havioral Sciences (McGraw-Hill, New York, 1956), p. 202 (Spearman's rank).
5. A. P. Szumlewicz, unpublished data.
6. I thank H. L. Andrews for suggestions on radiation and D. W. Alling for performing the statistical tests and for revising the manuscript.
* Visiting scientist. Permanent address: Instituto Nacional de Endemias Rurais, Belo Horizonte, Brasil.

10 March 1964

Audiogenic Seizures, the Dilute Locus, and Phenylalanine Hydroxylase in DBA/1 Mice

Abstract. *Reduced audiogenic seizure susceptibility in dilute mice was associated with enhancement of phenylalanine hydroxylase activity, previously reported as low in dilute mice. Though nutritional changes complicate the interpretation, evidence exists for a mutation linked with dilute which modifies susceptibility.*

The mechanism of genetic control of susceptibility to audiogenic seizures has not been satisfactorily explained. One hypothesis was suggested by Coleman (*1*) who demonstrated a relationship between the dilute locus and phenylalanine hydroxylase activity. The relatively low activity of this enzyme in dilute mice could reduce synthesis of serotonin or other neurohormones, thus modifying seizure susceptibility. For example, reduced enzyme activity could directly decrease production of serotonin from tryptophan (*2*); or an increase in phenylacetic acid (*1*), resulting from the lowered conversion of phenylalanine to tyrosine, could decrease synthesis of serotonin, adrenalin, and γ-aminobutyric acid through inhibition of their respective decarboxylases. Large amounts of phenylalanine have been shown to decrease the concentration of serotonin in the brain (*3*). This postulated mechanism is similar to that known to occur in the human condition, phenylketonuria. Busnel and Lehmann (*4*) have shown that a number of drugs that lower serotonin metabolism potentiate audiogenic seizures.

We, therefore, hypothesized that dilute (*dd*) mice would be more prone to seizures than nondilute (*Dd* or *DD*) mice which were maintained constant at other loci. The multiple factor determination of seizure susceptibility has been demonstrated (*5*), so that the effects of a single locus might be difficult to demonstrate in genetically heterogeneous populations. Nevertheless, some evidence for association of increased susceptibility and dilute coat color has been obtained (*6*).

Investigations were undertaken to determine the effect of two alleles, *D* and *d*, at the dilute locus maintained on a common genetic background by repeated backcrossing in stock mice of strain DBA/1 (*dd*) (*7*). The *D* gene, which is phenotypically indistinguishable from wild type, arose as a mutation from *d* in this stock. Matings of, *DD* × *DD*, *Dd* × *dd*, and *DD* × *dd* were made in order to obtain a large group of animals that were tested for seizure susceptibility at 30 days of age. Each subject was exposed once for 90 seconds to a door bell (sound level approximately 100 db above 0.0002 dyne/cm^2). Only actual convulsions were counted as positive responses.

The results of this experiment, which are shown in Table 1, did not support the original hypothesis. However, the low incidence of seizure in all three genotypes compared with the 80 percent expected in mice of the DBA/1 strain suggested that unknown factors were operating. Measurement of phenylalanine hydroxylase (*8*) in all three genotypes showed that individuals had enzyme activity ranging between 48 and 100 percent of the "standard" C57BR/cd strain. Most of them were close to 100 percent. Previously, enzyme activity in all DBA/1 mice had been low, only 51 percent of the standard activity found in *dd* individuals.

The association in these mice of an increase in enzyme activity with decreased susceptibility to audiogenic seizures is consistent with the original hypothesis, but is not conclusive proof of it. One can only be certain that the dilute phenotype is not invariably a marker for low phenylalanine hydroxylase activity.

Further experiments were conducted to determine the physiological nature of the change in susceptibility. A group of mice not convulsing at 30 days was tested at 40 days. The population incidence was similar to that found previously when subjects were classified as susceptible if they convulsed on either the first or the second trial (Table 2). The increase on the second trial could have been caused by a sensitization produced by the first trial, or by an age shift in susceptibility. In order to distinguish between these possibilities mice were tested first at 40 days and later at 50 days with the results shown in Table 3. First-trial seizure incidence was similar at 30 and 40 days; both groups showed enhanced susceptibility after 10 days, although the increase was less for the 40- to 50-day subjects. These DBA/1 mice differed from the usual stock, not in age of maximum sensitivity, but in requiring a sensitizing stimulation before becoming highly susceptible. A similar phenomenon was observed in the HS strain formerly maintained by one of us (J.L.F.).

