expanding gases within the float. These will create additional sound-reflecting targets which may well be an added cause of the marked increase in scattering intensity observed as the scattering layers rise.

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3. A. K. Totton has inspected our poorly relaxed material and rendered a tentative identification of *Nanomia bijuga* (Delle Chiaje) 1841. The genus was formerly known as *Stephanomia*.
4. Total pressure of dissolved gases in the sea is taken as approximately 1 atm throughout the water column. Partial pressure of dissolved CO is taken as essentially zero. Diffusion gradient, as used in this report, refers to diffusion across the total float wall which varies in thickness generally within 20 to 50 μ.
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12. We thank Lt. R. L. McCampbell and the men of the *U.S.S. Marysville* and M. Sullivan, H. Kiner, and M. Taylor of the Navy Electronics Laboratory for assistance in obtaining data at sea. Dr. D. Jameson provided facilities at San Diego State College for histological sectioning. Drs. T. Enns, E. Hemmingsen, and P. Hansen gave helpful advice and discussion, and P. F. Scholander, E. Douglas, G. Curl, and E. C. Buffington critically read the manuscript. Contribution from Scripps Institution of Oceanography.

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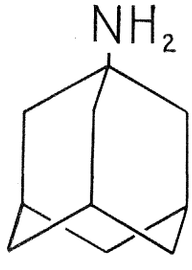
Antiviral Activity of 1-Adamantanamine (Amantadine)

Abstract. *1-Adamantanamine (amantadine) causes a selective, reproducible, dose-related inhibition of influenza infections in tissue culture, chick embryos, and mice. The compound is not virucidal and appears to act by interfering with the penetration of the host cell by the virus. In influenza infections of mice, greatest efficacy occurs with treatment at the time of infection; however, there is significant antiviral activity with treatment delayed up to 72 hours after infection. Virus inhibition is not complete and survivors are immune to a challenge infection with the original infecting virus.*

1-Adamantanamine (amantadine), a stable, colorless, crystalline amine with an unusual symmetrical structure,



reproducibly and selectively inhibits influenza viruses in tissue culture, chick embryos, and mice. The compound studied is the water-soluble salt, amantadine hydrochloride.

Most of the tissue culture studies were carried out by plaque-inhibition techniques similar to those described (*1*), although hemagglutination inhibition and hemadsorption inhibition methods were also used. Amantadine hydrochloride inhibited virus multiplication of four strains of influenza A, one of A′, three of A-2, one of C, and the Sendai strain of parainfluenza. Certain other myxoviruses were resistant, including two strains of influenza B, Newcastle disease virus, mumps, and parainfluenza strains 1, 2, and 3. A variety of RNA and DNA viruses were also insensitive. Amantadine hydrochloride showed a dose-responsive relationship in its effect on sensitive influenza strains propagated in chick fibroblasts with a maximum inhibition of 25 μg per milliliter and significant inhibition at 1 to 2 μg per milliliter. In one-step multiplication cycle experiments the maximum effect of the compound was a one-log drop in virus production. No toxicity to chick-embryo fibroblasts was seen at 30 μg per milliliter; at 100 μg per milliliter toxicity was manifested only by retardation of cell growth rate. Antiviral activity was also demonstrated in cultures of monkey kidney cells, canine kidney cells and human amnion cells.

Table 1. Length of treatment and effect of specific antiserum on the antiviral activity of amantadine hydrochloride against influenza A-2, Jap. 305.

Amantadine-HCl (20 μg/ml)	Duration of treatment (hr)	Specific antiserum*	Virus increase at 24 hours −(− log HA titer)
−	−	−	2.29
+	2 †	−	1.77
+	24 ‡	−	1.21
−	−	+	1.90
+	2 †	+	1.08

* Added 1.75 hours after virus. Cultures were washed 2 hours after virus and incubated at 37°C for 24 hours. † Compound added 15 minutes before virus. Cultures were washed 2 hours after virus to remove compound and incubated at 37°C for 24 hours. ‡ Added 15 minutes before virus and present for whole incubation period.

Amantadine hydrochloride caused no inactivation of viruses held in contact with concentrations of 1 to 25 μg per milliliter for 24 hours at 37°C; at 100 μg or more per milliliter, a number of viruses were inactivated, including some whose multiplication in tissue culture was not inhibited by 25 μg per milliliter. Thus direct inactivation of virus by amantadine hydrochloride is not a major factor in the activity of the compound in tissue culture. The adsorption of influenza A to chick embryo fibroblasts and red blood cells was not affected by 25 μg of amantadine hydrochloride per milliliter nor was the enzymatic release of virus from red blood cells affected by the compound.

Preliminary tissue culture studies have indicated that amantadine hydrochloride blocks or slows penetration of the host cells by the virus. The evidence for this is as follows. Cultures of chick embryo cells were infected with a multiplicity of 20 so that all cells were infected at the beginning of the experiment. To obtain high antiviral activity amantadine hydrochloride had to be present within 5 minutes with cells and virus held at 37°C and within 60 minutes with cells and virus held at 23°C. All cultures were incubated for 24 hours at 37°C immediately after adding compound, and after 24 hours virus production was measured by hemagglutinin titration on cells and culture fluid. Similar results were obtained with homologous antiserum under identical conditions. Partial reversal of the antiviral activity of amantadine hydrochloride was accomplished by washing the infected cell layers during the incubation period. The greatest antiviral activity occurred when the compound was present during the entire period of incubation.

