EVIDENCE THAT TWO LOCI PREDOMINANTLY DETERMINE THE DIFFERENCE IN SUSCEPTIBILITY TO THE HIGH PRESSURE NEUROLOGIC SYNDROME TYPE I SEIZURE IN MICE

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ABSTRACT

Most mammals tested, when exposed to increasing pressure in helium/oxygen atmospheres, exhibit progressive motor disturbances culminating in two, usually successive, well-differentiated convulsive seizures. The seizures are highly reproducible components of the constellation of events that collectively constitute the High Pressure Neurologic Syndrome (HPNS). In the present study, we present evidence that the mean difference in seizure threshold pressures of the first seizure to occur (HPNS Type I) between inbred mouse strains DBA/2J and C57BL/6J is predominantly determined (> 60%) by the expression of a major locus—possibly linked to the *H-2* locus on chromosome *17*—and a minor locus, probably unlinked. This outcome is derived from applications of the maximum likelihood modeling procedure of ELSTON and STEWART (1973) and STEWART and ELSTON (1973) to eleven models of genetic determinacy and tests (including breeding tests) of "preferred" models so derived using BXD recombinant inbred strains that show the following: The major locus exhibits conditional dominance characteristics depending upon compression rate and minor locus genotype. At a constant mean compression rate of 100 atm hr^{-1}, the major locus manifests strong, though incomplete, dominance apparently independent of minor locus genotype. Its expression is, however, highly sensitive to compression rate, losing its dominance altogether at a linear rate of 1,000 atm hr^{-1}. The major locus interacts with the weakly dominant and relatively compression-rate-insensitive minor locus to retain dominance at fast compression only when the dominant alleles of both loci are present. A principal finding of this study is that employing two compression rates permits fuller genetic characterization of murine high-pressure seizure susceptibility differences than could be achieved by use of a single compression rate.

MAMMALS exposed to increasing pressure in helium-oxygen (heliox) atmospheres exhibit progressively greater motor disturbances culminating, in most species tested, in two successive and distinct types of seizures. The seizures and preseizure behavior and the constellation of associated CNS events collectively constitute the High Pressure Neurologic Syndrome (HPNS). The HPNS is one of the best characterized of all CNS syndromes from a number of perspectives including ontogenetic development (MANSFIELD *et al.* 1980), comparative

aspects among vertebrates (BRAUER *et al.* 1974), electrophysiological properties (LUNDGREN and ØRNHAGEN 1976), pharmacologic properties (LEVER *et al.* 1971), neuroanatomy (MANSFIELD, GILLEN and BRAUER 1979) and relation to time course of pressure increase (BRAUER *et al.* 1979).

One aspect of the syndrome, which until recently had received little attention, is the extent to which the variation insusceptibility to HPNS phenomena is heritable. While the preseizure phenomena at the behavioral level are graded responses and therefore unsuited to genetic analysis, seizure susceptibility measured by the threshold pressure P_c at which the seizure occurs, is a test parameter well-suited to genetic analysis: the seizures are sudden, dramatic events that are highly reproducible in experimental animals; they are stable for a significant portion of an animal's lifespan; and among 12 inbred mouse strains tested, the intrastrain variance in seizure threshold was small compared to interstrain variance (BRAUER *et al.* 1979).

The aim of the present study is to refine and extend the results contained in our previous report (MCCALL and FRIERSON 1981) concerning the heritable nature of differences in the first seizure to occur in mice during compression in heliox (HPNS Type I seizure). The analytical approach used must be adequate for the case in which the test parameter is continuously distributed, since unambiguous assignment of an animal to a phenotypic class in the segregating generations is not possible. In considering ways to achieve such an analysis, we were mindful of the considerable and growing number of reported instances in which major portions of the heritable differences in continuously variable biological parameters have been shown to be associated with one or two genes. We therefore thought it essential, as a step in analysis, to employ a method with the potential to discriminate cases of relatively simple inheritance from those more complex. ELSTON and STEWART (1973) and STEWART and ELSTON (1973) devised a mathematical modeling procedure that has the necessary discriminatory capability when applied to data of the sort available for the high-pressure induced seizure. In essence, the procedure entails deriving, for a number of different models of genetic determinacy, the likelihoods of these models, while maximizing population parameter estimates from data for two inbred parental strains widely separated in the character of interest, their F_1 hybrids and the backcross generations. This, then, is the method applied to the analysis of genetic determinacy of Type I HPNS convulsions in the present study. The conclusions reached were further tested by devising breeding tests of "preferred" models of inheritance.

MATERIALS AND METHODS

Compression conditions: The mice used in this study were exposed to compression in a heliox atmosphere under general conditions described previously (BRAUER *et al.* 1977) until a convulsive seizure was observed. The pressure expressed in atmospheres (atm) at which the first seizure occurred is denoted by ${}_1P_c$. Key compression parameters were: $P_{O_2} = 0.5 \pm 0.05$ atm; $P_{CO_2} < 0.005$ atm throughout; and chamber temperature $= 34.5 \pm 0.5°$ except where noted otherwise. A constant mean compression rate of 100 atm hr^{-1} applied in stepwise increments of 2 atm used in some experiments represented a compromise among reproducibility, resolution and convenience. In other experiments, the mice were compressed continuously at a linear rate of 1,000 atm hr^{-1}. To minimize the effect on the animals of a 4° adiabatic temperature excursion resulting

from rapid compression, those experiments were begun at a chamber temperature of 31°. Male and female mice were used in all experiments, but, as no significant difference in ${}_IP_c$ with respect to sex was found, the results for the sexes were combined in all analyses.

Mice and breeding program: All mouse stocks were obtained from the Jackson Laboratory and maintained in the Institute's colony in plastic cages at a temperature of 24 ± 2° on a 12-hr light, 12-hr dark cycle. Their diet was Charles River mouse chow and water provided *ad libitum*. Mated pairs were housed separately. When the female was judged pregnant by observation, the male was removed. Offspring were separated by sex at weaning and maintained as sibling groups with no more than four sibs to a cage. The experiments were performed on mice 35–80 days old. No systematic effect of age on ${}_IP_c$ has been detected in mature mice, up to one year of age (*cf.* MANSFIELD *et al.* 1980).

Seizure threshold pressures recorded for the 100 atm hr^{-1} rate (${}^{100}_{I}P_c$) and/or the 1,000 atm hr^{-1} rate (${}^{1000}_{I}P_c$) were determined for five experimental breeding systems each comprising two inbred parental strains, some of which were TAYLOR's BXD recombinant inbred (RI) strains derived from C57BL/6J and DBA/2J progenitor strains; F_1 hybrids produced by reciprocal parental crosses; the four backcrosses, which included each of the reciprocal F_1's and either the parent strain or F_1 as mother, to each of the parental strains. There were no significant differences between any of the reciprocal crosses, and they were combined in the presentation of data.

