

Rhinovirus Inactivation by Aqueous Iodine *in Vitro* and on Skin (40990)

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Abstract. Iodine has been found to be virucidal against rhinovirus on human skin. The kinetics of *in vitro* rhinovirus inactivation by iodine and the residual virucidal activity of iodine on skin were examined in this study. Maximum inactivation of rhinovirus *in vitro* occurred within 3 sec with aqueous iodine concentrations of ≥ 100 $\mu\text{g/ml}$. Aqueous iodine concentrations of ≤ 10 $\mu\text{g/ml}$ were ineffective. Inactivation of rhinovirus on the skin of the hands was accomplished by applying iodine in concentrations of 20,000 μg (2%) or 10,000 μg (1%) iodine/ml. Viral inactivation took place after 3 min of exposure to skin treated with the higher concentration, but took up to 20 min with the 1% solution. Viral inactivation with 1% iodine followed first-order kinetics. The virucidal activity of 2% iodine on hands persisted for up to 2 hr. Iodine will be used to attempt to interrupt hand contact transmission of rhinovirus infection.

Recently, rhinovirus has been recovered from the hands of persons with colds and from objects in their environment (1,2). This has led to the suggestion that rhinovirus infections may spread by contamination of the hands of susceptible individuals followed by accidental self-inoculation of the nose or eye. If true, it may be possible to interrupt spread of rhinovirus colds by disinfection of the hands of infected and susceptible persons.

In a previous study, a variety of germicidal compounds applied to the hands were tested for virucidal activity against rhinovirus (3). Of the compounds examined, aqueous iodine exhibited the most effective virucidal activity against rhinovirus on the skin. The current study investigates the conditions under which aqueous iodine is effective in inactivating rhinovirus and examines how long after application to skin iodine maintains virucidal activity. This was done in preparation for trying to interrupt rhinovirus transmission in volunteers and under natural conditions by treatment of the hands with iodine.

Materials and methods. *Virus.* A laboratory strain of rhinovirus type 29 (in passages WI 6, HeLa 3 or WI 7, HeLa 4) was diluted 1:10 in Hanks' balanced salt solution (HBSS) for use in the experiments. Infectivity titrations were performed in

HeLa cell cultures in screw-capped tubes. The cultures were maintained on 2% fetal calf serum in minimum essential medium and were incubated in roller drums at 34°. Tests were read daily for viral cytopathic effect.

Aqueous iodine. A stock of aqueous iodine solution containing 5 g of iodine and 10 g of potassium iodide/100 ml of water was diluted in water to the desired concentrations of elemental iodine.

Experimental design. In vitro studies. One part rhinovirus in HBSS was added to nine parts aqueous iodine in desired concentrations. After incubation at 24° for varying intervals of time, aliquots were removed for infectivity titrations. Virus suspended in HBSS was used as a control.

In a second experiment, the effect of temperature was examined. One part rhinovirus was added to nine parts aqueous iodine solution and the mixtures were incubated for 5 min at varying temperatures. Infectivity titrations were then performed on test solutions and a control solution of virus in HBSS.

Rhinovirus inactivation on skin. Volunteers washed their hands with Ivory soap and dried them with paper towels. One milliliter of aqueous iodine solution was placed in the palm of one hand. The volunteer then rubbed his hands vigorously together in the

same fashion as applying hand lotion. One milliliter of iodine was then placed in the other palm and the rubbing was repeated. The solution was allowed to dry for 3 min. As a control treatment, water was applied to the hands of other volunteers in the same way.

Three minutes after application of iodine or water, contamination of the hand was accomplished by rubbing the fingers up to the second interphalangeal joint in 0.3 ml of rhinovirus in HBSS. The procedure was repeated for the other hand. Virus was left in contact with the pretreated skin for varying time intervals ("Duration of Virus/Treated Skin Contact"). Then the presence of virus was determined by rinsing the fingers of each hand into a sterile petri dish with 2.5 ml beef infusion broth containing 1% bovine serum albumin. Approximately 1.5 ml was recovered and then added to 1 ml of collecting broth containing penicillin (50 units/ml) and kanamycin (50 $\mu\text{g}/\text{ml}$). Infectivity titers were performed on the rinse fluids.

