/5	0.4	0/10	6.4
	100	5/5	1.2	6/10**	7.0*
	50	5/5	-1.8	3/10	6.4
Ribavirin ^c	300	5/5	0.0 ^d	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

^dWeight 2 days latter.

*P<0.05

**P<0.01

Conclusions: AVS3587 is the 5-chloro analog of ABPP (AVS2776), which was highly active vs PTV when administered i.p. once only 24 hr pre-virus inoculation. AVS3587 was considered moderately active by this treatment regimen.

Table IX-82. Expt. PtA122. Effect of AVS3588 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS3588	400	5/5	1.6	5/10**	8.4**
	200	4/5 ^d	-0.2	7/10**	8.0**
	100	5/5	0.7	5/10**	7.4*
	50	5/5	1.4	0/10	6.9
Ribavirin ^c	300	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

^dOne mouse found dead on day 20 with cross bite.

*P<0.05

**P<0.01

Conclusions: AVS3588 is the meta fluoro derivative of ABPP (AVS2776). The activity seen in the present experiment closely resembles that exhibited by ABPP.

Table IX-83. Expt. PtA123. Effect of AVS3588 on Punta Toro Virus Infections in Mice (Initial Test, Single Shot).

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage (mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/ Total</u>	<u>Host Wt. Change (g)^a</u>	<u>Surv/ Total</u>	<u>MST^b (days)</u>
AVS3588	400	5/5	-1.0	9/10**	7.0**
	200	5/5	-0.8	6/10**	6.5
	100	5/5	-0.2	5/10**	7.8**
	50	5/5	0.5	2/10	6.9
Ribavirin ^c	300	5/5	0.0 ^d	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

^dWeight 2 days latter.

*P<0.05

**P<0.01

Conclusions: AVS3588 is the meta fluoro derivative of ABPP (AVS2776). The activity seen in the present experiment closely resembles that exhibited by ABPP.

Table IX-84. Expt. PtA124. Effect of AVS3589 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.1-13.6 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 3, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg/day)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3589	400	5/5	1.0	5/10**	8.0**
	200	5/5	1.0	6/10**	8.0**
	100	5/5	1.9	0/10	7.0
	50	5/5	0.8	1/10	7.2
Ribavirin	300 ^c	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS3589 is the 5-chloro-2,3-difluorophenyl derivative of ABPP (AVS2776). The anti-PTV activity seen in the present experiment is similar, but somewhat weaker than that exhibited by ABPP.

Table IX-85. Expt. PtA125. Effect of AVS3589 on Punta Toro Virus Infections in Mice (Initial Test, Single Shot).

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: CMC. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3589	400	5/5	-0.3	3/10	6.3
	200	5/5	0.5	2/10	6.4
	100	5/5	0.0	1/10	7.3
	50	5/5	0.1	1/10	6.3
Ribavirin ^c	300	5/5	0.0	6/10**	10.0**
CMC	-	-	-	1/20	6.2
Normals	-	10/10	0.5,0.6	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cRibavirin given in a single shot 4 hr post-virus inoculation.

*P<0.05

**P<0.01

Conclusions: AVS3589 is the 5-chloro-2,3-difluorophenyl derivative of ABPP (AVS2776). No activity was seen by this single treatment schedule, in contrast to strong activity seen by ABPP.

Table IX-86. Expt. PtA189. Effect of AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: DMSO, Methocel. Experiment Duration: 21 days.

Compound	Dosage (mg/kg)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS3925	200	3/5	-0.9	0/10 ^c	3.9
	100	5/5	-1.6	0/10 ^c	6.0*
	50	5/5	-0.3	0/10	5.4
	25	5/5	0.4	0/10	5.5
Ribavirin	350	5/5	0.0	4/10	7.8
Methocel (0.25%)		-	-	6/20	5.9
DMSO +Tween 80		-	-	0/20 ^c	5.0
Normals	-	5/5	0.8	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cMice seemed very sick after first treatment. One mouse in 200 dose and one in DMSO controls died with first treatment.

*P<0.05

**P<0.01

Conclusions: This compound is du Pont A2222-1, it was prepared and used per instructions from du Pont, with initial solutions prepared in 100% DMSO + a small quantity of Tween 80, then diluted in methocel. The compound appeared inactive in this experiment, and the DMSO + Tween 80 appeared somewhat toxic to the mice. A further experiment is being run to confirm the latter observation.

Table IX-87. Expt. PtA190. Effect of AVS3926 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: DMSO, Methocel. Experiment Duration: 21 days.

Compound	Dosage (mg/kg)	Tox. Control		Infected, Treated	
		Surv/ Total	Host Wt. Change (g) ^a	Surv/ Total	MST ^b (days)
AVS3926	200	0/5	-	0/10	1.0
	100	0/5	0.6	0/10 ^d	2.6
	50	5/5	-0.9	0/10	5.3
	25	5/5	-0.1	0/10	6.0*
Ribavirin	350	5/5	0.0	4/10	7.8
Methocel (0.25%)	-	-	-	6/20	5.9
DMSO + Tween 80	-	-	-	0/20 ^d	5.0
Normals	-	5/5	0.8	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cThree mice died with first treatment.

*P<0.05

**P<0.01

Conclusions: This compound is du Pont A2227-1. It was prepared and used per instructions from du Pont, with initial solutions prepared in 100% DMSO + a small quantity of Tween 80, then diluted in methocel. The compound appeared inactive in this experiment, and the DMSO + Tween 80 appeared somewhat toxic to the mice. A further experiment is being run to confirm the latter observation.

Table IX-88. Expt. PtA191. Effect of AVS3927 on Punta Toro Virus Infections in Mice (initial test).

Animals: 10.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once only, 24 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: i.p.
 Drug Diluent: DMSO, Methocel. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> <u>(mg/kg)</u>	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3927	200	2/5	-1.1	0/10	4.8
	100	5/5	-0.1	0/10	5.9*
	50	5/5	0.1	0/10	5.5
	25	5/5	0.6	0/10	5.5
Ribavirin	350	5/5	0.0	4/10	7.8
Methocel (0.25%)	-	-	-	6/20	5.9
DMSO + Tween 80	-	-	-	0/20	5.0
Normals	-	5/5	0.8	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: This compound is du Pont A754-1. It was prepared and used per instructions from du Pont, with initial solutions prepared in 100% DMSO + a small quantity of Tween 80, then diluted in methocel. The compound appeared inactive in this experiment, and the DMSO + Tween 80 appeared somewhat toxic to the mice. A further experiment is being run to confirm the latter observation.