In the breeding system code used in the results section, CD stands for the parental strains C57BL/6J and DBA/2J and the derivative first and second generation hybrids. Similarly, the D23 system involved DBA/2J and RI strain BXD-23 as parents; in the D14 system, DBA/2J and RI strain BXD-14 were the parental strains. The rate of compression used in each system was designated by Slow (100 atm hr^{-1}) or Fast (1000 atm hr^{-1}). The various breeding tests of the genetic modeling procedure results are briefly described in the presentation of results.

Maximum likelihood modeling: The 100 atm hr^{-1} Type I seizure threshold pressure data for the C57BL/6J and DBA/2J strains, their F_1 hybrids and both backcrosses were utilized in deriving the likelihoods of eleven models of genetic determinacy by the maximum likelihood procedure of ELSTON and STEWART (1973). The number of models tested do not, of course, exhaust the possibilities, but were adequate, we believe, to differentiate the cases involving a simple inheritance pattern from the complex "multifactorial" patterns. A general description of the version of the procedure used in the present study follows: Let $N(\mu, \sigma^2)$ represent a normal distribution with mean μ and variance σ^2 and assume in each model that the C57BL/6J distribution is $N(\mu_1, \sigma^2)$, the DBA/2J distribution is $N(\mu_3, \sigma^2)$, and the F_1 distribution is $N(\mu_2, \sigma^2)$. The theoretical backcross distributions vary from model to model, but in each case are assumed to be mixtures of normal distributions. For all the models, the values of μ_1, μ_2, μ_3, and σ^2 are regarded as unknown parameters to be estimated. First, maximum likelihood estimates of the unknown parameters are generated in each model, using a computer program that includes a subroutine developed for this purpose by KAPLAN and ELSTON (1972). The natural logarithm of each model's likelihood, maximized with respect to the various parameters, is then obtained. The functions describing the expected distributions for each of the 11 models tested in this manner are summarized in Table 1. The model code presented in the first column of Table 1 is the same as that used by STEWART and ELSTON (1973).

RESULTS

The present genetic analysis of high-pressure induced seizure threshold differences contains a large number of elements in an extended argument. As an aid to following the development of the argument, we have included in Figure 1 a diagrammatic representation of the analytical sequence.

Development of model inheritance modes in the CD systems

The CD Slow system: The distributions of ${}^{100}_{I}P_c$ in the DBA/2J and C57BL/6J parental strains, their F_1 and F_2 hybrids, and both backcrosses are shown in Figure 2. Summary statistics of the distributions are presented in Table 2. The

TABLE 1

Inheritance modes of variability in susceptibility to a high pressure induced seizure: eleven models used in a maximum likelihood study

Model Code	Model description	Theoretical distribution of backcross to C57BL/6J[1]
A-1	Single Locus	$\frac{1}{2}N(\mu_1,\sigma^2) + \frac{1}{2}N(\mu_2,\sigma^2)$
A-2	Two unlinked additive loci	$\frac{1}{4}N(\mu_1,\sigma^2) + \frac{1}{2}N((\mu_1 + \mu_2)/2,\sigma^2) + \frac{1}{4}N(\mu_2,\sigma^2)$
A-LC	Many equal additive unlinked loci, $\sigma^2_\infty = \sigma^2 + C \cdot (\mu_1 - \mu_3)^2$, $C > 0$	$N((\mu_1 + \mu_2)/2, \sigma^2_\infty)$
A-LO	Same as A-LC except C=0	$N((\mu_1 + \mu_2)/2, \sigma^2)$
B-AO	Two linked loci with additivity restriction ($\mu_{12} + \mu_{21} = \mu_1 + \mu_2$), r = recombination fraction, μ_{12} and μ_{21} means of recombinant genotypes	$\frac{1}{2}(1-r)N(\mu_1,\sigma^2) + \frac{1}{2}rN(\mu_{12},\sigma^2) + \frac{1}{2}rN(\mu_{21},\sigma^2) + \frac{1}{2}(1-r)N(\mu_2,\sigma^2)$
B-OS	Same as B-AO except replace additivity restriction with symmetry restriction, $(\mu_{12}-\mu_{21})/(\mu_1-\mu_2) = (\mu_{32}-\mu_{23})/(\mu_3-\mu_2)$, μ_{23} and μ_{32} are means of recombinant genotypes for backcrosses to DBA/2J	Same as in B-AO
B-OO	Same as B-OS except no symmetry restriction is imposed	Same as in B-AO
C-OO	One major locus with a large number of equal additive minor loci	$\frac{1}{2}N(\mu_{12},\sigma^2) + \frac{1}{2}N(\mu_{21},\sigma^2)$
C-OC	Same as C-OO except σ^2_∞ is as in model A-LC	$\frac{1}{2}N(\mu_{21},\sigma^2_\infty) + \frac{1}{2}N(\mu_{21},\sigma^2_\infty)$
C-AO	Same as C-OO except that the additivity restriction of B-AO is imposed	Same as in C-OO
C-AC	Same as C-OC except that the additivity restriction of B-AO is imposed	Same as in C-OC

[1] $N(\mu, \sigma^2)$ is a normal probability distribution with mean μ and variance σ^2; μ_1, μ_2, and μ_3 are means of P_c for C57BL/6J, F1, and DBA/2J respectively; C is a constant the value of which determines the additional variance in the backcrosses due to the magnitude of the difference between parental means; r = recombination fraction, $0 \leq r \leq 0.5$. Backcross to DBA/2J may be obtained by replacing the subscript 1 by 3.

mean seizure threshold value of the F_1 hybrids is significantly higher than that of DBA and lower than that of C57, although closer to the latter, indicating a degree of dominance. Tests of the hypothesis of homogeneity of variances among the distributions show the following: among C57, DBA, F_1, BC C57, BC DBA and F_2, at least one of the variances of the distributions is different. Inspection of the distributions of variances in Table 2 indicates that the segregating generations (BC C57, BC DBA and F_2) are different from (variances are larger than) the genetically homogeneous generations. This result is supported by the finding that the variances of C57, DBA and F_1 are homogeneous, as are those of BC C57, BC DBA and F_2. These results establish that the ${}^{100}_{I}P_c$ mean difference between the parental strains is genetic in origin.

