Lethal Infection of Adult Mice with Coxsackie B-1 Virus. (28712)

E. GRUNBERG AND H. N. PRINCE
Department of Chemotherapy, Hoffmann-La Roche, Inc., Nutley, N. J.

Since their discovery by Dalldorf and Sickles(1), viruses of the Coxsackie group have been characterized by their ability to kill suckling mice and by the inability to kill weanling or adult mice. When propagation of the virus within adult mice has been shown to occur, as with the Connecticut-5 strain of Coxsackie B-1, the animals have experienced a self-limiting pancreatitis(2). Other investigators have been able to demonstrate that this otherwise inapparent pancreatitis could be converted to a fully lethal infection if the mice were treated with cortisone(3) or exposed to a cold environment(4). Moreover, even though Dalldorf(5) was able to adapt a strain of Coxsackie A-14 virus to produce paralysis in nonconditioned adult mice by intracerebral or intraspinal inoculation, recovery from paralysis was frequent and death was inconsistently produced.

Thus, a rapid and consistently lethal infection of adult mice with Coxsackie viruses has not been accomplished without the use of stress or conditioning agents. Therefore, the emergence of a strain of Coxsackie B-1 virus that became lethal for weanling (9-12 g) and adult (17-21 g) mice seems worthy of publication.

Materials and methods. Virus. The strain of Coxsackie B-1 virus used was obtained from Drs. Leon Rosen and Karl Johnson, Nat. Inst. Health.

Cell cultures. Monkey kidney cell cultures obtained from Microbiological Associates, Bethesda, Md., were fed with mixture No. 199 supplemented with penicillin (50 units/ml) and streptomycin (50 mcg/ml). Cultures (1.0 ml/tube) were inoculated with 0.1 ml of a 10% suspension of minced brain or skeletal muscle tissue prepared with mixture No. 199.

Antisera. Prototype reference antiserum (rabbit) against ECHO #9 (Hill) and Coxsackie B-1 (Connecticut-5) viruses were obtained from Microbiological Associates.

Mice. Swiss albino mice were employed throughout these experiments. Suckling mice were 2-4 days old when infected subcutaneously with virus. Adult mice (CD-1 strain) obtained from the Charles River Breeding Laboratories, North Wilmington, Mass., were infected intraperitoneally.

Results and discussion. The tissue culture and animal passage history of the strain, from the time it was received until the time that its virulence for weanling and adult mice became apparent, is shown in Table I.

Upon production of pool no. 2, an experiment was conducted to determine the effect of cortisone on susceptibility of weanling mice to the strain of Coxsackie B-1 virus. Ten Swiss albino mice weighing 9-12 g were administered cortisone (50 mg/kg subcutaneously $\times$ 4) and then infected intraperitoneally with 0.25 ml of an undiluted culture of Coxsackie B-1 virus propagated in monkey kidney cells. At the same time, 5 mice which had not received cortisone, were infected as controls. After 4 days, 10/10 of the conditioned mice and 2/5 of the control mice had

TABLE I. Initial Passage History of the GP Strain of Coxsackie B-1 Virus.

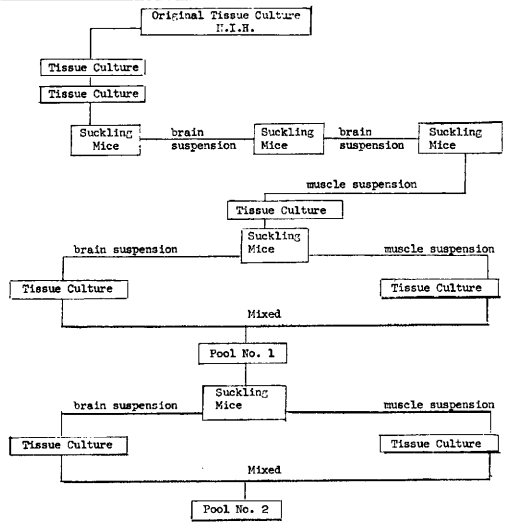

TABLE II. Serum Neutralization of Coxsackie B-1 Infection in Weanling Mice (9-12 g).

Pool	Inoculum*	Incubation time of virus-serum mixture *in vitro*	Survivors at 14 days: Undil. antiserum† Cox. B-1	Survivors at 14 days: Undil. antiserum‡ ECHO 9 (Cox. A-23)	Survivors at 14 days: Saline control
3	10^{-1} ip	15 min room temperature	6/8	1/8	1/8
5	10^{-1} ip	60 " " "	8/10	0/6	0/9
11	10^{-2} ip	60 " " "	9/10	—	1/10

* $LD_{50} = 10^{-4}$.
† Antiserum obtained from Microbiological Associates, Bethesda, Md., Type B-1 (Conn.-5).
‡ Antiserum obtained from Microbiological Associates, Bethesda, Md.

TABLE III. Serum Neutralization of Coxsackie B-1 Infection in Adult (17-19 g) CD-1 Mice, Pool 15.

