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Rubella Virus: Inhibition in vitro by Amantadine Hydrochloride

Abstract. *Amantadine (or 1-adamantanamine) hydrochloride, a compound reported to be active against influenza viruses and Sendai virus, inhibited the growth of rubella virus in tissue culture. The antiviral activity appears at an early phase of the infection and is not due to direct inactivation of the virus.*

Amantadine hydrochloride (1-adamantanamine hydrochloride, EXP 105-1) inhibits influenza (A, A_2, and C) and Sendai viruses in vitro and in vivo (*1*). Although only a limited number of viruses from the rather extensive group examined were susceptible, nevertheless we tested the activity of amantadine hydrochloride against certain other viruses and observed that rubella virus was inhibited in vitro.

Continuous cultures of monkey kidney cells of the LLC MK_2 line (*2*) were grown and maintained with Medium 199 (*3*) containing 1 percent horse serum. Cell cultures, untreated or treated with amantadine hydrochloride (*4*), were infected with the WM strain of rubella virus (*5*), and at appropriate times samples were withdrawn and titrated. Rubella virus was assayed by its capacity to interfere with the development of the more easily recognizable, typical cytopathology seen after superinfection with 100 tissue-culture 50-percent infective doses ($TCID_{50}$) of ECHO-11 virus (*6*) applied as previously described (*5*).

Judged by visible microscopic morphology, LLC MK_2 cells could tolerate amantadine hydrochloride at a concentration of 31.2 μg/ml but not 62.5 μg/ml. In view of the complex, indirect method of assaying rubella virus by its capacity to interfere with ECHO-11 cytopathology it was also necessary to establish that ECHO-11 virus was not inhibited by amantadine hydrochloride. Typically, in 4 days an inoculum of approximately 100 $TCID_{50}$ of ECHO-11 virus grew equally in the presence or absence of amantadine hydrochloride. The final $TCID_{50}$ was $10^{-7.5}$ in the presence of 31.2 μg of amantadine hydrochloride per milliliter compared with $10^{-7.3}$ in control cultures.

Data for three separate experiments showing the inhibitory effect of amantadine hydrochloride on the growth of rubella virus in vitro are presented in Table 1. Drug was added just before inoculation of the cell cultures with rubella virus. A period of 5 hours was allowed for virus adsorption before the cells were washed and the medium was replaced to maintain appropriate concentrations of inhibitor. Samples for titration were taken at 0, 3, and 6 days, these times being based on the growth characteristics of rubella virus described elsewhere (*7*). Concentrations of amantadine hydrochloride inhibiting virus growth did not have detectable direct action against free rubella virus when tested after a contact period of 12 hours. Since the time these data were presented (*8*) the finding has been confirmed elsewhere (*9*). It should also be mentioned that, although the results in Table 1 are presented as interference with the cytopathogenic effect of ECHO-11 virus, inhibition of the less obvious cytopathology directly caused by rubella virus, which has also been observed (*10*), parallels the interference findings. Thus the inhibition of rubella virus demonstrated in Table 1 appears not to be an artifact of the indirect assay system.

The influence of time of treatment on the antiviral effectiveness of amantadine hydrochloride was also studied (Table 2). Results are given for two separate experiments showing that when added during the first 3 hours amantadine hydrochloride clearly inhibits replication of rubella virus. When treatment was initiated 5 or more hours after virus inoculation, no protection was observed; this suggests that, as is the case with influenza virus (*1*), amantadine hydrochloride may be inhibiting rubella virus in vitro at an early stage in the infectious process. On the other hand the compound failed to inhibit Chang's strain of lipovirus or the Edmonston strain of measles virus (*11*).

Amantadine hydrochloride appears to have a fairly circumscribed range of antiviral activity embracing several types of influenza virus (*1*), Sendai virus (*1*), and rubella virus. This evidence, as well as its size (*12*) and nucleic acid species (*7*), suggests that rubella virus is related to the myxoviruses. Also amantadine hydrochloride may possibly prove effective in the chemotherapy of rubella as a disease.

Table 1. Amantadine hydrochloride (EXP 105-1) inhibition of rubella virus in tissue culture. Virus yields from three experiments are expressed as negative $\log_{10}$ of the 50 percent interfering dose (Int. D_{50}) (*5*, *6*). Designated concentrations of drug were added before rubella virus and maintained thereafter. After a virus adsorption period of 5 hours cultures were washed, and the medium including drug was replaced.

Day	Rubella virus yield (neg. $\log_{10}$ Int. D_{50})		
	EXP 105-1		Untreated
	31.2 μg/ml	15.6 μg/ml	
0	<1, 1, 2*	1, <1, 2*	<1, 1, 1.8*
3	<1, 1, 1*	1.8, 1, 2.3*	2.3, 2, 2.5*
6	1, 1.3, <1*	3, 1.8, 2.7*	3.3, 3.5, 3.5*

* Virus inoculum was not removed.

Table 2. Effect of delayed addition of amantadine hydrochloride (31.2 μg/ml) at various times after rubella virus infection. First samples were removed at the indicated time of drug addition. Values are given for two experiments, a and b.

