JOURNAL OF BACTERIOLOGY, Aug., 1965
Vol. 90, No. 2

NOTES

Multiplication of Boston Strains of Coxsackievirus A9 in the Adult Mouse Heart

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Received for publication 31 March 1965

Strains of coxsackievirus group A type 9 were isolated in Boston from 1959 through 1961 from patients with rash, aseptic meningitis, or pneumonia (Table 1). One of these strains (no. 13) multiplied in the myocardium of 7- to 12-month-old mice, producing focal myocarditis (Lerner et al., New Engl. J. Med. **269**:678, 736, 1963; Lerner, Levin, and Finland, J. Exptl. Med. **115**: 745, 1962; Lerner, Progr. Med. Virol., *in press*). Since this property, to our knowledge, had not been noted in other strains of coxsackievirus A9 recovered elsewhere, or in the other serotypes of the group A coxsackieviruses, the other strains of coxsackie A9 virus isolated in this laboratory during the same period were examined for virus replication in the myocardium, and for resultant myocarditis.

Stock viruses prepared from rhesus kidney cell cultures which had been frozen (−20 C) for several years were thawed, and 0.1 ml of a 10^{-1} dilution of each of them was inoculated into three monkey-kidney tissue-culture tubes per specimen. The cultures were incubated in stationary racks (37 C), and the fluids were harvested 24 to 72 hr later when cytopathic effects were maximal. These new virus pools were two to six tissue culture passages beyond their primary isolation. Freshly prepared viruses titered $10^{6.5}$ to $10^{7.5}$ TCD_{50} per milliliter.

Amounts of 0.03 to 0.05 ml of each virus strain containing approximately 10^5 TCD_{50} were inoculated intraperitoneally into 7 to 15 white male mice (8 to 12 months old; supplied by Charles River Breeding Laboratories, Inc., Boston, Mass.). The animals (which all appeared well) were killed 5 days later by exsanguination, and the hearts were examined simultaneously for the presence of virus and for focal myocarditis by light microscopy of tissue sections stained with hematoxylin and eosin. Four of these same mice were studied for simultaneous viremia, and the amount of coxsackievirus A9 in their hearts at the time was determined. The methods used were standard, and have been described previously (Lerner and Shaka, Proc. Soc. Exptl. Biol. Med. **111**:804, 1962).

Hearts of mice which were killed on day 5, and which had been inoculated with strains 13, 186, 711, and 713, contained $10^{4.5}$, $10^{2.5}$, $10^{4.5}$, and $10^{3.0}$ TCD_{50} per gram of coxsackievirus A9. Although the sera of the inoculated mice tested were free from virus on the 5th day after infection, 6 of 15, 3 of 3, 3 of 3, and 3 of 3 mouse hearts which had been inoculated with these respective virus strains contained virus. Although virus replication was evident in these hearts 5 days after infection, all of the myocardiums appeared normal under light microscopy.

At 9 days after inoculation, however, strain 711 (the only strain tested) induced a focal myocarditis (Fig. 1). With this same strain, none of 10, 4 of 10, 2 of 10, and none of 10 hearts of mice showed focal myocarditis at 5, 9, 20, and 30 days after infection. These data are similar to results obtained previously with coxsackievirus A9 strain 13.

Another of these strains (186) was passaged 20 times in monkey-kidney tissue culture. At the 3rd and the 20th passages, crude virus suspensions of tissue-culture fluids were inoculated intraperitoneally into 10 mice each. The animals were killed 5 days later, and the hearts were processed for the presence of virus. Of 10 hearts, 8 contained virus at the third passage, but only 1 of them was positive 17 rhesus-kidney passages later.