The concurrent change in seizure susceptibility and enzyme activity could be fortuitous, or both could be dependent upon some common factor. Between the time of Coleman's study (*1*) and ours, the standard colony diet was shifted from Purina Lab Chow to

Table 1. Occurrence of convulsions on first trial in DBA/1 mice at 30 days of age.

Genotype	Male		Female	
	N	Convulsions (%)	*N*	Convulsions (%)
DD	50	26	63	26
Dd	114	17	96	15
dd	78	8	63	14

Table 2. The number of DBA/1 mice that convulsed when tested at 30 days and 40 days (first and second trials combined).

Genotype	Male		Female	
	N	Convulsed (%)	*N*	Convulsed (%)
DD	28	88	33	81
Dd	62	83	50	85
dd	39	67	31	87

Table 3. The number of DBA/1 mice that convulsed when tested at 40 days and 50 days.

Genotype	*N*	Convulsed at 1st trial (%)	Convulsed at 1st and 2nd trial (%)
DD	29	28	36
Dd	24	13	52
dd	7	0	57

Table 4. Effect of selection based on the occurrence of audiogenic seizures during the first trial.

	Genotype	
	DD	*dd*
Generation O		
Number of mice	113	141
Seizures (%)	26	11
F1 selected for resistance		
Number of mice	35	23
Percentage susceptible	26	13
F1 selected for susceptibility		
Number of mice	28	33
Percentage susceptible	71	15
Mice not selected		
Number of mice	29	97
Percentage susceptible	34	11

Old Guilford, and it is possible that the reduction in susceptibility is partially explained by this change. However, individual variations in enzyme activity led us to suspect an additional genetic factor. Specifically, we hypothesized a mutation which suppressed seizures, possibly by modifying the enzyme inhibitory properties of *d*. Susceptibility of DBA/1 mice (*dd*) from lines (in the Russell-Wolfe colony) which had never been crossed to *D*-carrying animals was only 18 percent (33 subjects). Heterozygotes from the colony showed an incidence of 21 percent and their dilute siblings, 17 percent. Therefore, the change of seizure incidence was not mediated by the presence of the *D* allele in the pedigree. If a genetic change had taken place, it must have occurred originally in a dilute mouse.

The data in Table 1 show that the ranking of the three genotypes for seizure susceptibility was actually the reverse of that originally predicted. Further analysis demonstrated that *Dd* mice from *DD* × *dd* matings showed a higher incidence of seizures (25 percent of 81) than *Dd* offspring from *Dd* × *dd* matings (14 percent of 168) ($\chi^2 = 4.608$, $p < .05$). The finding is compatible with the existence of a seizure suppressor *Sz*, originally occurring in the dilute stock in the same linkage group as *d*. Dense mice from *D sz/D sz* × *d Sz/d Sz* would all be *D sz/d Sz*. Dense mice from *Dd* × *dd* matings could be of two classes, *D sz/d Sz* (noncrossover) and *D Sz/d Sz* (crossover). The latter type, homozygous for the suppressor, would be less susceptible to seizures. For verification of the original data, crosses were made as follows, *DD* × *dd*, *dd* × *DD*, *Dd* × *dd*, and *dd* × *Dd*. No seizures occurred in 27 *Dd* offspring from *Dd* × *dd* matings; 19 seizures in the first trial at 30 days were obtained in 138 offspring from *DD* × *dd* matings. The lowering of seizures incidence was found in other groups from different experiments conducted during the summer months and appears to be an environmental effect. Comparisons between seizure frequencies made at different periods must be made with caution.

