Effect of Heat and of pH on Strains of Coxsackie Virus. (18272)

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This report describes studies of the thermostability and resistance to pH of three strains of Coxsackie virus of Group A and two strains of Group B. Results of earlier studies of MM and Lansing poliomyelitis virus are included for comparison.

Procedure. Infected mouse brains or leg muscles that had been stored in a dry-ice box were suspended and clarified by centrifugation. Immediately after preparation, 0.5 ml volumes of the suspensions were sealed in 3 ml lyophile bulbs for immersion in units of 3, in the heating bath. Immediately before and after heating they were chilled in an ice and water bath. For each suspension, one unit was held without heating. The samples were pooled on the day heated and tested in mice on the following day. To determine the time required for the samples to reach the temperature of the bath, a constantan-nickel thermocouple in 0.5 ml of distilled water in a lyophile bulb was used. The lag time was relatively constant about 60 seconds. The pH values of each original suspension and of a heated sample were determined electrometrically. The values lay between 6.3 and 7.4, many between 6.9 and 7.2. Heating of a given suspension did not change the pH. Suspensions were prepared in a 10% solution of beef-infusion broth in physiologic salt solution (broth-salt solution) and in distilled water. They were heated for 30 minutes.

Infectivity was tested by intraperitoneal injection of 0.05-ml amounts into 2- to 6-day-old suckling mice of the Albany standard strain; from 6 to 9 for each dilution tested. They were observed for 2 weeks, but only those affected from the second to the seventh day (for those receiving No. 5096 (Group B), from the second to the tenth day) are included in the results reported.*

MM virus was suspended in distilled water or in physiologic salt solution containing 10% normal horse serum. Infectivity was tested by intracerebral injection of 0.03 ml into 10- to 12-gram mice which were observed for 7 days. Mice dying on the day of injection or the following day were excluded from the results. Lansing virus was suspended in distilled water and tested by intracerebral injection of 0.03 ml into 10- to 12-gram mice which were observed for 21 days. For the tests of pH effect, 10^{-1} mouse-brain suspensions in broth-salt solution were further diluted to 10^{-2} by adding 0.5 to 4.5 ml of solutions of controlled pH. The diluents were Michaelis's veronal-acetate and Sorensen's glycocoll-sodium hydroxide buffers and 0.1 N and 0.01 N hydrochloric acid solutions. They were prepared at approximately the desired pH, and sterilized in a boiling water bath. In each test a 10^{-2} suspension in broth-salt solution was included as a control. The suspensions were held at room temperature under a dark cloth. Tests were made after 1 and 7 days, a fresh container being opened for

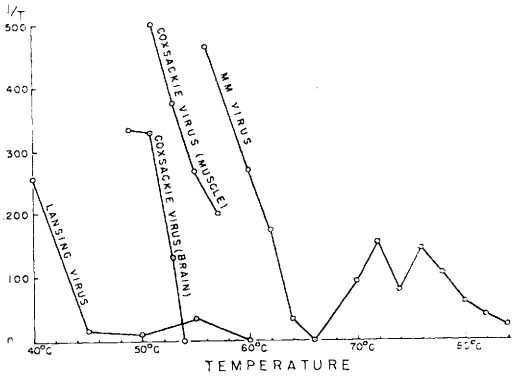

FIG. 1.

Effect of heat on infectivity of Coxsackie virus (Group A, Type 1, No. 48249) as compared with MM and Lansing viruses. 10^{-1} dilutions were heated for 30 min.

* Impressions of the activity of virus based on late and early effects were not significantly different from those based on early effects alone. Late paralysis and death were not entirely nonspecific, however. In nearly 900 mice injected with No. 49190 (Group A, Type 2) no effects were observed after the seventh day, whereas roughly 2 mice of every 100 injected with Nos. 48249 and 49191 (Group A, Types 1 and 2) and No. 5096 (Group B), were found paralyzed or dead on the 8th to the 14th day.

each test. The method of testing for infectivity was that described for the heated material. The pH of a sample withdrawn from each suspension on the day of the infectivity test was determined electrometrically. Toxicity of the diluents was ruled out by tests in suckling mice of virus-free samples of each at the extremes of pH used. No growth occurred on blood plates streaked with each test mixture at the time the sample for the pH test was taken.

Results. Effect of heat. The thermosusceptibility of 5 strains of Coxsackie virus was very nearly the same and is in the range of that of poliomyelitis, Y-SK, and mouse encephalomyelitis viruses(1). After 30 minutes at temperatures between 53° and 55°C no infectivity for suckling mice was detected in 10^{-1} brain tissue suspensions in physiologic salt solution containing 10% beef infusion broth. With the exception of 1 strain, No. 49190 (Group A, Type 2), all withstood heating at 49°C with no apparent loss. The viruses were somewhat more susceptible when suspended in distilled water. Muscle-tissue suspensions lost activity at about the same rate per increment of temperature as the brain-tissue suspensions in the range 49° to 54°C, but retained definite activity at 57°C. (Tables I and II.)

MM virus was resistant to higher temperatures, resembling Columbia SK(2) and Mengo(3) viruses. A brain-tissue suspension of MM in distilled water lost a relatively small part of its activity at 56°C. Heating at 62° to 64°C destroyed most of the activity but traces remained even at 86°C. Lansing virus, on the other hand, lost much of its activity at 45°C but retained a trace at 55°C. The apparently greater resistance of muscle than brain suspensions of Coxsackie virus may be due in part to greater concentration. If that is true the results with Lansing virus are not directly comparable. To facilitate comparison the value 1/T(4) has been cal-

TABLE I. Effect of Heat on Coxsackie Virus. Group A. 10^{-1} Brain Tissue Suspension Heated for 30 Minutes.

