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)

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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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pH 7 with N NaOH, from which lower concentrations were obtained by further dilution. Dosages were administered i.p. in volumes of 0.3-0.6 ml (on occasions up to 1.2 ml).

Preliminary determinations of individual convulsive threshold to MOB6 were made weekly on all mice 15 minutes after i.p. physiologic saline (0.3-0.6 ml). For challenge, the threshold dose was employed, or in cases in which only minimal reactions occurred a slightly higher dosage level was used. Minimum protective dose of i.p. pyridoxine was titrated by injection 15 minutes prior to individual challenge dose of MOB6. Periodic checks of MOB6 threshold were made during drug-testing period of 3-4 months.

Seizures were classified as minor clonic or generalized. In the minor clonic seizure mice exhibited uncontrolled coarse clonic movements confined chiefly to front paws and head, usually with salivation, for 5-20 seconds, often while standing on hind legs and base of tail. In the typical generalized seizure all extremities and body showed pronounced rapid clonic movements frequently of such intensity as to cause the animal to rebound from the cage walls. Seizures frequently started as minor clonic and later progressed to generalized; often generalized seizures were followed by a minor clonic phase.

Statistical evaluation. Data were analyzed with t tests. A log transformation of the data was employed, since in the double dosage method used for testing protective action of pyridoxine error increases logarithmically.

Results. 1. *Methoxypyridoxine thresholds in epileptic and non-epileptic mice.* The convulsive (threshold) dose of MOB6 which produced clinical seizures in the epileptic (cobalt) mice averaged about one-third that of the controls (Table I). Within the latter group, no significant difference was noted in threshold levels of brain-operated (magnesium trisilicate) and normal unoperated mice.

A latent period of 10-66 minutes elapsed before onset of clinical seizures following injection of MOB6 at threshold convulsant levels. The mean latent time for 12 epileptic mice was 24 ± 7 min, for 25 control operated mice 31 ± 7 min, and for 37 unoperated controls 34 ± 10 min. The latent interval for the epileptic mice was significantly shorter ($P<.01$) when compared to each of the 2 control non-epileptic groups. No significant difference was found between the brain-operated and unoperated controls.

TABLE I. Convulsant Threshold Dose of Methoxypyridoxine (MOB6) in Epileptic and Non-epileptic Mice.

Brain operation	No. of mice	Range (mg/kg)	Mean (mg/kg)
Epileptic			
Cobalt	12	.5– 5	3.3 ± 1.6
Non-epileptic			
Magnesium trisilicate	25	8 –14	10.0 ± 1.7
Unoperated	37	8 –12	9.5 ± 1.3

MOB6 i.p. 15 min after physiologic saline i.p.

The character and quality of induced seizures was similar for convulsing mice of the non-epileptic brain-operated and unoperated normal groups. At threshold levels both groups of controls displayed short generalized seizures of several seconds' duration. At higher dosages there was noted either a train of short generalized seizures followed by generalized convulsions, minor clonic followed by generalized, or sequences of minor clonic, short generalized and generalized reactions. With cobalt-treated (epileptic) mice, threshold dosages induced minor clonic seizures, and only very rarely the short generalized type. At higher levels a series of minor clonic reactions was followed by one or more generalized convulsions.

2. *Effectiveness of initial threshold dose of methoxypyridoxine after 3-4 months.* The response of 33 mice to their initial threshold convulsant dose of MOB6 was retested during the 3-4 month experimental period. No apparent change in reactivity was noted in 8, slightly more intense convulsions occurred in 13 and an increase of 1-4 mg/kg over the initial threshold dose was found necessary to induce seizures in the remaining 12.

3. *Anticonvulsant action of pyridoxine on methoxypyridoxine seizures.*

TABLE II. Minimum Protective Dose of Pyridoxine in Relation to Threshold Dose of MOB6.