A confirmation of the genetic origin of seizure suppression found in our DBA/1 colony was sought through selection. Four groups of matings were made: *DD* susceptible; *DD* nonsusceptible; *dd* susceptible; and *dd* nonsusceptible. Families were classified as having high or low susceptibility to seizures, based on the testing of at least two litters. All individuals selected as parents had been tested and were phenotypically characteristic of their family category. From five to eight matings were made in each category and the results are given in Table 4. The change in seizure incidence among offspring of *DD*-susceptible matings compared with nonselected mice is significant ($\chi^2 = 7.05$; $p < .01$), but no change was observed in the other groups. Whatever effects nutritional and other environmental factors are exerting, appropriate selection restored the expected high level of susceptibility characteristic of the DBA/1 strain. It seems reasonable to interpret these results as follows: *dd* mice chosen for the selection experiment are not segregating at the suppressor locus, and variability in susceptibility is therefore largely environmental. The asymmetry of selection in the *DD* groups suggests that the heterozygote *Sz sz* is phenotypically closer to the resistant *Sz Sz* than to the susceptible *sz sz*. If the nonsusceptible *DD* mice are chiefly heterozygous with respect to the suppressor locus, a likely condition according to the data presented here, one would expect little change in one generation of selection.

The high susceptibility of appropriately selected *DD* mice indicates that the *d* gene has little effect on proneness to seizures within this particular genetic and environmental background. Further biochemical studies are necessary for conclusions regarding the original hypothesis of a positive relationship between seizure susceptibility and deficient phenylalanine hydroxylase activity. The coincidence of the decreased susceptibility in our DBA/1 colony with an increase of enzyme activity is highly suggestive of such relationship. The possible role of nutritional changes in modifying both seizure susceptibility and enzyme activity is acknowledged, but was not tested in this experiment. Evidence was found that variation in susceptibility within the colony was associated with a genetic change distinct from the dilute locus, but linked with it.

SALLY D. HUFF*
JOHN L. FULLER

Jackson Laboratory, Bar Harbor, Maine

References and Notes

1. D. L. Coleman, *Arch. Biochem. Biophys.* **91**, 300 (1960).
2. R. A. Friedland, I. M. Wadzinski, H. A. Waisman, *Biochem. Biophys. Res. Commun.* **5**, 94 (1961).
3. H. L. Wang, V. H. Harwalkar, H. A. Waisman, *Arch. Biochem. Biophys.* **97**, 181 (1962).
4. R. G. Busnel and A. Lehmann, *J. Physiol., Paris* **52**, 37 (1960).
5. J. L. Fuller, C. Easler, M. E. Smith, *Genetics* **35**, 622 (1950).
6. S. D. Huff and R. L. Huff, *Science* **136**, 318 (1962).
7. The original stocks were provided by H. G. Wolfe and E. S. Russell.
8. Liver phenylalanine hydroxylase activities were analyzed by D. L. Coleman.
9. We thank Jane Harris, Frank Clark, Roy Hostetter, and Gayle Hostetter for their aid in testing mice. This work was supported in part by USPHS grant MY-1775 from the National Institute of Mental Health.
* Present address: Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City.

10 February 1964

Inherited Male-Producing Factor in an Insect That Produces Its Males from Unfertilized Eggs

Abstract. *An inherited factor causing the normal sex ratio of 92 percent females to drop to about 5 percent has been produced by selective breeding in a laboratory strain of the arrhenotokous parasitic wasp* Dahlbominus fuliginosus *(Nees) (Hymenoptera, Eulophidae). The factor is known to be of genetic origin and is sex-limited, being transmitted by females to their sons. Its effect on the sex ratio is constant and not influenced by the female parent, the host, or the environment.*

In the hymenopteran *Dahlbominus fuliginosus*, as with most other arrhenotokous insects, maleness has previously been considered to be determined solely by the lack of fertilization of the egg. Haploid males arise from unfertilized eggs and diploid females from fertilized eggs. The underlying genetic mechanism of sex determination is as yet unknown. In *D. fuliginosus* there are no known series of sex alleles, no diploid males resulting from close inbreeding, such as in *Habrobracon juglandis* (*1*), and no apparent sex chromosomes. Unlike what Flanders (*2*) believes to be the situation in arrhenotokous species, females of *D. fuliginosus* cannot control fertilization of their eggs, and the sex ratio of progeny from females producing offspring of both sexes is usually rather uniform, except when environmental conditions