Table IX-89. Expt. PtA192. Effect of AVS3934 on Punta Toro Virus Infections in Mice (initial test).

Animals: 10.1-12.0 g (3 wk) C57BL/6 Mice. Treatment Schedule: Once daily x 7, 36 hr pre-virus inoculation.
 Virus: Adames strain Punta Toro virus, s.c. injected. Treatment Route: p.o.
 Drug Diluent: H₂O. Experiment Duration: 21 days.

<u>Compound</u>	<u>Dosage</u> (mg/kg/day)	<u>Tox. Control</u>		<u>Infected, Treated</u>	
		<u>Surv/</u> <u>Total</u>	<u>Host Wt.</u> <u>Change (g)^a</u>	<u>Surv/</u> <u>Total</u>	<u>MST^b</u> <u>(days)</u>
AVS3934	300	5/5	2.4	1/10	5.3
	150	5/5	3.7	0/10	5.3
	75	5/5	4.2	0/10	5.5
	37.5	5/5	2.7	0/10	5.7
	18.8	5/5	3.9	0/10	5.5
	9.4	5/5	3.3	2/10	6.9**
Ribavirin	75	5/5	4.0	10/10**	>21.0**
H ₂ O	-	-	-	0/20	5.2
Normals	-	5/5	4.3	-	-

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

*P<0.05

**P<0.01

Conclusions: AVS3934 (Ge132) was considered inactive vs PTV using this treatment regimen. The regimen was used based on data provided to us by the manufacturer.

X. EFFECT OF A DRUG COMBINATION ON IN VIVO PUNTA TORO VIRUS INFECTIONS.

Introduction

It has been one goal of this project to investigate the potential for use of combinations of compounds in the treatment of PTV infections. A concept in this use of combinations has been to use materials which inhibit PTV by known different mechanisms of action. We have progressed sufficiently in this project to have found compounds which have highly significant inhibitory effects on *in vivo* PTV infections.

As reviewed in Section IX of this Report, ribavirin (AVS01) and ampligen (polyI-polyC12U, AVS2149) have demonstrated marked PTV-inhibitory activity. Ribavirin has a multifaceted antiviral mechanism of action. The 5'-monophosphate is an inhibitor of IMP dehydrogenase, thus inhibiting DNA synthesis (1); a phosphorylated form of the compound is also inhibitory to structural peptides (2). Ribavirin 5'-triphosphate is selectively inhibitory to the RNA polymerase of certain viruses (3). Finally, ribavirin 5'-triphosphate has also been shown to be incorporated into the cap of messenger RNA, presumably in place of the terminal GMP (4). The specific mechanism(s) of action vs PTV for ribavirin has yet to be determined. Ampligen is a known immunomodulator, inducing interferon, enhancing natural killer cell function, and stimulating macrophage activity. Compounds related to ampligen, poly(ICLC) and poly(G):poly(C), have been reported to have significant enhanced therapeutic antiviral efficacy when used in combination with ribavirin (5, 6).

These materials thus appeared logical candidates for an initial combination chemotherapy trial, which is described in this Section.

Materials and Methods

Virus: The Adames strain of PTV used in our standard *in vivo* experiments (Section VI) was used. A 100LD50 dose of virus was used for this study.

Mice: Three week-old C57BL/6 mice as described in Section I were used.

Compound: Ribavirin (AVS01) and ampligen (AVS2149) were provided by Technassociates, Inc. The ampligen was provided in vials in 10 ml quantities. To each vial was added 20 ml sterile pyrogen-free water, which made a solution containing 2.5 mg/ml. The vial was placed at 65°C in a waterbath for 30 minutes, allowed to sit at room temperature for 1 hr, then refrigerated until use. Dilutions of this material were made in sterile pyrogen-free water.

Experiment Method: An expanded-parameter anti-PTV experiment was run with these compounds, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus titer and serum virus titer. The experiment was run essentially as a standard confirmatory anti-PTV experiment, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated infected controls, 20 mice as normal controls, and 5 animals in each treatment group as toxicity controls.

Five separate experiments were run in parallel, as follows:

- #1. (PtA 162): AVS01 only, at dosages of 150, 100, 32, 10, 3.2, 1, and 0.32 mg/kg/day.
- #2. (PtA 166): AVS2149 only, at dosages of 5, 0.5, and 0.05 mg/kg/day.
- #3. (PtA 163): AVS01 at dosages used in #1, + AVS2149 at 5 mg/kg/day used with each AVS01 dose.
- #4. (PtA 164): AVS01 at dosages used in #1, + AVS2149 at 0.5 mg/kg/day used with each AVS01 dose.

#5. (PtA 165): AVS01 at dosages used in #1, + AVS2149 at 0.05 mg/kg/day used with each AVS01 dose.

Treatment with AVS01 was p.o., twice daily for 5 days beginning 24 hr pre-virus inoculation. Treatment with AVS2149 was i.p. once daily for 5 days beginning 24 hr post-virus inoculation. One-half of each treatment group, virus controls and normal controls were killed 3 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT and PTV virus titers. The remainder of all groups was held 21 days post-virus inoculation with deaths noted daily.

Toxicity and normal control mice were weighed immediately prior to treatment and again 18 hr after final treatment.

Statistical Evaluations: The major points to be demonstrated in this study were whether 1) A near toxic dose of AVS01 would be more tolerated by the animal when also treated with AVS2149; 2) Low, usually ineffective antiviral doses of either AVS01 or AVS2149 would be made more antiviral through the use of their combination. Increased survivors per total and increased numbers of serum samples having negative SGOT and SGPT values were evaluated using chi square analysis with Yate's correction; decreased mean liver scores were analyzed using Wilcoxon ranked sum analysis; increased mean survival times of animals dying on or before day 21 and decreased SGOT, SGPT, serum virus, and liver virus were analyzed using *t* test.

