Effects of Pyridoxine Deficiency on Audiogenic Seizure Susceptibility in Inbred Mice.* (30154)

D. L. COLEMAN AND K. SCHLESINGER (Introduced by J. B. Storer)
Jackson Laboratory, Bar Harbor, Maine, and Department of Psychology, University of North Carolina, Chapel Hill

Lyon(1) studying the effects of diets containing varying amounts of pyridoxine (vit. B_6) on body weight gains, food intake and organ levels of pyridoxine in C57BL/FnLn and I/FnLn mice incidentally observed that mice of the I/FnLn strain, but not of the C57BL/FnLn strain were susceptible to audiogenic seizures when maintained on pyridoxine deficient diets. The I/FnLn strain used by Lyon, while carrying numerous recessive genes, is homozygous for the dilute *d/d* gene as are the strains of mice (DBA/1J and DBA/2J) which have been utilized most in audiogenic seizure studies(2). The presence of these recessive alleles has been associated with a higher seizure incidence(3) and lowered levels of the enzyme phenylalanine hydroxylase(4). The purpose of this experiment was to investigate the effects of dietary pyridoxine on audiogenic seizure susceptibility in dilute and non-dilute strains of mice.

Methods. One hundred and ninety-four mice, 60 C57BL/6J, 60 DBA/2J, and 48 B6D2F_1, (all males) and 14 BDP/J and 12 P/J, (both males and females) were used as experimental subjects. All animals were obtained from stocks maintained by the Jackson Laboratory, Bar Harbor, Maine, and were between 3 and 4 weeks of age at the beginning of experiments. A metal washtub on the sides of which was hung a battery operated bell was used to induce the seizures.

The diets contained 0, 10 and 30 mg of pyridoxine per kg of complete diet. The percentage composition of the complete diet was sucrose 67%, casein 20%, corn oil 5%, Wesson salts 5%, non-nutritive bulk 3% and adequate amounts of all known vitamins(5). The C57BL/6J, DBA/2J and B6D2F_1 mice were randomly assigned to 3 groups which were fed the 3 diets. The BDP/J and P/J mice were divided randomly by strain and sex into 2 equal groups and fed either the high-pyridoxine or zero-pyridoxine rations.

After 3 weeks on these diets half of the mice in each dietary group were tested for audiogenic seizures. All BDP/J and P/J animals were tested at this time. After 2 additional weeks on the respective diets the remaining half of the mice were tested for seizure susceptibility while all of the BDP/J and P/J animals were tested again. After another 2 weeks all mice in each group were again tested.

For seizure testing the animals were taken individually from the animal room and brought to the laboratory where they were placed into the washtub and subjected to the ringing of the bell for 90 seconds. During this period 3 events were recorded: the incidence of the wild running phase (a behavior which usually but not always precedes onset of the seizure) and the incidences of both clonic and tonic seizures. Animals which did seize were revived by artificial respiration.

Results and discussion. The growth rate of DBA/2J mice was severely affected by the diets devoid of pyridoxine (total weight gain 3.5 g after 35 days on the diet) and was somewhat suboptimal on the diet containing 1 mg/kg of pyridoxine (weight gain 6 g *vs* 10 g on diet 1). The growth curves for the other 2 dilute strains of mice (P/J and BDP/J) were similar to that seen for DBA/2J mice (total gain 2.5 g, on diet 3, *vs* 9 g, on diet 1). In contrast the C57BL/6J and B6D2F_1 mice showed decreased growth only on the zero pyridoxine diet and were not affected by diet 2 containing 10 mg of pyridoxine per kg of diet (Fig. 1).

The C57BL/6J strain of mice is not nor-

* The assistance of Miss Ruth Ellen Rosenblatt and Mr. R. Benes is gratefully acknowledged. This investigation was supported by USPHS Research Grants CA-05873 from Nat. Cancer Inst.; MH 1775 from Nat. Inst. of Mental Health; and by Grant NSF GE-3792 from Nat. Science Foundation.