Brain operation	No. of mice	MOB6 Threshold dose (mg/kg)	MOB6 Threshold dose (log mg/kg)	Pyridoxine Range (mg/kg)	Pyridoxine Mean (mg/kg)	Pyridoxine Mean (log mg/kg)
Cobalt	2	2	.30	22.5 – 180	101.3	1.86 ± .43
	4	4	.60	22.5 – 720	253.1	2.11 ± .55
	2	5	.70	5.63 – 45	25.3	1.20 ± .45
	8	Mean	.55 ± .15		158.2	1.80 ± .62
Mg	10	10	1.00	5.63 –>720	239.1	2.02 ± .66
	12	12	1.08	5.63 – 360	105.9	1.68 ± .63
	2	14	1.15	22.5 – 90	56.3	1.65 ± .03
	24	Mean	1.05 ± .05		157.3	1.82 ± .65
Unop	12	10	1.00	5.63 –>720	155.2	1.78 ± .62
	19	12	1.08	5.63 –>720	82.9	1.48 ± .56
	4	14	1.15	11.25 – 45	30.9	1.43 ± .25
	35	Mean	1.06 ± .05		107.9	1.58 ± .57

In all tables >720 was treated as 720. Mg = magnesium trisilicate; unop = unoperated.

TABLE III. Comparison of Minimum Protective Dosages of Pyridoxine for Non-epileptic Mice with Different MOB6 Thresholds.

Brain operation	No. of mice	MOB6 Threshold dose (mg/kg)	Pyridoxine Range (mg/kg)	Pyridoxine Mean (mg/kg)	Pyridoxine Mean (log mg/kg)
Mg + unop	22	10	5.63–>720	193.3	1.89*± .65
Mg + unop	37	12–14	5.63–>720	83.3	1.55*± .56

* Significant at P <.05. Data derived from Table II.

a. *Minimal protective dose of intraperitoneal pyridoxine.* The minimum protective dose of pyridoxine capable of preventing the clinical convulsions of i.p. MOB6 was not related to the size of threshold convulsant dose for the group of mice as a whole (Table II). The mean protective dose of pyridoxine was similar for the epileptic and non-epileptic mice. However, a significant difference was found among the non-epileptic mice between those with MOB6 thresholds of 10 mg/kg and those with 12-14 mg/kg (Table III), suggesting that more pyridoxine was needed for protection in mice with lower threshold.

b. *Minimal protective dose of pyridoxine after 3-4 months.* The minimal protective dose of pyridoxine against MOB6 was increased during the 3-4 month test period when compared to the initial values (Table IV). (In the interim, thresholds for pyridoxine administered intramuscularly were also determined.) A t test indicated that this was significant (P<.01) for the entire series (2 cobalt, 4 magnesium trisilicate, 11 unoperated). The increase was significantly greater (P<.01) for the unoperated controls when compared with the operated controls or the operated controls combined with the cobalt treated mice.

TABLE IV. Comparison of Minimum Protective Dose of Pyridoxine at Beginning (A) and End (B) of 3-4 Month Test Period.

Brain treatment	Mouse No.	MOB6 challenge (mg/kg)	Pyridoxine A (mg/kg)	Pyridoxine B (mg/kg)
Cobalt	1	5	5.63	45
	2	2	180	360
Mg	3	12	11.25	45
	4	10	22.5	45
	5	12	22.5	90
	6	12	180	180
Unop	7	12	5.63	22.5
	8	12	11.25	180
	9	12	11.25	180
	10	10	11.25	>720
	11	14	11.25	>720
	12	12	22.5	180
	13	14	22.5	360
	14	12	22.5	>720
	15	12	45	>720
	16	12	45	>720
	17	12	180	360

Discussion. Epileptic mice were found to

be more susceptible to the convulsant effects of intraperitoneal methoxypyridoxine (MOB6) than non-epileptic mice. The mean dose of the epileptic group was about one-third the mean dose of the non-epileptic group, with no significant difference between the brain-operated non-epileptic and normal unoperated mice. The finding of increased convulsive susceptibility of epileptic mice agrees with our previous reports in chronic epileptic monkeys, rats, and mice where the convulsant threshold level to various analeptics is lower for the epileptic than the non-epileptic animals(5-8).

The convulsive thresholds to intraperitoneal MOB6 showed some variation in both epileptic and non-epileptic mice during the test period of 3-4 months.

Pyridoxine's protective action against MOB6 seizures was as effective for epileptic as for non-epileptic mice. However, in the non-epileptic control mice significantly more pyridoxine was required to protect mice with lower thresholds to MOB6. Over the 3-4 month test period most mice required a larger dose for protection than on initial testing. This increased need for pyridoxine did not appear to be based on change in epileptic status since relatively little difference was noted in this respect between mice which remained the same, slightly more or slightly less reactive to the threshold convulsant dose of MOB6. The finding that the pyridoxine requirement for i.p. protective effect increased so markedly over the relatively short period of 3-4 months indicates that for future studies a randomized experimental design would be essential.