Results and Discussion

The results of this study are summarized in Tables X-1 to X-5. AVS01 was surprisingly well tolerated when used alone at 150 mg/kg/day (Table X-1); we had anticipated this dose to be probably a 10% lethal dose based on earlier studies with it (see Table IX-2). The lowest effective dose of AVS01 used alone was approximately 32 mg/kg/day, which compared well with the 25 mg/kg/day dose shown earlier (Table IX-2). The efficacy of this orally administered AVS01 was seen primarily as increased survivors, decreased mean liver scores and decreased SGOT and SGPT titers. Virus titers were not reduced substantially except for liver virus from mice treated with the 150 mg/kg/day dose. AVS2149 used alone was well tolerated at all dosages used (Table X-2). Marked activity seen by all parameters was seen at 5 and 0.5 mg/kg/day dose levels. The 0.05 mg/kg/day dose was less effective, with moderate survivor increases, decreased liver score and decreased SGOT and SGPT. Liver and serum virus was not reduced at this lowest AVS2149 dose level to the extent seen at the higher dose levels.

Based on these data with each compound used alone, we would anticipate the combination of AVS01 at all dosages + 0.5 and 5 mg/kg/day of AVS2149 to be highly active, primarily because of the strong activity of AVS2149 used alone. This indeed was seen (Tables X-3, X-4), although it is significant that the virus titer reduction seen with the combinations was greater than with either drug used alone (Figures X-1-3).

The greatest potentiation of antiviral effect was seen using AVS2149 at 0.05 mg/kg/day in combination with the usually ineffective doses of AVS01 (Table X-5). This is seen particularly as increased liver and serum virus titer reductions (Figures X-4, 5) and increased total survivors (Figure X-6). The effect was not uniform at all doses; e.g., the 0.32 mg/kg/day dose of AVS01 in combination with this lowest dose of AVS2149 was more effective on total survivors and reductions in liver and serum virus than were the 1 or 3.2 mg/kg/day AVS01 doses used in combination. Such inconsistency would be more concerning if we were dealing with two non-immunomodulating antiviral compounds. Since AVS2149 is an immunomodulating agent, however, we might anticipate possible erraticism in antiviral response.

Toxicity control mice treated with the various drug combinations or with either drug alone appeared to tolerate all treatments well, with host weight change within acceptable limits.

The treatment schedules selected for AVS01 and AVS2149 were selected to be more therapeutic than prophylactic in order to relate more to the human situation. The oral treatment was selected to again relate more closely to actual human use. Our previous work (Table IX-57A) indicated AVS2149 was only slightly active when administered orally, so the i.p. route, which is

known to provide strong antiviral activity with this compound, was used in this study. It may be of interest in a future study to determine if AVS01 would enhance the oral efficacy of AVS2149, however.

Since AVS2149 at the lowest dosage used was still moderately effective, we have repeated this exact combination therapy experiment, but using a 0.005 mg/kg/day dose of AVS2149. That experiment is now underway, but was not sufficiently completed to include with this report.

These data indicate this combination of an antiviral drug (AVS01, ribavirin) and an immunomodulating agent (AVS2149, ampliten) had definite enhanced anti-PTV effect when compared to either substance used alone.

Conclusions

The combination of AVS01 (ribavirin) and AVS2149 (ampliten) had a definitely enhanced effect against PTV infections in mice compared to either compound used alone. The enhanced activity was particularly seen as increased serum and liver virus titer reductions and survivor increases at usually ineffective or marginally effective doses of either compound used alone.

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Table X-1. Expt. PtA162. Effect of AVS01 Therapy on Punta Toro Virus Infections in Mice (Part I of a Combination Study with AVS2149).

Animals: 11.0-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H₂O.

Treatment Schedule: Twice daily X 5, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Toxicity controls				Infected/Treated						
Compound	Dosage (mg/kg/day)	Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)
AVS01	150	5/5	2.0	10/10**	>21.0**	0.0**	9/9**(66**)	9/9**(15**)	1.7**	3.3**
	100	5/5	2.1	10/10**	>21.0**	0.2**	10/10**(71**)	10/10**(30**)	4.2**	4.6**
	32	5/5	2.2	4/10*	7.5*	0.7**	5/9*(1410**)	5/9**(849**)	5.7	5.7
	10	5/5	2.6	0/10	4.4	1.9	0/8(10,738)	0/8(9331)	6.5	6.5
	3.2	5/5	1.9	0/10	4.2	0.2**	0/9(4650)	0/9(3856)	6.5	6.5
150	1	5/5	1.9	0/10	3.9	1.4	1/6(7967)	1/6(6781)	6.9	6.5
	0.32	5/5	2.6	0/10	4.2	2.0	0/10(11,050)	0/10(11,080)	7.2	6.2
H ₂ O	-	-	-	0/20	4.0	2.2	2/18(6067)	0/18(7496)	6.5	6.2
Normals	-	5/5	2.8	-	-	0.3	5/5(52)	5/5(18)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This is the first experiment in a series to determine the effects of combination therapy using AVS01 (ribavirin) and AVS2149 (ampligen). In this experiment ribavirin used alone was active vs PTV at doses down to 32 mg/kg/day.

*P<0.05

**P<0.01

Table X-2. Expt. P1A166. Effect of AVS2149 Therapy on Punta Toro Virus Infections in Mice (Part II of a Combination Study with AVS01).

Animals: 11.0-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H₂O.

Treatment Schedule: Once daily x 5 beginning 24 hr post virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

Toxicity controls				Infected/Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	Surv/ Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	Mean Serum Virus Titer ^f (log ₁₀)	
AVS2149	5	5/5	1.2	8/8**g	>21.0**	0.0**	10/10**(87**)	10/10**(13**)	1.4**	0.4**	
	0.5	5/5	2.4	10/10**	>21.0**	0.1**	9/10**(203**)	9/10**(143**)	3.2**	4.3**	
	0.05	5/5	2.0	5/10**	6.6**	0.0**	8/10**(487**)	8/10**(354**)	4.5**	5.6	
H ₂ O	-	-	-	0/20	4.0	2.2	2/18(6067)	0/18(7496)	6.5	6.2	
Normals	-	5/5	2.8	-	-	0.3	5/5(52)	5/5(18)	0.0	0.0	

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

^g Two mice missing.