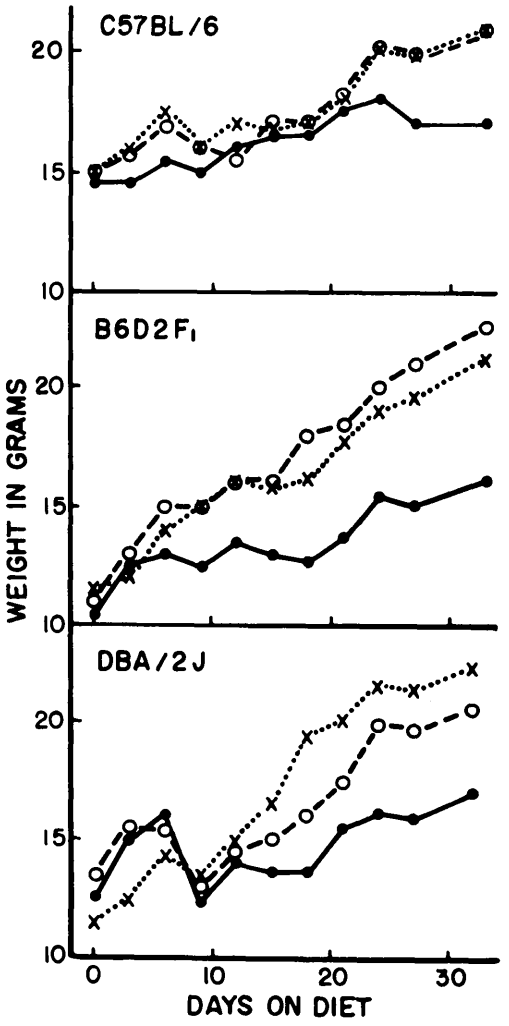

FIG. 1. Effect of varying amounts of pyridoxine on growth in normal and dilute strains of mice. Diet 1 (30 mg pyridoxine per kg) ×····×. Diet 2 (10 mg pyridoxine per kg) O– – –O. Diet 3 (0 pyridoxine) ●——●.

mally susceptible to audiogenic seizures and indeed showed no incidence of seizures or wild running on audiogenic stimulus even after 7 weeks on the most deficient ration. In contrast are the data shown in Table I for the 3 dilute strains of mice. It is apparent that after 3 weeks on the respective dietary regimens all dilute strain mice showed an incidence of seizures which was proportional to the degree of their pyridoxine deficiency. After 5 weeks on the diets the dilute strains still showed a seizure incidence roughly proportional to the degree of the deficiency. At this time the $B6D2F_1$ hybrid mice (not shown in Table I) on the pyridoxine-free diet showed the first suggestion of seizure susceptibility exhibiting a 22% incidence of wild running behavior but having no actual seizures. At 7 weeks (not shown on Table I) the incidence of seizures was still highest (13-33%) in dilute animals maintained on the zero pyridoxine ration although a marked decrease in overall susceptibility for the DBA/2J strain was noted. Some incidence of tonic seizures (11%) was observed in the $B6D2F_1$ hybrid mice maintained on the zero pyridoxine ration but the C57BL/6J animals were still completely resistant to audiogenically induced seizures.

This decreased incidence of seizures in the DBA/2J strain of mice after 7 weeks on the experiment (12 weeks of age) was not unexpected since seizure susceptibility in the DBA strains of mice has been shown to be a function of age reaching a maximum at 30 days of age and then decreasing rapidly (6). The results of these dietary studies demonstrate that susceptibility to audiogenic seizures can be maintained at a fairly high level for a greatly extended period if animals are maintained on diets deficient in pyridoxine and emphasize the need for strict dietary control in all audiogenic seizure studies. All dilute strains tested were found to be more susceptible to the pyridoxine deficiency with respect to both seizure incidences and growth rate than the non-dilute strains. This could represent either a higher pyridoxine requirement or an inability to utilize dietary pyridoxine efficiently by the dilute strain mice and would implicate the dilute gene as responsible for the similar observations made by Lyon(1) in the I strain of mice. Recently(7) two new metabolites of phenylalanine have been isolated from the phenylketonuric urine. Both of these have been identified as conjugates with pyridoxine. Dilute mice, which are similar in many respects to human phenylketonurics, may require excess pyridoxine in order to offset the amounts being excreted with these abnormal phenylalanine metabolites.