Results with 2-hour and 24-hour amantadine hydrochloride treatment of virus in tissue culture are shown in Table 1. The results indicate that susceptible virus strains are absorbed normally to cells treated with amantadine hydrochloride and nontreated cells. However, in the presence of the compound, the viruses do not penetrate the cell and thus remain susceptible to inactivation by antibody for a greater length of time than the virus on control cells. In plaque inhibition experiments, amantadine hydrochloride could be added as late as 24 to 48 hours after virus inoculation and still prevent the formation of normal-sized plaques because further spread of infection to noninfected cells was halted.

The antiviral activity of amantadine hydrochloride was demonstrated by injecting the compound into either the yolk sac or the allantoic cavity 30 minutes before the injection of approximately one EID_{50} (egg infectious dose) of influenza virus into the allantoic cavity. With groups of 13 to 60 eggs per treatment, the number of eggs showing hemagglutination in a spot test 48 hours after infection with influenza A, strain PR-8, was decreased from 76 percent for the controls to 23 percent by the well-tolerated dose of 500 μg of amantadine hydrochloride per egg injected into the yolk sac. With influenza A, strain WS, the corresponding change was from 65 percent for the controls to 33 percent for the treated group. With no virus multiplication the infecting EID_{50} of virus did not cause hemagglutination in the spot test. When the death of the embryo was used as the criterion of virus infection, the survivors of an infection with influenza A-2, strain Jap. 305, increased from zero for the control group to 50 percent for the treated group. Similar results were found in tests in which the compound was administered by way of the allantoic cavity.

Table 2. Effects of graded doses of amantadine hydrochloride on experimental influenza infections in mice. The mice were observed for 14 days after infection. The virus (0.05 ml) was administered intranasally in white mice lightly anesthetized with ether. There were 50 to 100 mice in each group for strains S-15 and A^2/AA, and 20 to 60 mice in each group for strain WS.

Amantadine HCl * (mg/kg)	Survivors (%)	MSD †
Influenza A, S-15 (Swine)		
40	70	12.5
10	62	12.4
2.5	43	10.7
0.62	25	9.1
0	13	8.1
Influenza A WS		
40	74	12.8
10	56	11.4
2.5	21	9.7
0.62	21	8.3
0	15	7.8
Influenza A-2 A^2/AA (Asian)		
40	58	11.9
10	38	10.7
2.5	33	10.3
0.62	26	9.4
0	14	8.7

* The amantadine hydrochloride was given intraperitoneally 30 minutes before virus at 4-hour intervals for 48 hours. † Mean survival day $= \frac{[\Sigma(\text{No. of mice alive each day to day 14})]}{(\text{Total No. of mice in the group})}$

Table 3. Effect of delayed treatment with amantadine hydrochloride on experimental infections of mice with influenza A-2 strain A^2/AA.

Treatment time* (hr)	Route of compound	Group (No.)	Survivors (%)	P†	MSD	P‡
24–72	IP	60	50	0.01	10.9	
Control		119	28		9.2	
48–160	IP	60	27	0.01	9.0	0.1
	Oral	60	45	0.001	10.6	0.01
	Oral §	60	43	0.001	10.6	0.01
Control		60	5		7.2	
72–120	IP	57	58	0.05	11.2	
Control		117	41		9.8	
96–192	IP	160	42	0.2	11.0	
Control		160	34		9.2	

* Intranasal instillation of virus (Table 2) at the start; 40 mg of compound per kilogram of body weight every 4 hours after infection as indicated. † Chi-square analysis. ‡ Analysis of variance. § 10 mg/kg every 4 hours.

The activity of amantadine hydrochloride in mice was similar to that in tissue culture and in chick embryos. When administered intraperitoneally (IP), subcutaneously, or orally, starting 30 minutes before infection, one or more injections of the compound at 0.6 to 40 mg per kilogram of body weight provided significant protection of mice infected intranasally with influenza virus. Typical dose-related responses of three influenza viruses to multiple-dose treatment are shown in Table 2. Comparable results were obtained with a single treatment of the compound administered 30 minutes before infection. At these infecting doses (3 to 4 LD_{50}) of virus, the protection was shown both as an increase in survivors and as an increase in survival time of those mice eventually succumbing to the disease. At higher virus concentrations, 20 LD_{50} or greater, amantadine hydrochloride no longer increased the rate of survival, but there was still a significant increase in survival time.

Significant protection of mice infected with influenza A-2 (strain A^2/AA) was obtained by both intraperitoneal and oral treatment with amantadine hydrochloride after infection. Results of tests with delayed treatment are shown in Table 3 and indicate that significant protection was obtained with treatment begun up to 72 hours but not 96 hours after infection. As shown by the doses administered at 48 to 160 hours, oral administration provided greater antiviral efficacy than did intraperitoneal injection. Treatment after infection with a single dose was not effective. In some experiments the survivors from the "infected and treated" group were challenged again with the original strain of infecting virus and were found to be immune to reinfection. Noninfected controls treated with amantadine hydrochloride were fully susceptible to subsequent challenge with virus. This, plus examination of lung tissue, indicated that amantadine hydrochloride had not completely suppressed the disease but had modified the infection to a less severe one in which normal antibody development resulted in immunity of the survivors.

The acute lethal doses (LD_{50}) of amantadine hydrochloride for mice used in these studies was 233 mg per kilogram of body weight intravenously and 1080 mg by mouth. As shown in Tables 2 and 3, significant antiviral effects were obtained at concentrations below the maximum well-tolerated doses of 50 mg per kilogram of body weight intraperitoneally and 60 mg orally when given 13 times at 4-hour intervals.

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