Conclusions: This is the second experiment in a series to determine the effect of combination therapy with ribavirin and ampicillin on PTV infections. Ampicillin was highly active at the 5 and 0.5 mg/kg/day dosages, but only moderately active at the 0.05 mg/kg/day dosage.

*P<0.05

**P<0.01

Table X-3. Expt. PtA163. Effect of AVS01 and AVS2149 Therapy on Punta Toro Virus Infections in Mice (Part III of a Combination Study).

Animals: 11.0-13.3 g (3 wk) C57BL/6 Mice.

Inoculation:

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: H₂O.

Treatment Schedule: 01: bid x 5, 2149: qd x 5 beginning 24 hr post virus

Combination Study).

Treatment Route: 01: p.o., 2149: i.p.

Experiment Duration: 21 days.

Compound	Toxicity controls			Infected Treated						Mean Serum Virus Titer ^f (log ₁₀)
	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change ^a (g)	MST ^b (days)	Surv/ Total	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Mean Liver Virus Titer ^f (log ₁₀)	
AVS01 +	150 + 5	5/5	1.3	12.0	9/10**	0.3**	8/8** (54**)	8/8** (12**)	0.9**	0.0**
AVS2149	100 + 5	5/5	1.3	>21.0**	10/10**	0.2**	10/10** (38**)	10/10** (13**)	1.3**	0.0**
	32 + 5	5/5	1.5	>21.0**	10/10**	0.4**	10/10** (36**)	10/10** (8**)	1.3**	0.9**
	10 + 5	5/5	0.9	>21.0**	10/10**	0.5**	10/10** (35**)	10/10** (10**)	0.6**	1.5**
	3.2 + 5	5/5	1.1	>21.0**	9/9**	0.5**	9/9** (37**)	99** (15**)	0.8**	1.6**
	1 + 5	5/5	1.8	>21.0**	10/10**	0.7**	8/8** (51**)	8/8** (23**)	0.4**	2.1**
	0.32 + 5	5/5	1.7	>21.0**	10/10**	0.5**	10/10** (67**)	10/10** (19**)	0.0**	1.3**
H ₂ O	-	-	-	4.0	0/20	2.2	2/18 (6067)	0/18 (7496)	6.5	6.2
Normals	-	5/5	2.8	-	-	0.3	5/5 (52)	5/5 (18)	0.0	0.0

^aDifference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^bMean survival time of mice dying on or before day 21.

^cScores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^dSerum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^eSerum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^fGeometric mean.

Conclusions: This is the third experiment in a series to determine the effect of combination therapy with ribavirin and ampicillin on PTV infections. Marked and uniform activity was seen at all dosages of ribavirin, we presume due to the ampicillin also administered at a known active dose.

*P<0.05

**P<0.01

Table X-4. Expt. PtA164. Effect of AVS01 and AVS2149 Therapy on Punta Toro Virus Infections in Mice (Part IV of a Combination Study).

Animals: 11.0-13.3 g (3 wk) C57BL/6 Mice.

inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: H₂O.

Treatment Schedule: 01: bid x 5, 2149: qd x 5 beginning 24 hr post virus

Combination Study).

Treatment Route: 01: p.o., 2149: i.p.

Experiment Duration: 21 days.

Toxicity controls				Infected, Treated				Mean Liver		Mean Serum	
Dosage	Surv/	Host Wt.	Surv/	SGOT	SGPT	Mean	Neg/Total ^d	Virus Titer ^f	(log ₁₀)	Virus Titer ^f	(log ₁₀)
Compound (mg/kg/day)	Total	Change ^a (g)	Total	Mean	(Mean)	Liver Score ^c	(Mean)	(log ₁₀)		(log ₁₀)	
AVS01 +	150 + 0.5	5/5	5/5	1.6	10/10**	>21.0**	8/8** (58**)	0.0**	8/8** (17**)	0.0**	0.5**
AVS2149	100 + 0.5	5/5	5/5	1.8	10/10**	>21.0**	10/10** (102**)	0.0**	10/10** (23**)	0.0**	0.8**
	32 + 0.5	5/5	5/5	1.7	10/10**	>21.0**	10/10** (74**)	0.0**	10/10** (24**)	1.6**	2.6**
	10 + 0.5	5/5	5/5	2.4	10/10**	>21.0**	10/10** (158**)	0.9	10/10** (77**)	1.3**	4.3**
	3.2 + 0.5	5/5	5/5	2.0	10/10**	>21.0**	10/10** (179**)	1.3	9/10** (108**)	1.6**	3.1**
	1 + 0.5	5/5	5/5	1.9	10/10**	>21.0**	9/9** (169**)	1.1	8/9** (98**)	2.4**	3.3**
	0.32 + 0.5	5/5	5/5	2.5	10/10**	>21.0**	9/9** (124**)	0.8	9/9** (23**)	3.1**	4.6**
H ₂ O	-	-	-	-	0/20	4.0	2/18 (6067)	2.2	0/18 (7496)	6.5	6.2
Normals	-	5/5	5/5	2.8	-	-	5/5 (52)	0.3	5/5 (18)	0.0	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

^f Geometric mean.

Conclusions: This is the fourth experiment in a series to determine the effect of combination therapy with ribavirin and ampicillin on PTV infections. Marked, but somewhat less uniform activity was seen at the various dosages of ribavirin, with least effect seen at lowest doses. We presume the 0.5 mg/kg/day of ampicillin was only moderately effective, with ribavirin contributing to the antiviral effects seen.

*P<0.05

**P<0.01

Table X-5. Expt. P1A165. Effect of AVS01 and AVS2149 Therapy on Punta Toro Virus Infections in Mice (Part V of a Combination Study).

Animals: 11.0-13.3 g (3 wk) C57BL/6 Mice.

inoculation.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: H₂O.

Treatment Schedule: 01: bid x 5, 2149: qd x 5 beginning 24 hr post virus

Combination Study).

Treatment Route: 01: p.o., 2149: i.p.

Experiment Duration: 21 days.