Inoculum*	Neutralization	Time of neutralization: *In vitro* room temp (min)	Time of neutralization: *In vivo* passive† (hr)	Survivors at 14 days
10^{-1} ip	Coxsackie B-1 antiserum‡	15		10/10
Idem	Normal rabbit serum	15		0/10
"	Saline	15		2/10
10^{-2} ip	"	15		0/10
10^{-1} ip	Coxsackie B-1 antiserum, .1 ml ip		3	10/10
Idem	Normal rabbit serum, .1 ml ip		3	0/10
"	Coxsackie B-1 antiserum, .1 ml subcut		3	9/9
"	Normal rabbit serum, .1 ml subcut		3	0/10

* $LD_{50} = 10^{-4}$.
† Serum administered before infection with virus.
‡ Antiserum obtained from Microbiological Associates, Bethesda, Md., Type B-1 (Conn.-5).

succumbed. The brains of the 2 mice from the control group were passed into monkey kidney cell cultures and a typical cytopathogenic effect was noted. The supernatants from these cultures were combined and designated as pool no. 3. The experiment with pool no. 2 was repeated with weanling mice and then with adult mice and in both cases the results were essentially similar to the first experiment.

At this point, pool no. 3 was passed (0.1 ml undiluted intraperitoneally) into 6 mice which had not received cortisone and 2/6 died. The brains from the dead mice were passed into monkey kidney cell cultures and a typical cytopathogenic effect was produced. The supernatants from these cultures were combined and designated as pool no. 4. This pool was then injected (0.1 ml undiluted intraperitoneally) into 9-12 g Swiss albino mice which had not received cortisone and the brains of 3 paralyzed mice passed after 3 days into monkey kidney cell cultures to produce pool no. 5. Titration of pool no. 5 by the intraperitoneal route gave an LD_{50} of 10^{-4} for the weanling mice and 10^{-2} to 10^{-3} for normal adult mice.

Intraperitoneal inoculation of weanling or adult mice with pool no. 5 produced an infection characterized by rapid death, frequently without any signs of paralysis. Rate of death was not dose dependent since mean survival time of weanling mice infected with 10 LD_{50} ranged from 3.3 to 5.4 days while that for mice infected with 10,000 LD_{50} ranged from 3.3 to 4.7 days. This degree of virulence has not changed appreciably after 13 subsequent passages (mouse to tissue culture) through nonconditioned weanling mice and the virus has been designated as the GP strain.

To substantiate that we are dealing with the Coxsackie B-1 virus, a series of neutralization tests were performed in weanling and adult mice. *In vitro* neutralization tests were performed with undiluted antiserum in which the virus was diluted. The virus-serum mixture was incubated at room temperature for

the stated length of time and then injected by the intraperitoneal route. *In vivo* neutralization tests were performed by administration of undiluted antiserum by the subcutaneous or intraperitoneal route 3 hours prior to infection with the virus. The results are shown in Tables II and III.

The results of the neutralization tests demonstrate that the viral agent under discussion is serologically identical with the Coxsackie B-1 (Connecticut-5) virus.

Summary. Coxsackie B-1 virus can become lethal for weanling and adult nonconditioned mice. This property is not gained at the expense of other characteristics, specifically cytopathogenic effect in monkey kidney cell cultures and ability to be neutralized by specific antiserum.

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Methoxypyridoxine Convulsions in Epileptic and Non-Epileptic Mice. Protective Action of Pyridoxine.* (28713)

LENORE M. KOPELOFF AND JOSEPH G. CHUSID†

Department of Bacteriology, New York State Psychiatric Institute, New York City

Studies reported on drug-induced seizures have been based for the most part on acute experiments in normal animals. The use of a method for producing chronic epilepsy in mice(1) has permitted investigation of some of the actions of various agents on epileptic as well as on non-epileptic brain-operated and normal mice. Much of the data on effects in mice of the pyridoxine antagonist methoxypyridoxine has been derived from acute experiments on normal animals(2-4). We have therefore recently attempted to obtain additional comparable data on methoxypyridoxine by use of chronic experiments on epileptic as well as non-epileptic mice.

Method. CF #1 female mice (Carworth Farms) were prepared at 2 months of age. Cobalt powder or powdered magnesium trisilicate, sterilized in autoclave, was placed at 2 cerebral sites unilaterally by the implantation technique described previously(1). Other mice were left unoperated. During the subsequent 3-6 months subcutaneous challenge with 30-35 mg/kg pentamethylenetatrazol (Metrazol) induced seizures in a high proportion of those prepared with intracerebral cobalt without causing any similar reactions in mice prepared with magnesium trisilicate or in unoperated controls. Three groups were selected for the present study: (a) 12 epileptic mice (cobalt-treated), (b) 25 control-operated (magnesium trisilicate), and (c) 37 normal unoperated. Body weight at time of drug testing ranged from 24-37 g, averaging 28.0, 28.8 and 30.1 g, respectively. Mice received only one injection of drug or drug-combination each week.

Methoxypyridoxine (MOB6). Purified 4-methoxy-methylpyridoxine hydrochloride‡ prepared freshly in physiologic saline was adjusted to pH 7 with N NaOH and diluted further from 0.08% stock solution as required, so that dosages were administered in volumes of 0.4-0.6 ml. All injections were made by the intraperitoneal route (i.p.).

Pyridoxine. Pyridoxine hydrochloride (Eastman Organic) was prepared daily as 2.72% stock solution in saline after adjustment to

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† Associate Director, Neurological Division, St. Vincent's Hospital, New York City.

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