Of the 15 new strains of coxsackievirus A9, 11 multiplied in the myocardium of adult mice, and, in the one strain tested, a focal myocarditis was seen on the 9th day of infection. It would be

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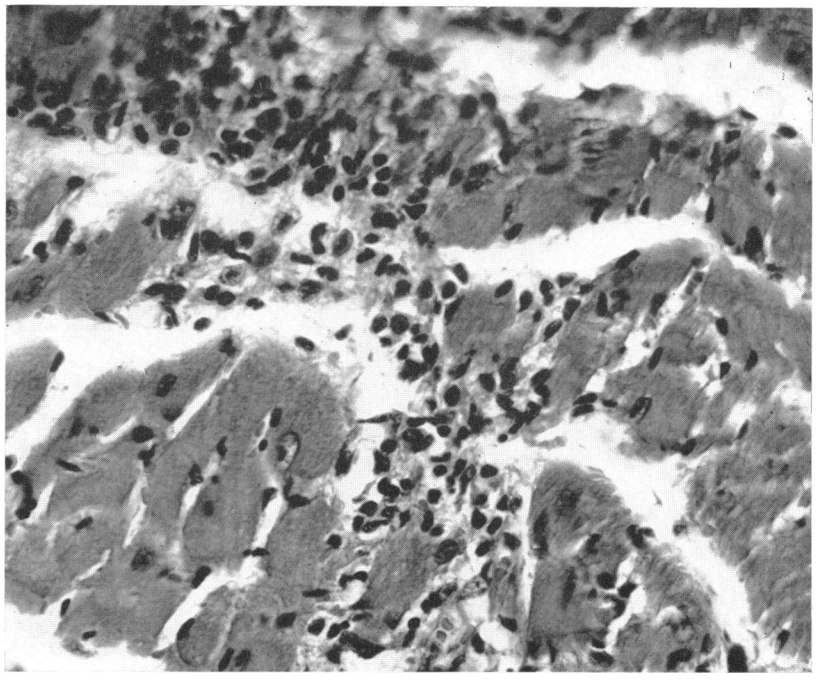

FIG. 1. *Myocarditis induced by coxsackievirus A9 strain 711 in mice 9 days after intraperitoneal inoculation of 10^5 TCD_{50}, showing an area of moderate focal inflammation.*

TABLE 1. *Presence of "myocarditis trait" in strains of coxsackievirus A9 recovered at the Boston City Hospital (1959–1961)*

Year of outbreak	Patient		Date seen	Source[a]	Diagnosis	Strain no.	Passage history[b]	Virus titer (TCD_{50}/ml)	Virus recovery from mouse hearts[c]
	Age	Sex							
1959	20 months	M	7/31	T	Aseptic meningitis,	65	MK_4	$10^{6.5}$	0/10
				R	exanthem	66	MK_4	$10^{7.0}$	0/10
	7.5 months	M	8/7	T	Aseptic meningitis,	225	A_3, MK	ND[d]	3/9
				R	exanthem	226	A, MK_2	$10^{7.0}$	0/9
				CSF		233	A, MK_2	ND	2/9
				S		S-88	MK_3	ND	7/10
	5 years	F	8/15	R	Exanthem	257	MK_5	$10^{6.5}$	0/10
	5 years	M	8/27	R	Aseptic meningitis	186	MK_3	$10^{7.5}$	8/9
						186	MK_{10}	$10^{7.5}$	2/10
						186	MK_{20}	ND	1/10
	2 years	F	9/2	T	Exanthem	197	MK_3	ND	5/9
	16 months	F	9/5	Lung	Exanthem and	13	MK_3	$10^{7.5}$	6/15
				Liver	pneumonia	14	MK_3	ND	4/7
1960[e]	24 years	F	4/8	T	Exanthem	712	MK_3	ND	8/10
				R		713	MK_3	$10^{6.5}$	7/9
	22 months	F	4/13	T	Exanthem	725	MK_3	$10^{7.0}$	1/9
	9 months	M	4/15	T	Exanthem	711	MK_3	ND	9/9
1961	17 months	M	8/17	T	Exanthem	1810	MK_6	ND	6/8

[a] T, throat; R, rectal swab; CSF, cerebrospinal fluid; S, serum.
[b] MK, rhesus kidney tube culture; A, human amnion tube culture.
[c] Number positive per total number of mice inoculated.
[d] Not done.
[e] Laboratory outbreak.

of interest to note whether other strains of coxsackievirus A9 isolated elswehere possess this "myocarditis trait."

This investigation was supported by Public Health Service grant 1 T1 AI 261-01 from the National Institute of Allergy and Infectious Diseases and by grant 64 G 184 from the American Heart Association.

The technical assistance of Nydia Tarrant is gratefully acknowledged.