The finding that pyridoxine is related to audiogenic seizure susceptibility is of par-

TABLE I. Incidence of Audiogenic Seizures in Mice Fed Diets Containing Varying Amounts of Vitamin B_6.

Strain	Diet*	Seizure incidence: 3 weeks† Wild running	3 weeks† Clonic seizure	3 weeks† Tonic seizure	5 weeks Wild running	5 weeks Clonic seizure	5 weeks Tonic seizure
		%	%	%	%	%	%
DBA/2J	1	88 (9)	11 (9)	11 (9)	11 (9)	0 (9)	0 (9)
	2	80 (10)	20 (10)	10 (10)	40 (10)	20 (10)	0 (10)
	3	100 (9)	55 (9)	44 (9)	77 (9)	44 (9)	44 (9)
BDP/J	1	17 (6)	0 (6)	0 (6)	0 (6)	0 (6)	0 (6)
	3	71 (7)	0 (7)	0 (7)	66 (6)	33 (6)	33 (6)
P/J	1	0 (6)	0 (6)	0 (6)	17 (6)	17 (6)	0 (6)
	3	66 (6)	33 (6)	33 (6)	100 (6)	50 (6)	17 (6)

* Diets 1, 2, and 3 contained 30 mg, 10 mg or 0 mg of pyridoxine/kg of diet respectively.
† Numbers of weeks refers to time on dietary regimens before seizure tests were conducted.
Numbers in parentheses refer to number of mice tested.

ticular interest inasmuch as it is an essential co-factor in the metabolism of many supposed "neurohumors." For example, pyridoxine is a co-factor in the production of such substances as serotonin, epinephrine, norepinephrine and γ-aminobutyric acid. Whether any of these substances, and particularly which one of these substances, is related to the audiogenic seizure phenomenon remains to be determined.

Summary. Mice of inbred strains DBA/2J, P/J, BDP/J, C57BL/6J, and F_1 hybrid B6D2F_1 (C57BL/6J × DBA/2J) mice were maintained on diets containing either 0, 10, or 30 mg of pyridoxine (vitamin B_6) per kg of diet. Animals were placed on these diets when 3 to 4 weeks old and maintained on the diets for 7 weeks. Susceptibility to audiogenic seizures was determined 3, 5, and 7 weeks after the mice had been put on these respective dietary regimens. Mice of the dilute strain (homozygous *d/d*), *i.e.*, strains DBA/2J, BDP/J, and P/J were found to be more susceptible to audiogenic seizures in the pyridoxine deficient state. Body weights were also recorded every 3 to 4 days. Dilute strains (DBA/2J, BDP/J and P/J) and the hybrid B6D2F_1 showed smaller increments in body weights on the pyridoxine deficient diets than did C57BL/6J mice.

1. Lyon, J. B., Jr., J. Nutr., 1958, v66, 261.
2. Fuller, J. L., Thompson, W. R., Behavior Genetics, John Wiley & Sons, 1960.
3. Huff, Sally D., Huff, R. L., Science, 1962, v136, 318.
4. Coleman, D. L., Arch. Biochem. & Biophys., 1960, v91, 300.
5. Fenton, P. F., Carr, C. J., J. Nutr., 1951, v43, 441.
6. Bevan, W., Psychol. Bull., 1955, v54, 473.
7. Yoo, Y. H., Ritman, Pauletta, Nature, 1964, v203, 1237.

Received January 22, 1965. P.S.E.B.M., 1965, v119.