Toxicity controls				Infected/Treated				Mean Serum	
Compound	Dosage (mg/kg/day)	Surv/Total	Host Wt. Change ^a (g)	Surv/Total	MST ^b (days)	Mean Liver Score ^c	SGOT Neg/Total ^d (Mean)	SGPT Neg/Total ^e (Mean)	Virus Titer ^f (log ₁₀)
AVS01 +	150 + 0.05	5/5	1.4	10/10**	>21.0**	0.0**	10/10** (108**)	10/10** (19**)	0.0**
AVS2149	100 + 0.05	5/5	1.6	10/10**	>21.0**	0.0**	9/9** (53**)	9/9** (9**)	2.3**
	32 + 0.05	5/5	2.0	10/10**	>21.0**	0.9	10/10** (102**)	10/10** (48**)	4.8
	10 + 0.05	5/5	1.8	9/10**	5.0	0.5**	9/10** (338**)	9/10** (278**)	3.0**
	3.2 + 0.05	5/5	1.8	4/10	5.8	0.5**	8/9** (319**)	8/9** (217**)	4.3**
	1 + 0.05	5/5	2.1	7/10**	6.3	0.3**	6/10** (1981)	6/10** (1867)	5.4
	0.32 + 0.05	5/5	2.3	9/10**	8.0	0.6**	9/9** (133**)	9/9** (69**)	3.1**
H ₂ O	-	-	-	0/20	4.0	2.2	2/18 (6067)	0/18 (7496)	6.5
Normals	-	5/5	2.8	-	-	0.3	5/5 (52)	5/5 (18)	0.0

^a Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

^b Mean survival time of mice dying on or before day 21.

^c Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

^d Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

^e Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

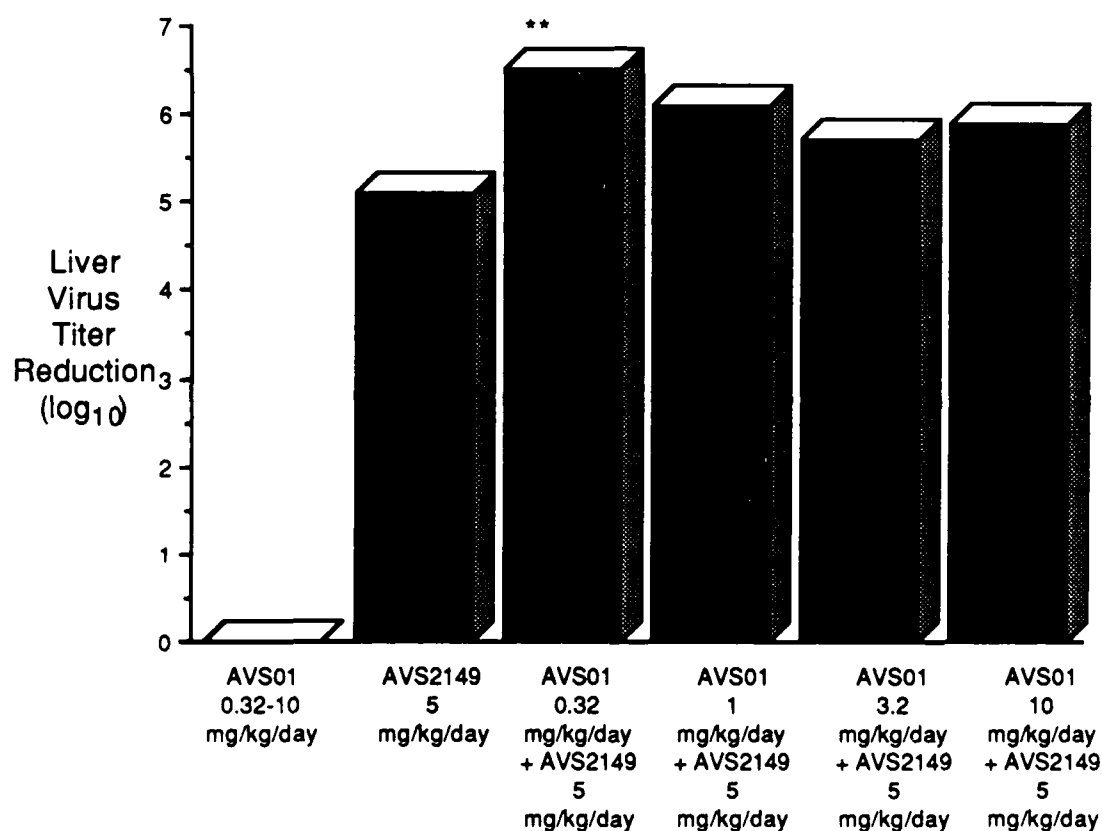
^f Geometric mean.

Conclusions: This is the fifth experiment in a series to determine the effect of combination therapy with ribavirin and ampicillin on PTV infections. Activity was seen at all ribavirin doses, including those inactive when used alone, when the 0.05 mg/kg ampicillin was used with the ribavirin. This dose of ampicillin was only weakly active by itself, so we presume the two compounds together have exerted an additive or synergistic anti-PTV effect.