Additional studies were performed in the same manner using virus suspended in nasal mucus. One part of undiluted virus was suspended in nine parts nasal mucus. The mucus had been shown in preliminary testing not to neutralize the virus. A total of 0.3 ml of the mixture was used to contami-

nate both hands of the volunteer which had been treated with either iodine or water.

Residual virucidal activity. The residual virucidal activity of iodine on skin was examined by allowing volunteers to continue normal activity (other than washing their hands) for 1 or 2 hr after hand treatment with iodine. The fingers were then contaminated with rhinovirus; the survival of virus was determined after 15 min of virus/treated skin contact. Control treatments of the hands with water accompanied each experiment.

Results. Effect of concentration, duration of exposure, and temperature. Concentrations of 1000 and 100 μg iodine/ml inactivated rhinovirus within 3 sec (Table I). With 10 and 1 $\mu\text{g}/\text{ml}$ concentrations, the virus was not inactivated even when exposed for the maximum period of 30 min. Temperatures ranging from 0 to 37° did not affect viral inactivation. At all temperatures there was complete inactivation by 100 $\mu\text{g}/\text{ml}$ of iodine, while virus persisted after exposure of 10 $\mu\text{g}/\text{ml}$.

Rate of rhinovirus inactivation on the hands. When the hands were treated with a concentration of 10,000 $\mu\text{g}/\text{ml}$ (1%) of iodine, the length of time that iodine was in contact with the treated skin was important (Table II). In only 1 of 28 tests with contact intervals of 10 min or less was virus com-

TABLE I. EFFECT OF DURATION OF EXPOSURE AND TEMPERATURE *IN VITRO* INACTIVATION OF T 29 RHINOVIRUS BY VARYING CONCENTRATIONS OF AQUEOUS IODINE

		Concentration (μg iodine/ml)				
		Control	1	10	100	1000
Duration of exposure	3 sec	5.0 ^a	3.5	4.0	0	0
	5 min	NT ^b	4.0	4.5	0	0
	10 min	NT	4.0	4.5	0	0
	15 min	NT	3.5	3.5	0	0
	30 min	3.5	3.5	4.0	0	0
Temperature (Centigrade)	0°	2.8 ^c	NT	2.3	0	0
	10°	3.0	NT	2.0	0	0
	20°	2.5	NT	1.5	0	0
	34°	2.0	NT	2.0	0	0
	37°	2.5	NT	1.4	0	0

^a Infectivity titer ($\log_{10}/\text{TCID}_{50}/\text{ml}$).

^b Not tested.

^c Geometric mean titer \log_{10}/ml of triplicate determinations (GMT = antilog of sum total of samples \times their respective log titers/total of all samples).

TABLE II. RATE OF RHINOVIRUS INACTIVATION BY AQUEOUS IODINE ON THE SKIN OF THE HANDS

	Duration of virus/treated skin contact (min)	No. of tests	Number of tests with indicated viral titers ^a						
			Undet. ^b	Undil. ^c	0.5	1.0	1.5	2.0	2.5
10,000 μ g iodine/ml	0.5	5			1		1	2	1
	1.5	3					1	1	1
	3.0	11			2	2	2	1	4
	5.0	3		1			1	1	
	7.0	2			1	1			
	10.0	4	1				3		
	15.0	6	4				1	1	
20,000 μ g iodine/ml	20.0	22	21		1				
	3.0	15	12	1	2				
	5.0	23	19	2	2				
	7.0	3	3						
	10.0	17	14	2	1				
Control (water)	15.0	2	2						
	0.5	6				1		3	2
	3.0	8			1		3	2	2
	5.0	3						1	2
	10.0	7			1		1	1	4
	15.0	4			1	1		2	
	20.0	4					3		1

^a Viral titers given as log₁₀ TCID₅₀/0.1 ml to allow comparisons with results with "undiluted" samples.

