Effects of Source, Sex and Gnotobiosis on Susceptibility of Adult Mice to Coxsackie B-1 Virus. (29433)

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It has previously been reported(1) that Coxsackie B-1 virus can become lethal for nonconditioned adult mice without alteration of such typical characteristics as cytopathogenicity and ability to be neutralized by specific antiserum. These experiments were carried out in a caesarian-derived strain of adult Swiss albino mice (Charles River CD-1). Since then we have had the opportunity to examine the virulence of this virus against other conventional random-bred strains of Swiss albino mice (Royalhart, Marland, Stocker and Scientific Breeders) as well as against a strain of adult germ-free mice derived from the conventional CD-1 animal. Information has also been obtained on the effect of sex on susceptibility of conventional animals to Coxsackie B-1 virus.

Materials and methods. Virus. The GP strain of Coxsackie B-1 virus(1) was used in all experiments. *Cell cultures.* Monkey kidney cell cultures were maintained in mixture 199 supplemented with penicillin G (50 units/ml) and streptomycin (50 μg/ml). Cultures (1.0 ml/tube) were inoculated with 0.1 ml of the supernatant from a 10% suspension of liver derived from paralyzed weanling mice infected 72 hours previously by the intraperitoneal route with 10 LD_{50} of virus (pool 24). Tubes showing typical cytopathogenic effects were combined, designated as pool 25, and stored frozen until used.

Mice. Conventional adult Swiss albino mice weighing 18-20 g were obtained from the following suppliers: Charles River Mouse Farms Inc., North Wilmington, Mass. (CD-1 = caesarian originated, barrier-sustained); Royalhart Labs., Inc., New Hampton, N. Y.; Marland Breeding Farms, West Caldwell, N. J.; Joseph Stocker, Ramsey, N. J.; and Scientific Breeders, Inc., East Orange, N. J.

Germ-free CD-1 mice were obtained from the Charles River Mouse Farms Inc., and were maintained in a plastic isolator on Ralston Purina 50-10-C ration. Frequent sterility tests were carried out during each gnotobiotic experiment by inoculating Brewer's thioglycollate broth, trypticase soy broth, and Sabouraud's broth with fecal material recovered from the bedding. Equal numbers of tubes were incubated at room temperature and at 37°C and observed for 10 days. The cultures were uniformly negative in the experiments being reported.

Infection of mice. Ten-fold dilutions of the tissue culture pool were made with saline. Mice were infected with 0.1 ml of the appropriate dilution by the intraperitoneal route and were observed daily for 10 days. Total survivors at each dilution were recorded and the LD_{50} values calculated according to the method of Reed and Muench (2).

Results and discussion. The susceptibility of the various strains of adult conventional Swiss albino mice to infection with the GP strain of Coxsackie B-1 virus is summarized in Table I. As much as a 2.1 log difference existed between the most sensitive and the least susceptible conventional strains. The differences in response of the strains were statistically analyzed and the results revealed a decreasing order of susceptibility as follows: Charles River>Royalhart = Marland >Stocker>Scientific. Thus the Charles River strain was the most susceptible, Royalhart and Marland, were essentially on the same order of susceptibility, Stocker was less susceptible and Scientific was the most resistant. Furthermore, the sex of the animal was not a biological determinant with regard to the response of the mice to the GP strain of Coxsackie B-1 virus.

Experiments were also carried out to determine the effects of the GP strain of Coxsackie B-1 virus in germ-free mice. Since only one of the breeders (Charles River) was a supplier of germ-free animals, a complete comparison between conventional and germ-free animals could not be made in each case.

TABLE I. Susceptibility of Different Strains of Conventional Swiss Albino Mice to Coxsackie B-1 Virus (GP Strain).

Supplier	Sex	No. of mice surviving 10 days after infection, Virus dilution 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	LD_{50}
Charles River (CD-1)	♂	0/50	0/50	1/50	1/50	12/50	26/50	$10^{-5.7}$
	♀	0/40	0/70	0/70	1/70	15/70	34/60	$10^{-5.7}$
Royalhart	♂	0/40	1/40	7/40	14/40	22/40	27/40	$10^{-4.7}$
	♀	0/40	2/40	2/40	9/40	21/40	26/40	$10^{-5.0}$
Marland	♂	2/40	3/50	8/50	11/50	24/50	29/40	$10^{-4.6}$
	♀	2/40	2/40	8/40	15/40	18/40	36/40	$10^{-4.6}$
Stocker	♂	6/40	5/40	4/40	22/40	27/40	32/40	$10^{-4.1}$
	♀	6/40	5/40	4/40	12/40	25/40	34/40	$10^{-4.4}$
Scientific	♂	3/40	10/40	16/40	25/40	26/40	34/40	$10^{-3.6}$
	♀	7/40	14/40	7/40	13/40	30/40	34/40	$10^{-4.1}$

Accordingly, the Charles River germ-free CD-1 mice were compared with the Charles River conventional CD-1 mice with respect to susceptibility to the same virus pool. Since the sex of the animals seemed to have no effect upon response to the virus and only a limited number of germ-free animals were available, the gnotobiotic mice were tested as a sexually heterogenous group. The survival data for the gnotobiotic mice were compared with the average results obtained with both sexes within the conventional group. These data, summarized for both groups, are presented in Table II.

It is apparent from the results in Table II that using the same strain from the same breeder, the germ-free animals were less susceptible to the lethal effects of the GP strain of Coxsackie B-1 virus than were the conventional animals (a 2.8 log difference, $P = < .00001$).

TABLE II. Comparison of Virulence of Coxsackie B-1 Virus (GP Strain) for Conventional and Germ-free Adult Swiss Albino Mice (Charles River CD-1 Strain).

Virus dilution	No. of mice surviving 10 days after infection, Conventional	Germ-free
10^{-1}	0/90	4/12
10^{-2}	0/120	19/49
10^{-3}	1/120	29/49
10^{-4}	2/120	40/51
10^{-5}	27/120	24/39
10^{-6}	60/110	—
LD_{50}	$10^{-5.7}$	$10^{-2.9}$

The decreased susceptibility of the germ-free animals to the GP strain of Coxsackie B-1 virus could possibly be due to the absence of a bacterial flora or differences in the immunological response of the strain. The role that bacterial flora may play in host-virus relationships, specifically with Coxsackie B-1 virus, is under investigation.

Summary. A distinct difference exists in the susceptibility of different strains of conventional 18-20 g Swiss albino mice to infection with the lethal GP strain of Coxsackie B-1 virus. The sex of the animal is not a determinant with regard to susceptibility to the infection. Germ-free Swiss albino mice (Charles River CD-1 strain) were significantly less susceptible to the infection with Coxsackie B-1 virus (GP strain) than were their conventional counterparts.

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1. Grunberg, E., Prince, H. N., PROC. SOC. EXP. BIOL. AND MED., 1963, v114, 494.

2. Reed, L. J., Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

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