*P<0.05

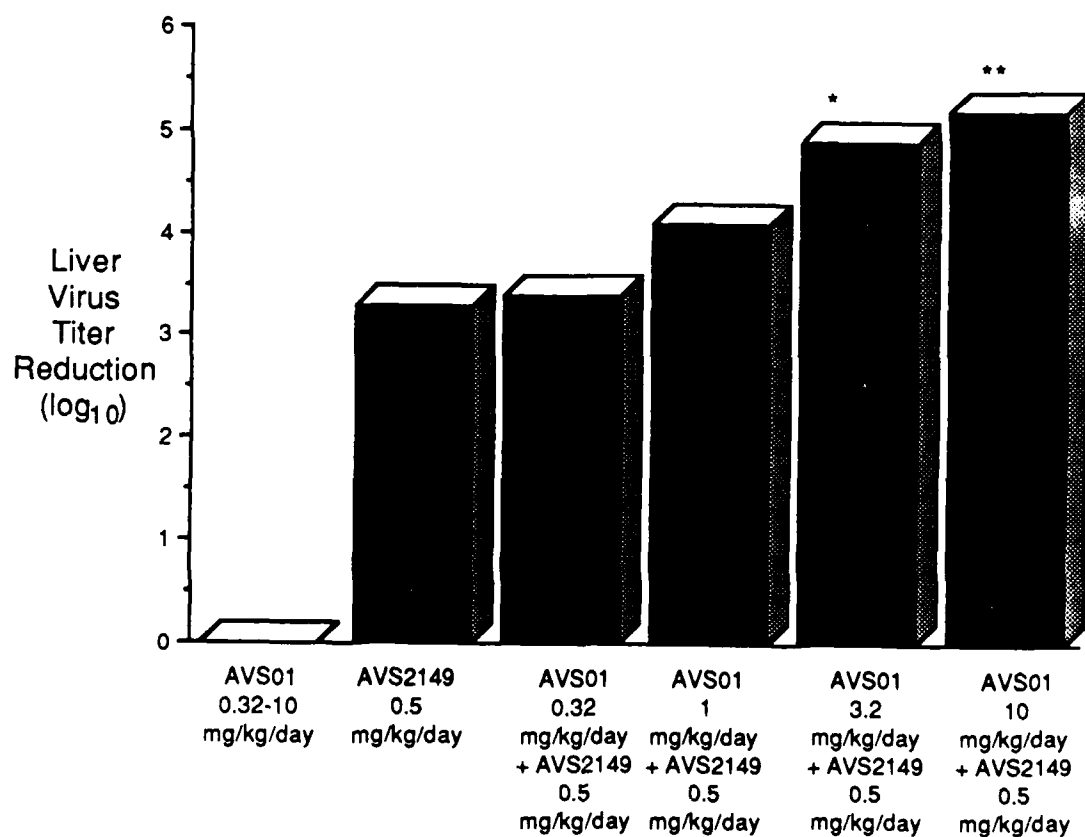
**P<0.01

Figure X-1. Effect of AVS01 (0.32-10 mg/kg/day) + AVS2149 (5 mg/kg/day) on PTV Titer Reduction in Livers of Infected Treated Mice



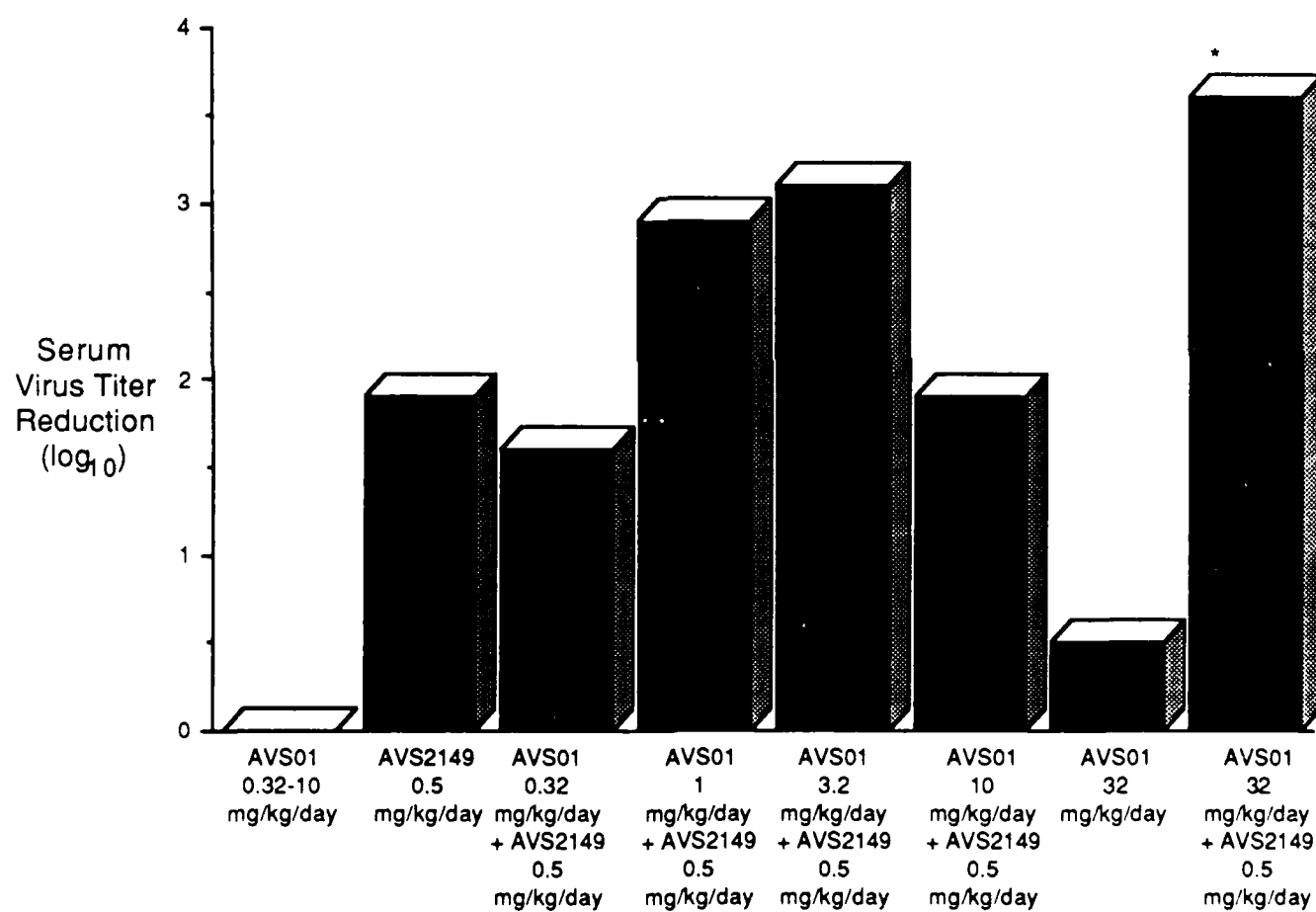
**P<0.01 compared to AVS2149 5 mg/kg/day.