^b No virus detected in tubes inoculated with 0.1 ml of undiluted hand rinse.

^c Virus detected in one of two tubes inoculated with 0.1 ml of undiluted hand rinse.

pletely eliminated from the skin. In contrast, no virus was recovered from 25 of 28 samples from hands in which there had been 15 or 20 min of contact ($P = <0.001$, Fisher exact test). Analysis (not shown) of these results showed a logarithmic decrease

in virus survival with time, indicating viral inactivation followed first-order kinetics.

With an iodine concentration of 20,000 μ g/ml (2%), virus was undetectable in 12 of 15 tests with a 3 min contact compared to none of 11 for this contact period with

TABLE III. RATE OF INACTIVATION OF RHINOVIRUS SUSPENDED IN NASAL MUCUS BY AQUEOUS IODINE ON THE SKIN OF THE HANDS

	Duration of virus/treated skin contact (min)	No. of tests	Number of tests with indicated viral titers ^a						
			Undet. ^b	Undil. ^c	0.5	1.0	1.5	2.0	2.5
20,000 μ g iodine/ml	3.0	2				1	1		
	5.0	2			1		1		
	10.0	6	4	1	1				
	15.0	6	4		2				
Control (water)	3.0	2							2
	10.0	2			1	1			
	15.0	2				1	1		

^a Viral titers given as log₁₀ TCID₅₀/0.1 ml to allow comparisons with results with "undiluted" samples.

^b No virus detected in tubes inoculated with 0.1 ml of undiluted hand rinse.

^c Virus detected in one of two tubes inoculated with 0.1 ml of undiluted hand rinse.

10,000 $\mu\text{g/ml}$ (1%) iodine ($P = <0.001$ Fisher exact test). Virus was undetectable in 50 of 60 tests performed with the higher iodine concentration at contact intervals from 3 to 15 min.

In none of 32 tests with water treatment as a control was there complete inactivation of virus.

Inactivation of rhinovirus in nasal mucus. When virus suspended in nasal mucus was exposed to hands which had been treated with iodine at a concentration of 20,000 $\mu\text{g/ml}$, 3 and 5 min were insufficient to reliably inactivate it (Table III). Virus was recovered in all of four tests done with 3 or 5 min of contact compared to 4 of 12 with contact intervals of 10 or 15 min ($P = <0.04$ Fisher exact test). Thus, longer periods of contact were required to inactivate rhinovirus suspended in nasal mucus than for virus suspended in HBSS.

Residual virucidal activity on skin. Residual activity was present for 1 and 2 hr following treatment of hands with iodine at a concentration of 20,000 $\mu\text{g/ml}$. No virus was recovered from 10 hands treated 1 hr before viral contamination and from 18 hands treated 2 hr before contamination. Virus was recovered from 4 of 4 water-treated control hands tested simultaneously.

Discussion. Iodine is one of the most potent of the available germicides (4). Recently, Hendley *et al.* (3) found that iodine inactivated rhinovirus on human skin. In the current investigations, kinetic studies showed that rhinovirus is maximally inacti-

vated within 3 sec *in vitro* by 100 μg iodine/ml. As a hand treatment, 10,000 μg iodine/ml exhibited first-order kinetics in inactivation of rhinovirus in HBSS. An iodine concentration of 20,000 $\mu\text{g/ml}$ applied to hands was effective in killing rhinovirus in nasal mucus within 15 min. Residual virucidal activity persisted on skin for up to 2 hr.

In future studies, the effectiveness of treatment of the hands with 20,000 $\mu\text{g/ml}$ of aqueous iodine in interrupting transmission of rhinovirus infection in volunteers will be examined. If iodine hand treatment prevents person-to-person spread of the virus in volunteers, it will then be tested in families during fall rhinovirus outbreaks in the home. In this way iodine will serve as a tool to investigate the theory of hand contamination/self-inoculation transmission of rhinovirus under natural conditions (1, 2). Iodine may also serve as a standard of comparison for more practical virucidal hand treatments with acceptable cosmetic properties.

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