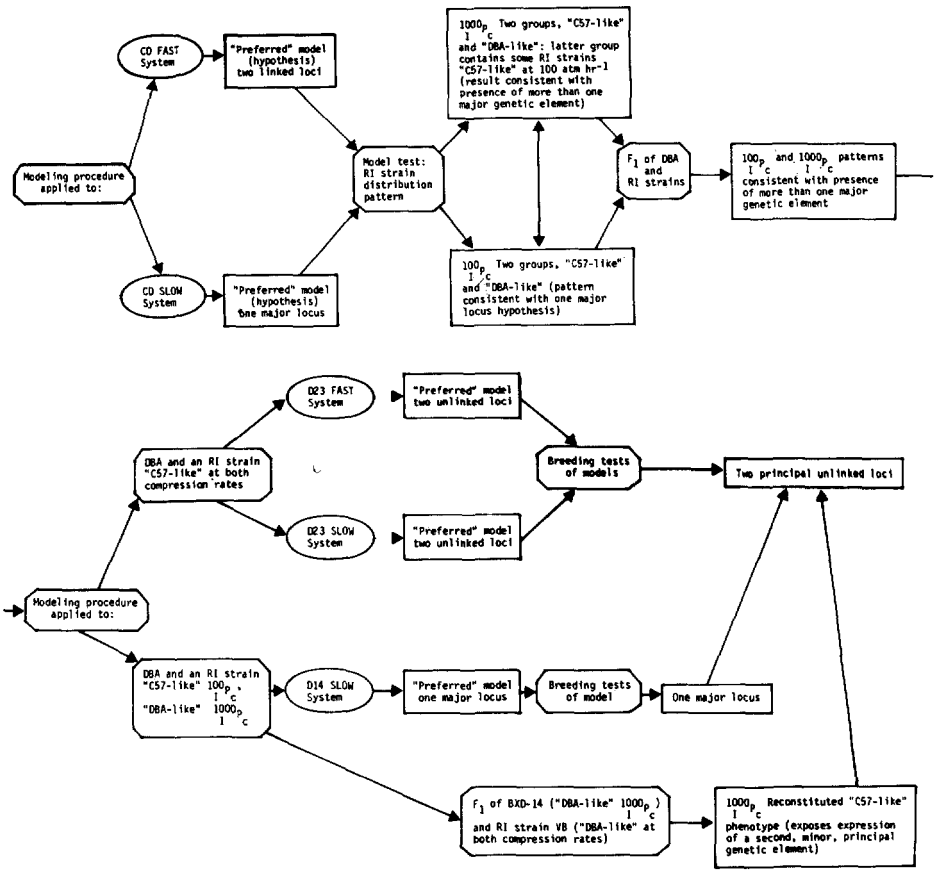

FIGURE 1.—Diagrammatic representation of the approach to genetic analysis of the difference between inbred mouse strains DBA/2J and C57BL/6J in susceptibility to the High Pressure Neurologic Syndrome (HPNS) Type I convulsive seizure. Explanation of terms and symbols is presented in MATERIALS AND METHODS. The bases for selection of "preferred" models are presented in RESULTS.

The results of the maximum likelihood modeling procedure applied to eleven models of genetic determinacy of the CD system at the 100 atm hr^{-1} compression rate are presented in the CD Slow column of Table 3. The ΔL value listed for each model is the difference in $\log_e$ likelihood from that of the model with highest likelihood. Our interpretations are based upon the approximate criteria for significance of STEWART and ELSTON (1973) in which a $\log_e$ likelihood difference between two models of less than 1.0 is considered "not significant", between 1.0 and 2.0 is "suggestive but not conclusive", and greater than 2.0 is "probably significant". On this basis, considering their associated $\log_e$ likelihoods, we exclude as candidates for the "preferred" model all except the major locus models C-OC and C-OO and possibly the two linked loci models B-OO and B-OS. Model B-OO, though it has a $\log_e$ likelihood only 1.02 lower than C-OC, is provisionally excluded as inadequate due to the tendency in maximization for extreme values in

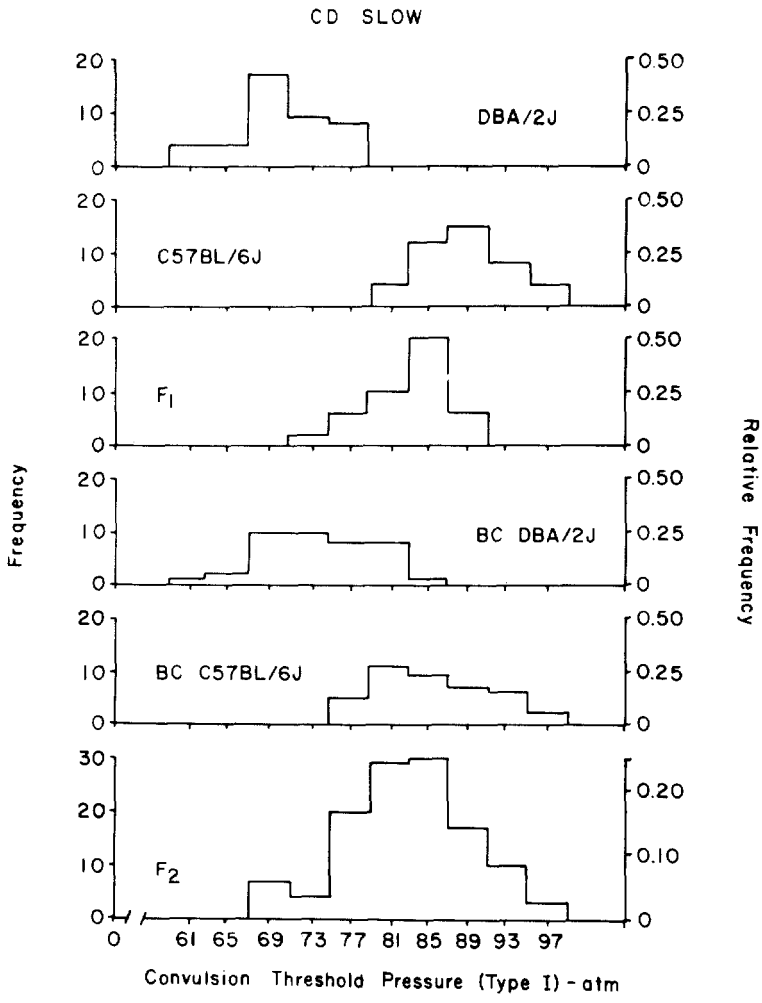

FIGURE 2.—CD Slow system: HPNS Type I seizure threshold pressure frequency distributions of inbred mouse strains DBA/2J and C57BL/6J and their first and second generation hybrids subjected to compression in heliox at a constant mean rate of 100 atm hr^{-1} in 2 atm increments.

the backcrosses to become the estimates of backcross means, μ_{12}, μ_{21}, μ_{23} and μ_{32}. Imposition of a symmetry restriction (B-OS) resulted in a further drop in likelihood to 1.89, thereby placing it close to the point of rejection.

Imposing additivity (C-AO, C-AC, B-AO) lowered the likelihoods significantly, indicating that genic interaction is occurring. The close similarity in ΔL of models B-AO and A-2 (3.55 and 3.50, respectively) is expected since all nonzero arbitrary initial estimates of the additional recombination coefficient parameter r quickly converged, by iteration, to 0.5 in all of the B- models.