Temp., °C	Diluent*	No. 48249 (Type 1)					No. 49190 (Type 2)						No. 49191 (Type 3)						
		No. of tests	Dilutions inj.				No. of tests	Dilutions inj.					No. of tests	Dilutions inj.					
			10^{-1}	10^{-2}	10^{-3}	10^{-4}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
N.H.†	B-S	5	38/38	38/38	25/37	3/31	7			45/45	52/54	35/54	5/29	7			39/39	53/54	31/53	12/45
	H_2O	8	61/61	52/59	37/58	9/61	3			23/23	22/23	11/24	1/24	3			24/24	22/23	15/24	5/22
49	B-S	3	23/23	20/22	18/22	2/16	2		15/15	9/15	3/15	0/15		2	8/8	7/7	14/14	14/16	6/8	0/7
	H_2O	2	15/15	15/15	10/16	1/10	1		2/8	0/8	0/8	0/7		1			7/7	7/7	2/8	0/7
51	B-S	4	20/20	22/23	6/22	1/15	2	10/13	3/14	1/4	0/7			2	17/17	15/15	9/16	4/8		
	H_2O	3	14/15	3/16	4/16	0/8	1	4/8	0/8	0/8				1	8/8	3/8	0/8	0/8		
53	B-S	4	16/30	4/32	0/22	0/5	3	10/22	1/23	0/24				4	0/31	0/31	0/32			
	H_2O	3	0/23	0/25	0/14									1	1/8	0/8	0/8			
54	B-S	1	0/8	0/8			2	2/15	1/8	0/14				2	0/16	0/16	1/14			
55	B-S	1	0/8	0/8			2	0/16	0/16	0/8				2	0/15	0/15	0/6			

Denominator = No. of mice inj.; numerator = No. dead, paralyzed, or missing during critical period.
* B-S = Physiologic salt solution containing 10% beef-infusion broth. H_2O = distilled water.
† Not heated.

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TABLE II. Effect of Heat on Coxsackie Virus. Group B. 10^{-1} Suspension Heated for 30 Min.

Temp., °C	Diluent*	No. 5096 No. of tests	No. 5096 Virus dil. inj. 10^{-1}	10^{-2}	10^{-3}	10^{-4}	No. 49683 No. of tests	No. 49683 Virus dil. inj. 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
N.H.†	B-S	4	23/23	31/31	5/8	2/8	2			16/16	14/16	6/16
	H_2O	1	21/21	23/23								
49	B-S	2	16/16	14/16	7/7		2		16/16	15/15	5/16	2/16
	H_2O	1	5/7	2/8								
51	B-S	1	5/7	1/7			2		8/15	5/16	1/16	
	H_2O	1	3/7	0/7								
53	B-S	2	2/16	0/16			2		0/16	0/16	0/8	
	H_2O	1	1/8	0/8								
54	B-S	1	0/8	0/8								
55	B-S	1	0/8	0/8			1		0/8	0/8		

Denominator = No. of mice injected; numerator = No. dead, paralyzed, or missing during critical period.

* B-S = Physiologic salt solution containing 10% beef-infusion broth.
H_2O = Distilled water.
† Not heated.

TABLE III. Effect of pH on Coxsackie Virus. Group B-Strain No. 5096. 10^{-2} Suspension Exposed at Room Temperature.

Diluent	No. of tests	Exposed 1 day pH	Exposed 1 day Dil. inj. 10^{-2}	10^{-3}	10^{-4}	10^{-5}	Exposed 7 days pH	Exposed 7 days Dil. inj. 10^{-2}	10^{-3}	10^{-4}	10^{-5}
HCl	2	1.12-1.16	0/16	0/16			1.14-1.15	1/15	0/15		
	2	2.28-2.29	16/16	16/16	13/16		2.25-2.28	16/16	12/16	2/16	
Veronal acetate	1	2.31	9/9	8/8	3/8		2.28	0/8	0/8	0/8	
	1	4.15	8/8	5/6	3/8		4.14	8/8	6/8	2/8	
Glycocoll-NaOH	2	8.02-8.28	16/16	14/16	12/16		8.10-8.11	15/15	11/16	7/16	
	2	9.31-9.38	16/16	15/15	12/15		9.06-9.15	6/16	4/16	0/8	
	1	9.47	8/8	6/8	4/8		9.70	0/8	1/8	0/8	
Broth-salt	3	7.07-7.25	24/24	16/16	16/24		7.20-7.42	23/23	17/24	7/24	

Denominator = No. of mice injected; numerator = No. dead, paralyzed, or missing during critical period.

culated and plotted against temperature for certain tests. Figure 1 shows the curves for a 10^{-1} suspension of Coxsackie, MM and Lansing viruses.

Results. Effect of pH. Coxsackie virus resembles poliomyelitis virus in its ability to withstand exposure to a wide range of pH(5, 6). All strains survived at room temperature for 1 day in suspensions at pH 2.3 to 9.4, and for 7 days at pH 4.0 to 8.0. No. 49190 (Group A, Type 2) and No. 5096 (Group B) survived for 7 days at pH 2.3 to 9.0. No. 48249 (Group A, Type 1) lost considerable activity at pH 2.3 in 1 day and all activity in 7 days. No. 49191 (Group A, Type 3) was more susceptible than the others at pH 9.4 to 9.6. The virus was more resistant in hydrochloric acid solution than in veronal-acetate buffers of the same pH. Table III presents the results with No. 5096 and is representative of the kind of data obtained.

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