The role of pyridoxine in experimental and human epilepsy remains obscure. Although pyridoxine has been shown to be a potent antidote for seizures resulting from various pyridoxine deficiency states of dietary, drug or other origin, the majority of human seizure patients do not benefit from pyridoxine treatment(9). Pyridoxine protects mice from the convulsant effects of MOB6 but does not prevent the decrease in brain gamma aminobutyric acid (GABA) which follows MOB6 treatment, so that the absolute level of brain GABA as an index of excitability state of brain has been questioned(4).

In chronic experiments, variability is to be expected because of aging, metabolic and endocrine changes, stimulus variability, post-operative effects, previous treatments, tolerance and adaptation, experimental method, etc. For example, Essig *et al*(10) recently called attention to the elevation of electroconvulsive threshold that occurs in cats and dogs during repeated daily measurements and concluded that this elevation which resembles tolerance was more likely the result of cerebral rather than extra-cerebral adaptation. The role of most of the factors cited above in chronic experiments is not well established although a variety of mechanisms might be applicable. Our experiences with brain-operated rats and mice suggest that similarly prepared small laboratory animals may be useful in chronic experiments designed to study many of these factors.

Summary. 1. Mice with chronic epilepsy (cobalt) had lower thresholds for the convulsant action of methoxypyridoxine (MOB6) than non-epileptic brain-operated (magnesium trisilicate) or normal mice. 2. Minimal effective dose of pyridoxine capable of preventing seizures varied widely among individuals. The mean protective dose was approximately the same for the epileptic and non-epileptic groups. Among the non-epileptic mice, those with lower convulsive thresholds to MOB6 appeared to require more pyridoxine for protection. 3. A significant increase in minimal effective anticonvulsant dose of pyridoxine occurred during the 3-4 month test period.

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Demonstration of Encephalomyocarditis Virus Antibody in Human Serums From Panama. (28714)

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The importance of the encephalomyocarditis (EMC) virus as a cause of disease in man is not known. Apparently unrecognized human infections occur inasmuch as antibody has been found in serums collected in many parts of the world. In 1958 EMC virus was recovered for the first time in Panama from swine dying with myocarditis(1). As a result of the isolation of this virus from a common domestic animal, we undertook an investigation to determine whether EMC serum antibody was present in the human population of the Isthmus. We report here results of studies on 180 sera and describe our experience with the hemagglutination inhibition (HI) test and neutralization (NT) tests employing HeLa cell cultures and mice.

Materials and methods. Serum. Blood specimens were obtained from inhabitants of 4 widely separated, geographically distinct areas of the Republic of Panama. Blood was aspirated with B-D vacutainer tubes or by syringe and was stored at environmental temperatures for 1 to 3 days. After transportation to the laboratory, serum was separated and stored for varying periods at –20°C.

Virus. The EMC virus was isolated in both mice and HeLa cells from the lung tissue of a naturally infected pig(1). It was identified in NT tests employing antiserum prepared against the American Type Culture strain of EMC virus.

Tissue culture (TC) NT tests. Virus which had been passaged 5 and, in some instances, 6 times in HeLa cell cultures was used in all tests. The medium overlying the cell monolayer was harvested after the appearance of cytopathic effect (CPE) and served as the virus pool. Tube cultures of HeLa cells which had been grown in Eagle's medium, containing 10% horse and 10% calf serum, were washed 2 times with Hanks' solution and maintained in Eagle's medium with 5% chicken serum. Antibiotics and heat-inactivated (56°C for 30 minutes) sera were used in all media. Cultures were incubated in stationary racks at 36-37°C.

Two types of NT tests were carried out. In the population survey, equal quantities of virus and inactivated whole serum were incubated together at 37°C for 30 minutes and 0.1 ml aliquot of the mixtures were introduced into each of 2 tube cultures of HeLa cells. Four virus dilutions were routinely used and CPE titers were determined on the 4th day after inoculation. The culture medium was not changed during the test. Several human sera lacking neutralizing activity were included in each test run and the titer(2) obtained with the majority of these controls was used to calculate the neutralization index (NI). In some instances sera were screened against 100 tissue culture infectious doses (TCD_{50}) of virus and those exhibiting neutralizing activity were retested against 4 virus dilutions.

In the second type of NT test, sera were diluted in 2-fold steps and tested against 100 TCD_{50} of virus. Serum-virus mixtures were

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