The models with the highest likelihoods, C-OC and C-00, differ by only 0.64 $\log_e$ units and are probably indistinguishable by this method. However, the in-

TABLE 2

Genetic analysis of a high pressure induced seizure strain difference:

$100p_c$ distributions of C57BL/6J, DBA/2J and their first and second generation hybrids

Generation	No. Animals	Mean (±S.D.)	Variance		Homogeneity of Variances*	
1. C57BL/6J	40	88.8 ± 4.5	20.3	1.	$B_c = 0.7$	$B_c = 16.4$
2. DBA/2J	40	70.0 ± 4.8	23.0	2.	$P = 0.695$	$P = 0.006$
3. F_1	44	83.0 ± 4.2	17.6	3.		
4. BC C57	40	85.4 ± 5.8	33.6	4.	$B_c = 2.4$	
5. BC DBA	40	73.8 ± 5.6	31.4	5.	$P = 0.308$	
6. F_2	120	82.9 ± 6.6	43.6	6.		

• Bartlett's test

TABLE 3

Reduced $\log_e$ likelihoods (ΔL)* of eleven genetic models of variability in susceptibility to a high pressure induced seizure for each of five breeding systems†. "Preferred" models are underlined.

BREEDING SYSTEMS									
CD SLOW		CD FAST		D23 SLOW		D23 FAST		D14 SLOW	
Model	ΔL	Model	ΔL	Model	ΔL	Model	ΔL	Model	ΔL
C-0C	0.00	B-00	0.00	B-00	0.00	B-00	0.00	C-00	0.00
C-00	0.64	B-0S	0.21	B-0S	0.35	B-0S	0.57	C-0C	0.01
B-00	1.02	C-0C	2.47	C-0C	1.80	C-0C	2.81	C-A0	0.33
B-0S	1.89	C-AC	5.15	C-00	1.80	C-00	5.30	C-AC	0.33
C-A0	1.99	C-00	5.44	C-AC	2.37	B-A0	7.31	B-0S	0.75
C-AC	2.33	B-A0	6.01	C-A0	2.42	C-AC	7.41	B-00	0.77
A-LC	3.02	C-A0	7.72	B-A0	2.98	C-A0	8.69	A-1	0.78
B-A0	3.50	A-2	8.07	A-1	2.98	A-2	9.67	B-A0	0.78
A-2	3.55	A-1	8.26	A-2	4.62	A-1	10.79	A-2	2.72
A-1	4.70	A-LC	17.17	A-LC	6.34	A-LC	17.34	A-LC	3.72
A-L0	4.77	A-L0	25.65	A-L0	9.38	A-L0	21.35	A-L0	7.04
{$\log_e$ likelihood of C-0C (L C-0C) = -444.82}		(L B-00 = -455.06)		(L B-00 = -474.28)		(L B-00 = -250.19)		(L C-00 = -336.69)	

• Values in the ΔL columns indicate $\log_e$ likelihood differences from models with the highest likelihoods.

† The breeding systems are defined in the text.

creased variance in the backcrosses is "accounted for" in C-OC with its positive estimate of $C = 0.033 \pm 0.02$, making it the "preferred" model. The maximum likelihood parameter estimates for all the breeding systems are presented in Table 4. The major locus specified by model C-OC is associated with about 64% of the difference in mean seizure susceptibility between the C57BL/6J and DBA/2J parental strains. The remainder of the mean difference is associated with an unspecified number of interacting "polygenes . It should perhaps be noted that by treating C as a parameter to be estimated with a lower bound of zero in the equation $\sigma^2_\infty = \sigma^2 + C\ (\mu_1 - \mu_3)^2$, we differ from ELSTON and STEWART (1973), who apparently treated σ^2_∞ as a parameter, rather than C, and did not restrict the value of σ^2_∞ to be $\geq \sigma^2$.

The CD Fast system: The distribution of ${}^{1000}_{I}P_c$ among the five CD Fast generations is depicted in Figure 3, using the "stem-and-leaf" method of TUKEY (1977). There appear to be more than two phenotypic classes in the BC DBA data. This may represent some gain in resolution, compared to the BC DBA data for ${}^{100}_{I}P_c$. If so, an inheritance pattern(s) other than the one major locus pattern is favored.

Results of the modeling procedure summarized in the CD Fast column of Table 3 indicate that the "two interacting loci" models B-OO and B-OS have significantly higher likelihoods than that of the next most likely model, C-OC. Because the symmetry restriction lowered the likelihood only slightly, B-OS was selected as the "preferred" model. As before, the B- model loci appear to be unlinked. Additivity seems to be an unrealistic restriction since, when imposed, it severely reduces the likelihood in all cases.

Another interesting result of repeating the experiment, changing only the rate of compression, is the very much larger spread of ΔL. In the CD slow system, the ratio between the most likely and least likely models is $e^{4.77}$, *i.e.*, model C-OC is about 120 times more likely to be an adequate "explanation" of the data than is

TABLE 4

"Preferred" model maximum likelihood parameter estimates for five breeding systems used in the genetic analysis of variability in high pressure seizure susceptibility

Breeding System	Preferred Model	MODEL PARAMETERS (±S.E.)									
		μ_1	μ_2	μ_3	σ^2	μ_{12}	μ_{21}	μ_{23}	μ_{32}	r	C
CD SLOW	C-OC	88.74 ±0.96	82.99 ±0.96	70.19 ±0.96	20.30 ±3.53	85.39 ±2.53	85.39 ±3.53	73.81 ±5.35	73.81 ±5.35	- -	0.033 ±0.02
CD FAST	B-OS	80.03 ±0.76	76.38 ±0.92	61.56 ±0.73	9.68 ±1.97	73.93 ±1.07	76.10 ±1.36	70.10 ±1.16	78.93 ±1.48	0.5 -	- -
D23 SLOW	B-OS	81.27 ±0.81	78.61 ±0.74	70.62 ±0.82	16.78 ±2.39	80.55 ±2.53	76.36 ±2.11	76.42 ±2.76	63.81 ±2.04	0.34 ±0.18	- -
D23 FAST	B-OS	80.57 ±0.74	78.38 ±0.75	62.52 ±0.84	6.21 ±1.35	76.34 ±1.09	75.21 ±1.02	69.52 ±1.05	61.26 ±1.26	0.5 -	- -
D14 SLOW	C-OO	82.64 ±0.87	78.57 ±0.98	68.78 ±0.85	16.19 ±2.59	81.85 ±1.25	77.53 ±1.22	77.69 ±1.12	67.27 ±1.26	- -	- -