Figure X-2. Effect of AVS01 (0.32-10 mg/kg/day) + AVS2149 (0.5 mg/kg/day) on PTV Titer Reduction in Livers of Infected Treated Mice



*P<0.05 **P<0.01 compared to AVS2149 0.5 mg/kg/day

Figure X-3. Effect of AVS01 (0.32-32 mg/kg/day) + AVS2149 (0.5 mg/kg/day) on PTV Titer Reduction in Sera of Infected Treated Mice



* $P < 0.05$ compared to AVS2149 0.5 mg/kg/day.

Figure X-4. Effect of AVS01 (0.32-10 mg/kg/day) + AVS2149 (0.05 mg/kg/day) on PTV Titer Reduction in Livers of Infected Treated Mice

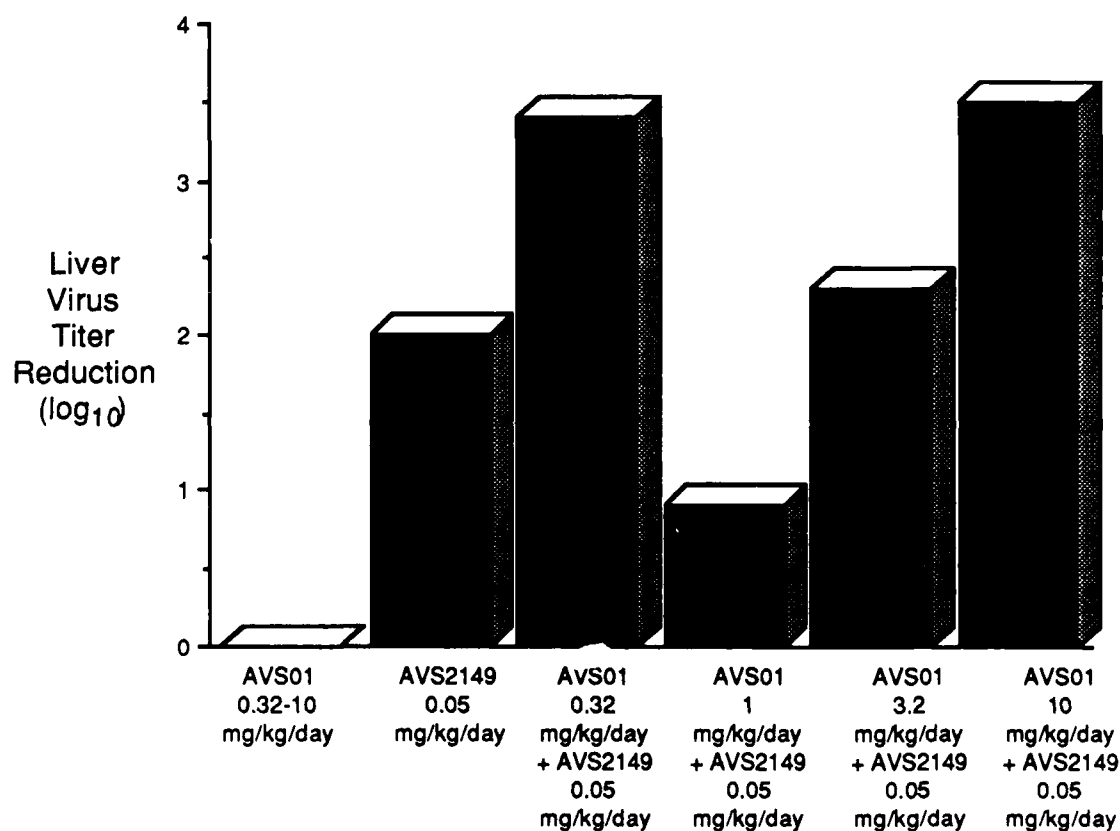
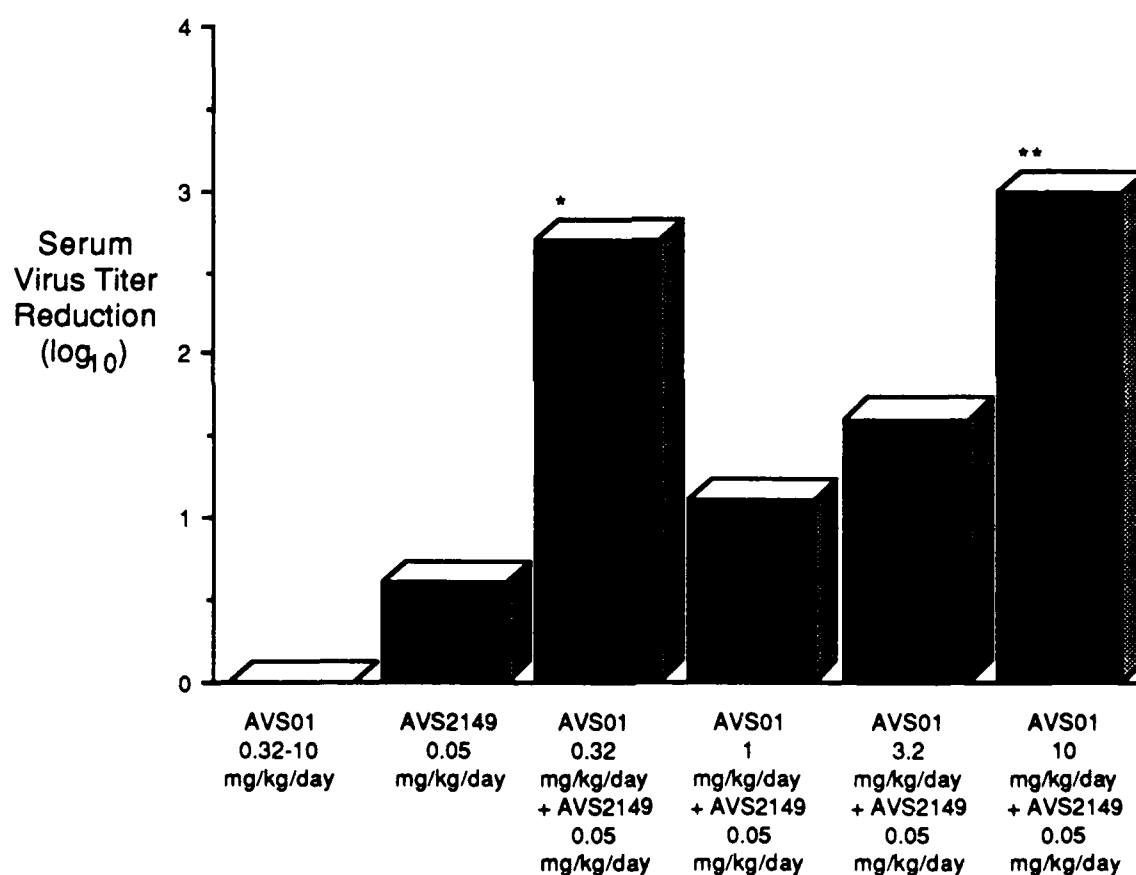
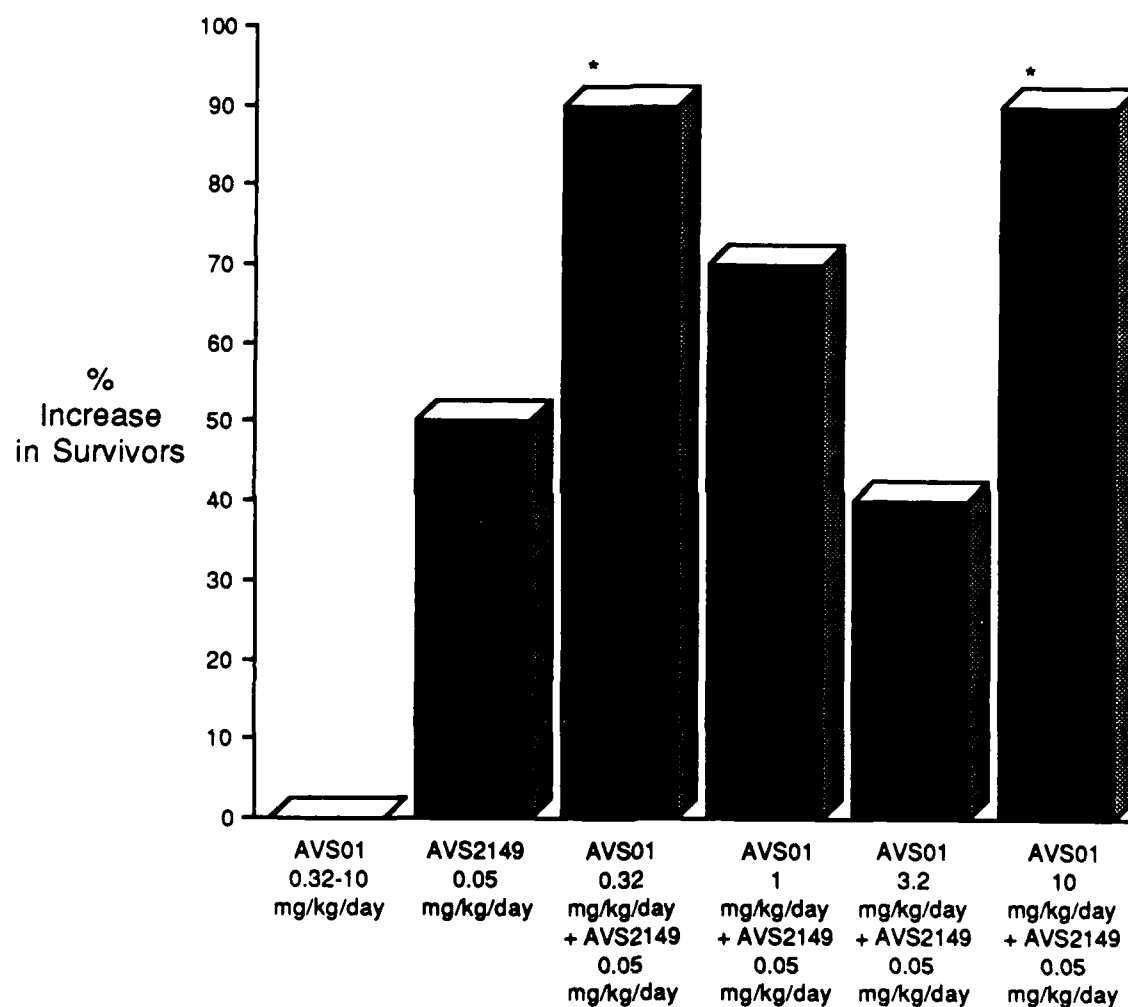