Time of addition (hr)	Rubella virus yield (neg. $\log_{10}$ Int. D_{50})			
	First sampling		96 hr	
	a	b	a	b
Control (5 hr)	1.5,	1.0	3.3,	4.0
0	1.3,	1.7	1.5,	1.5
1	1.5		2.0	
3	1.3		1.5	
5	1		3.3,	3.0
10, 12	1.3,	1.5	4.0,	3.3
24		1.7		3.3
36		2.0		3.5
48		2.5		3.3

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Compact Bone Deficiency in Protein-Calorie Malnutrition

Abstract. *Children hospitalized for acute protein-calorie malnutrition in Guatemala City were not delayed in ossification status as compared with Guatemalan Indian children on their customary low-protein diets, but were markedly and often dramatically deficient in cortical bone.*

In reviewing radiographs made on admission to the clinic of 95 infants and children hospitalized for protein-calorie malnutrition in Guatemala City, we were greatly impressed by the radiolucent quality of both round and tubular bones. Compared with the degree of skeletal maturity attained, these malnourished patients appeared to show the kind of bony rarefaction reported in experimental protein-calorie malnutrition in animals (*1*), and they presented a clinical picture of juvenile osteoporosis.

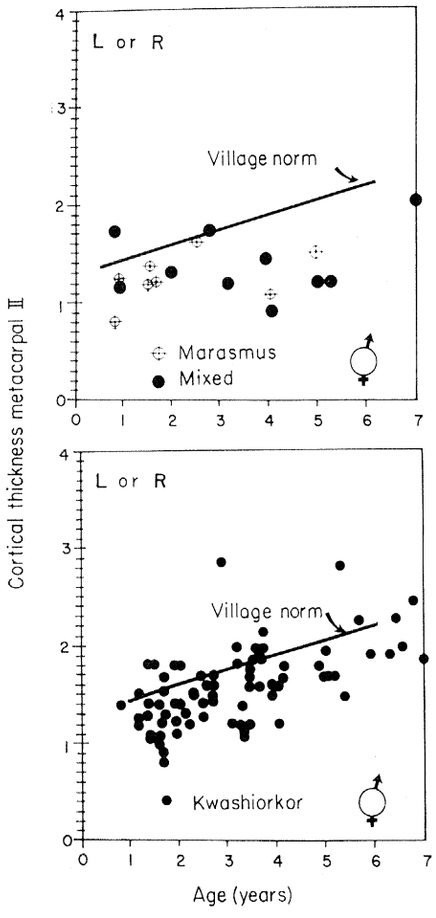

Fig. 1. Reduced thickness of cortical bone in the second metacarpal in 95 children with protein-calorie malnutrition.

For more critical comparison, we studied the children living in the two Guatemalan Indian villages of Santa Cruz Balanyá and Santa María Cauqué, where the diet was characterized by low average intake of animal protein (*2*). We studied these children simultaneously with the hospitalized patients during 1959–1963. Ossification status in both patients and controls was measured objectively as the number of postnatal ossification centers visible in postero-anterior hand-wrist radiographs (*3*). Compact bone was measured simply as the total thickness of cortical bone at midshaft on the second metacarpal (*4*). Radiographic enlargement approximated 1 percent throughout, and therefore no correction was used. Replicability of both ossification status and thickness of cortical bone exceeded 0.98.

Ossification status in the 95 boys and girls with protein-calorie malnutrition was not retarded compared with that in the 694 children from the two Indian control villages. By the Chi-squared (χ^2) test the number of hospitalized children above and below the sex-specific village trend lines did not differ significantly from chance.

In contrast to ossification status, the thickness of cortical bone was greatly diminished in the children hospitalized for protein-calorie malnutrition. As shown in Fig. 1, 75 percent of the hospitalized patients fell below the village trend for both sexes for cortical thickness. Deficiency of compact bone was observed for all three of the clinically-defined subgroups of protein-calorie malnutrition (kwashiorkor, kwashiorkor-marasmus, and marasmus) as defined by Scrimshaw and Béhar (*5*). The more marasmatic the child, the greater the degree of compact bone deficiency.

The fact that some of the hospitalized patients had no more compact bone at 4 to 6 years than might be expected for a 1-year-old Guatemalan Indian child, despite comparable ossification status, led us to consider that the cause might be an actual bone loss rather than simple failure to gain bone. While this supposition could not be tested retrospectively, it was confirmed by study of serial, longitudinal radiographs of the patients, taken during the period of hospitalization. Approximately 10 percent exhibited a concomitant bilateral increase in measurable bone length and decrease in cortical thickness during the recovery period, thus giving metrical support to the hypothesis of cortical dumping in acute protein-calorie malnutrition.

Guatemalan Indian children suffering from extreme protein-calorie malnutrition are therefore no further delayed in ossification status than children on low-protein diets in the control villages, though the latter are obviously retarded in comparison with middle-class Ohio-born American white children (*6*). The fact that in cases of acute protein-calorie malnutrition at comparable "bone age" there is a marked deficiency in compact bone, especially in the children with marasmus, suggests actual bone loss as well as simple failure to gain (*7*).

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