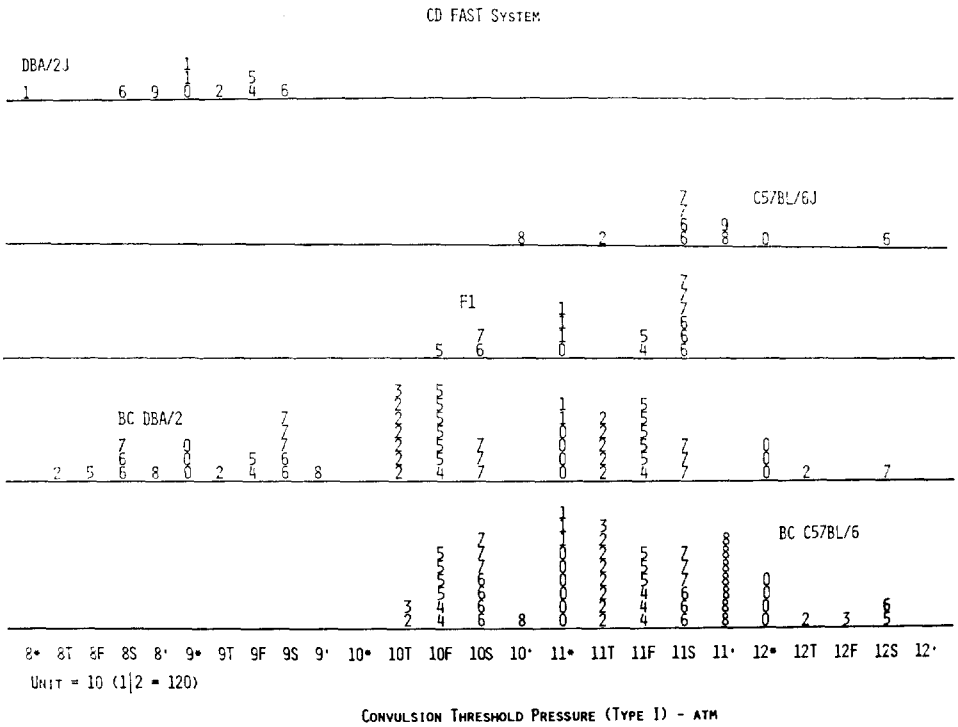

FIGURE 3.—CD Fast system: HPNS Type I seizure threshold pressure frequency distributions of mouse strains DBA/2J and C57BL/6J, their F_1 hybrids and the backcross generations subjected to compression in heliox at a continuous linear rate of 1,000 atm hr^{-1}. The data are displayed in the "stem-and-leaf" method of TUKEY (1977). The abscissa is divided into intervals of psi so that 8* represents 800 or 810 psi; 8T, 820 or 830; 8F, 840 or 850; 8S, 860 or 870; 8·, 880 or 890; and similarly for the remaining intervals.

model A-LO. The spread of ΔL values in the CD Fast system is 25.65; that is, B-OO is more than 10^{10} times more likely than A-LO, which again has the lowest likelihood.

The apparent gain in resolving power in the CD Fast system notwithstanding, the question of the relative superiority of the competing B- and C- models in describing the inheritance of variability in ${}_1P_c$ remains open. The change in relative position of the models with the change in compression profile suggests that the expression of the genetic elements is not uniformly sensitive to compression rate. If, as seems likely, some portion of the variation in ${}_1P_c$ is associated with segregation of minor modifying genes, the effect may be to blur distinctions between phenotypic classes in an unknown manner and to such a degree that further manipulation of compression rate in the CD system is unlikely to be productive.

In the following, we treat as competing working hypotheses of the inheritance mode of the HPNS Type I mean seizure threshold strain difference those spe-

cified by "preferred" models C-OC (one major locus and an unspecified large number of interacting loci each of small effect) and B-OS (two interacting unlinked loci).

A test of the competing "preferred" models (hypotheses) utilizing RI strains

The BXD strain distribution pattern (SDP) of $^{100}_{I}P_c$: The pattern of mean $^{100}_{I}P_c$ among 23 BXD RI strains is shown on the left half of Figure 4. Two distinct groups are present and the separation between them attains high statistical significance ($t = 26.8$, $p < 0.001$). The experimental distribution resembles that predicted by model C-OC, represented by the vertical lines on the left. These represent the 36% fraction of the C57-DBA mean difference in $^{100}_{I}P_c$ not asso-

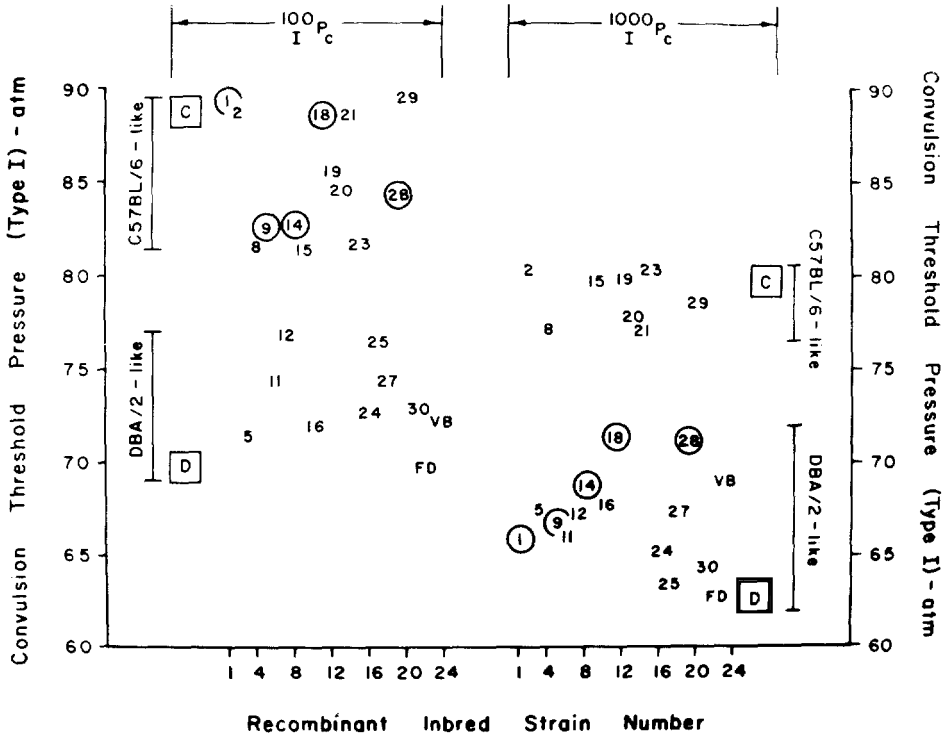

FIGURE 4.—Distributions of mean HPNS Type I seizure threshold pressure at compression rates of 100 atm hr^{-1} ($^{100}_{I}P_c$) and 1,000 atm hr^{-1} ($^{1000}_{I}P_c$) among progenitor mouse strains C57BL/6J (C), DBA/2J (D) and 23 derivative BXD recombinant inbred (RI) strains denoted by numbers or letters. RI strains encircled on the upper left are those that change status from "C57BL/6-like" to "DBA/2-like" (same strains encircled on the lower right) with a change in compression rate. Vertical lines on the left indicate the spread of RI strain means predicted from model results in the CD Slow system (see Figure 2) suggesting involvement of a major locus. Vertical lines on the right encompass the spread of RI strain mean $^{1000}_{I}P_c$ without reference to model results. Numbers on the abscissa refer to number of RI strains tested and not to RI strain designation.

ciated with the major locus, added to the mean for DBA mice ("DBA/2-like" group) and subtracted from the mean for C57 mice ("C57BL/6-like" group). The fact that the data for the recombinant inbred strains group themselves into two portions corresponding to the predicted ranges is consistent with the model assumptions that (1) genes of minor effect that raise the seizure threshold are concentrated in the C57 strain, and (2) minor threshold-modifying genes segregate independently of the major locus.