Figure X-5. Effect of AVS01 (0.32-10 mg/kg/day) + AVS2149 (0.05 mg/kg/day) on PTV Titer Reduction in Sera of Infected Treated Mice



*P<0.05 **P<0.01 compared to AVS2149 0.05 mg/kg/day.

Figure X-6. Effect of AVS01 (0.32-10 mg/kg/day) + AVS2149 (0.05 mg/kg/day) on Survivor Increases in Infected Treated Mice



*P<0.05 compared to AVS2149 0.05 mg/kg/day.

XI. PUBLICATIONS AND PRESENTATIONS.

The following summarizes the publications and presentations resulting from data generated in this project.

Publication

1. Sidwell, R.W., J.H. Huffman, B.B. Barnett, and D.Y. Pifat. (1988) In vitro and in vivo phlebovirus inhibition by ribavirin. *Antimicrob. Ag. Chemother.* (in press).

Presentations

1. Sidwell, R.W., J.H. Huffman, B.B. Barnett, and D.Y. Pifat. *In vitro* and *in vivo* phlebovirus-inhibitory activity of ribavirin. Abst. R32.14, VII International Congress of Virology, Edmonton, Alberta, Canada. August 9-14, 1987.
2. Sidwell, R.W., J.H. Huffman, B.B. Barnett, and D.Y. Pifat. Effects of a carboxamide derivative of ribavirin on *in vitro* and *in vivo* phlebovirus infections. Abst. C-3, Annual Mtg., Intermountain Branch, American Society for Microbiology, Provo, Utah. October 24, 1987.
3. Huffman, J.H., R.W. Sidwell, B.B. Barnett, and D.Y. Pifat. Effects of a 3-carboxamide derivative of ribavirin on phlebovirus infections. Abst. 16, IVth Annual Conference, Inter-American Society for Chemotherapy, Clearwater Beach, Florida. January 10-13, 1988.

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