The BXD strain distribution pattern of ${}^{1000}_{I}\mathrm{P_c}$: Displayed on the right half of Figure 4 is the BXD strain distribution pattern for mean ${}^{1000}_{I}P_c$. For all strains that are "DBA-like" at slower compression, seizure thresholds are lower at the higher compression rate. Among the strains that are "C57-like" at slow compression, two patterns of response are present. Eight of the strains show a much-compressed range of variation in mean ${}^{1000}_{I}P_c$ and constitute a high-convulsion threshold pressure "C57-like" group, distinct from the rapidly compressed "DBA-like" strains. The remaining five strains (encircled in Figure 4), while "C57-like" at the slower compression rate, change their character and become "DBA-like" under fast compression. While partitioning of the BXD strains into two groups is characteristic of cases of major-locus inheritance, their occurrence in a ratio of 1.9:1.0 for "DBA-like" may suggest some other inheritance mode(s).

A preliminary analysis of the BXD strain distribution patterns for ${}^{100}_{I}P_c$ and ${}^{1000}_{I}P_c$ reveals that, while the former accords with the SDP of the H-2 locus on chromosome *17* in 18 of 23 strains (BXD-1, 9, 18, 21 and 28 are discordant), the latter accords in 21 of 23 BXD strains (BXD-14 and 21 are discordant). The presence of two distinct groups in both SDP's suggests that predominant expression of a major locus is responsible. The same major locus may be associated with both SDP's, but nonidentity in the patterns introduced by the five BXD strains that change status with a change in compression rate may mean that one or more additional genetic element(s) is (are) expressed or fail(s) to be expressed, depending upon the rate of compression. A test of this inference involving the DBA strain, certain of the BXD strains and their F_1 hybrids is presented in the next section.

A test of the competing "preferred" models involving ${}^{100}_{I}\mathrm{P_c}$ *and* ${}^{1000}_{I}\mathrm{P_c}$ *of DBA-BXD* F_1 *hybrids*

Mean ${}^{100}_{I}P_c$ values among F_1 hybrids of DBA/2 and selected BXD strains are similar to those of the parent strain with the higher ${}^{100}_{I}P_c$, as shown in Figure 5. The model assumption that the hypothesized genes of minor effect in raising ${}^{100}_{I}P_c$ exhibit a degree of dominance is supported by the relative positions of the ${}^{100}_{I}P_c$ F_1 means—intermediate between the parental means. Under fast compression, the F_1's resemble the BXD parent strain rapidly compressed, a situation apparent in the essential symmetry of the left and right sides of Figure 5.

The apparent change in dominance relations with a change in compression conditions among some of the BXD strains demonstrates that they differ genetically from those strains for which there is no change in dominance associated with a

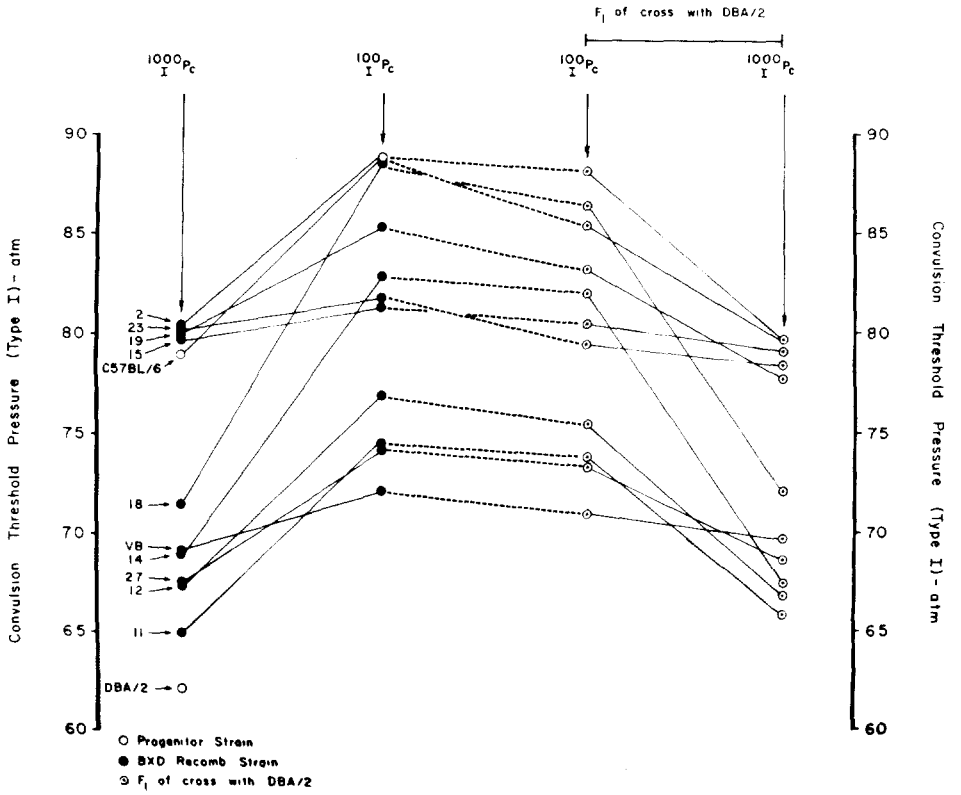

FIGURE 5.—Distributions of mean HPNS Type I seizure threshold pressure at compression rates of 100 atm hr^{-1} ($^{100}_{I}P_c$) and 1,000 atm hr^{-1} ($^{1000}_{I}P_c$) among C57BL/6J and DBA/2J and their F^1 hybrids, and 10 BXD RI strains and their F^1 hybrids with DBA/2J.

change in compression rate. This in turn implies that the observed presence of two groups of BXD strains with distinct responses to fast compression does not reflect an instance of segregation of one major locus.

The change-of-status phenomenon is readily compatible with the relative superiority of the "two interacting unlinked loci" hypothesis (B-OS model). And now the hypothesis can be modified to include the presence of conditional dominance in one of the loci, the expression of which is predominant at slow compression. The results further suggest the possibility of devising ways to follow segregation of the hypothesized principal loci separately. For example: BXD-23 and BXD-14 are similar in mean $^{100}_{I}P_c$ (81.7 ± 3.9 and 82.8 ± 3.7, respectively), but dissimilar in mean $^{1000}_{I}P_c$ (respectively, 80.0 ± 3.1 and 68.9 ± 5.7); mean $^{100}_{I}P_c$'s of F_1 hybrids of BXD-23 and DBA/2 and of BXD-14 and DBA/2 (78.5 ± 4.5 and 78.2 ± 4.5, respectively) are similar to the "C57-like" BXD parents' $^{100}_{I}P_c$'s and to each other; and at fast compression the D23 F_1 mean $^{1000}_{I}P_c$ (78.4 ± 2.7) is similar to that of BXD-23, but the D14 F_1 $^{1000}_{I}P_c$ (67.7 ± 4.5) is more like that of the DBA parent. The inference drawn from these data is that BXD-23 and BXD-

14 are genetically identical at one of the two principal loci and different at the other. On this basis, considering the pattern of ${}^{100}_{I}P_c$ and ${}^{1000}_{I}P_c$ among the BXD strains and their F_1's with DBA, we provisionally assign principal loci genotypes to the C57BL/6 and DBA/2 parental strains and to some of the BXD strains according to the convention in which *A* and *a* designate major locus alleles, *B* and *b* are the minor locus alleles and the upper case denotes dominance: BXD-23 mice, like C57BL/6 mice, bear dominant alleles at both loci (*AA BB*) and BXD-14 mice (*AA bb*) are dominant only at the locus associated with "C57-like" status at slow compression. The seizure behavior of the DBA strain and its F_1 hybrids with other strains suggests that DBA is the doubly recessive genotype *aa bb*. By inference, DBA and BXD-14 differ at the major ("slow compression") locus.

In the following, we present the results of additional applications of the modeling procedure, the purpose being to test the inferences made in this section about the inheritance of seizure susceptibility differences. In the first instance, DBA and BXD-23 (D23 system) were the parental strains. Selection of BXD-23 was based upon the assumption that the relative position of its mean ${}^{100}_{I}P_c$—at the lower extreme of the "C57-like" distribution—might represent a C57BL/6 seizure genotype minus the influence of the hypothesized genes of minor effect. The D23 Slow and D23 Fast model results are supplemented by experiments designed to test further the superiority of the two-loci inheritance pattern hypothesis. Our aim, in the second instance (D14 Slow system and breeding tests), was to follow segregation of the hypothesized major locus in a system the parental strains of which are assumed to have the same minor locus genotype.

Refinement of the "preferred" two-loci model of the ${}_{I}P_c$ difference involving the D23 systems

The D23 Slow system: The distribution of ${}^{100}_{I}P_c$ in the five generations of the D23 Slow system is shown in Figure 6. At least three overlapping phenotypic classes appear to be represented in the BC DBA data. This suggests that an inheritance mode other than one major locus is required adequately to represent the HPNS Type I seizure threshold difference.

The outcome of the modeling procedure for the D23 Slow system (Table 3) shows that the highest likelihood is associated with model B-OO, though imposition of the symmetry restriction results in an insignificant drop in likelihood so that B-OS is the preferred model. The recombination values of both models are closely similar and suggest loose linkage. It should be noted, though, that the B-models' error estimates of the recombination fraction are rather large and include 0.5. Discrimination between models B-OO and B-OS and the major locus models C-OO and C-OC remains relatively weak ($\Delta L < 2.0$), but since the two-loci models are also in better agreement with the results of the previous section and of the CD Fast system, they must be considered more representative of the inheritance pattern of ${}_{I}P_c$.

Breeding tests of the D23 Slow system inheritance model: Figure 7 shows the distributions of ${}^{100}_{I}P_c$ resulting from the mating of mice representing the extremes of the D23 Slow BC DBA distribution again crossed to DBA. ${}^{100}_{I}P_c$ for the offspring

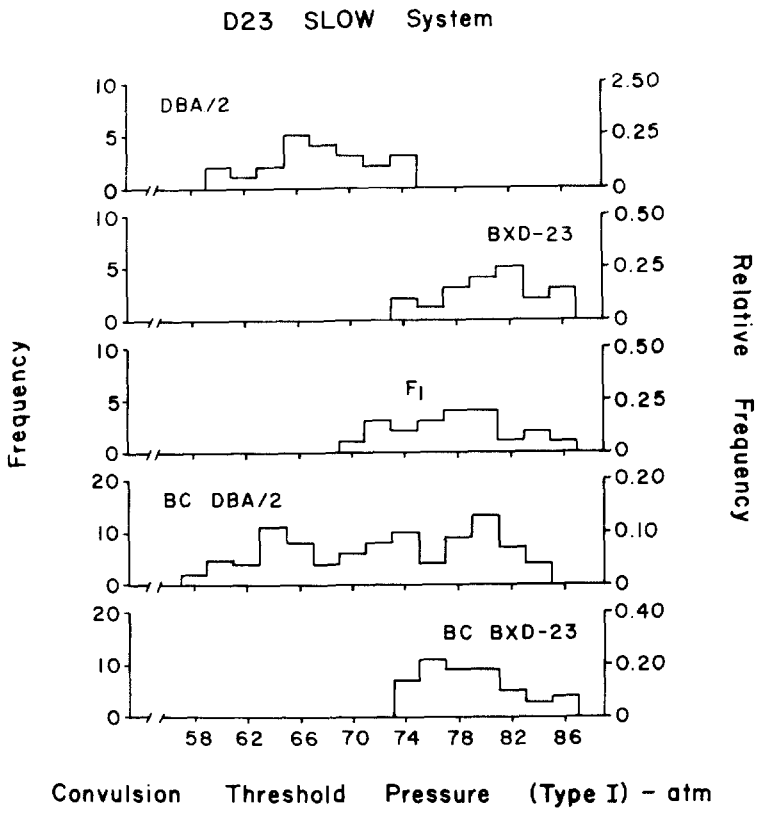

FIGURE 6.—D23 Slow system: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 2 among mouse strains DBA/2J and RI strain BXD-23, their F_1 hybrids and the backcross generations.

of mice selected from the low extreme of the BC DBA distribution crossed to DBA (BC_2 DBA_{low}) is indistinguishable from that of the DBA parent strain (67.1 ± 4.6 and 67.6 ± 3.7, respectively). This result suggests that no further segregation of the hypothesized principal loci was occurring. Neither is there apparent any hidden variation resulting from compression-rate-dependent expression of ${}^{100}_{I}P_c$ in a sample drawn at random from the BC_2 DBA_{low} mice and recompressed three weeks later to achieve ${}^{1000}_{I}P_c$ (64.1 ± 3.0; compare with DBA ${}^{1000}_{I}P_c = 62.5 \pm 2.2$). These results imply that the mice selected from the low extreme of the BC DBA ${}^{100}_{I}P_c$ distribution were probably homozygous at the principal loci.

Male mice drawn from the high extreme of the BC DBA ${}^{100}_{I}P_c$ distribution were allowed to mate with DBA females, and then were recompressed at 1,000 atm hr^{-1}, the purpose being to expose any hidden variability in the sample. The outcome for the sample (${}^{1000}_{I}P_c = 79.1 \pm 3.3$) resembles ${}^{1000}_{I}P_c$ for the F_1 of BXD-23 and DBA (78.4 ± 2.7), which suggests that, like the latter, it may also be genetically homogeneous, but not homozygous: among the offspring of mice selected from the high extreme of the BC DBA distribution and crossed again to DBA, ${}^{100}_{I}P_c$ is as variable as the BC DBA ${}^{100}_{I}P_c$ (73.9 ± 8.1 and 72.9 ± 7.6, respectively;

Figure 7). The variability among the former encompasses at least three observable phenotypic classes similar to those observed in the BC DBA distribution. We conclude from the results of these breeding tests that the low and high extremes of the BC DBA ${}^{100}_{I}P_c$ distribution differ probably in two principal genetic elements.

The D23 Fast system: A "stem-and-leaf" histogram display of the data for the D23 Fast system generations is shown in Figure 8. As was the case for the BC DBA data in the CD Fast and D23 Slow systems, the D23 Fast BC DBA data, which exhibit three distinct though overlapping groups, are compatible with a two-loci inheritance mode. An explanation for the observed preponderance of low seizure threshold pressures among the BC DBA mice is provided by the results of the breeding tests described in the next section.

Summary results of the modeling procedure applied to the data of the D23 experiment at fast compression are presented in Table 3. This time the likelihoods of the B-OO and B-OS models are significantly higher than that of the next most likely model C-OC. Model B-OS, though its likelihood is 0.57 $\log_e$ units lower than B-OO's, is again the preferred model for the same reason given in the

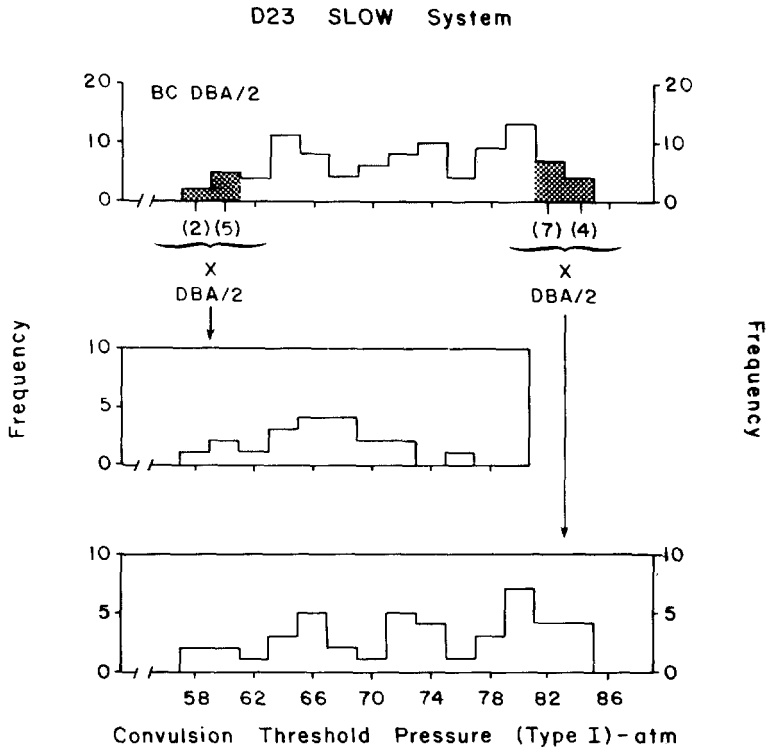

FIGURE 7.—D23 Slow system: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 2 among the BC DBA/2 in the DBA/2J-BXD-23 breeding system (see Figure 6) and the offspring of crosses between mice of the lower extreme of the BC DBA/2 distribution and DBA/2, and mice of the upper extreme of the BC DBA/2 distribution and DBA/2.

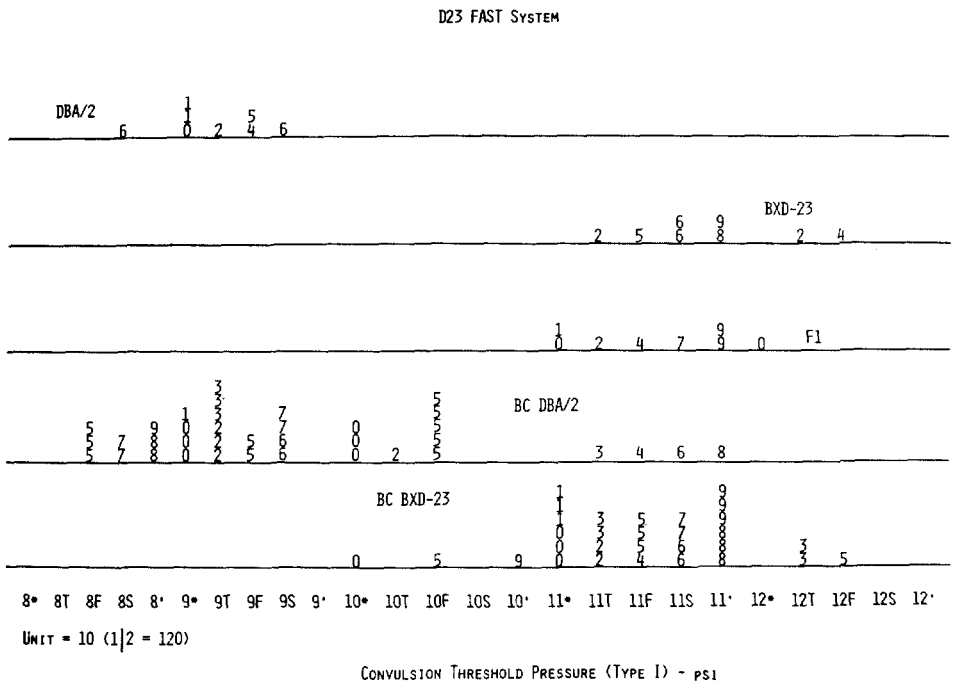

FIGURE 8.—D23 Fast System: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 3 among mouse strains DBA/2J and RI strain BXD-23, their F_1 hybrids and the backcross generations. The data are displayed in Figure 3.

presentation of results in the CD Slow system. Consistent with previous results, the B-OO and B-OS model loci appear to be unlinked. Similarly, the substantial decline in likelihood associated with the additivity restriction follows the pattern established for other breeding systems.

Tests of the D23 Fast system inheritance model: Based upon the pattern of segregation observed in the D23 Fast system BC DBA, mice that manifest the lowest seizure threshold pressures probably differ from those showing the highest ${}^{1000}_{I}P_c$ in at least one genetic element. As Figure 9 shows, when the four animals with the highest seizure threshold values were recompressed weeks later at 100 atm hr^{-1}, there was virtually no change in the mean ${}^{100}_{I}P_c$ (80.1) compared to that of ${}^{1000}_{I}P_c$ (78.4). In contrast, the sample selected from the low extreme of the BC DBA distribution, upon recompression at the slow rate, was highly variable, with some of the seizure threshold pressures attaining the level of the sample from the high extreme of the BC DBA distribution (Figure 9).

These results imply the following: (1) the D23 Fast BC DBA low ${}^{1000}_{I}P_c$ sample was composed of mice with different genotypes; and (2) the mice showing high ${}^{100}_{I}P_c$ also constitute a genetically heterogeneous group. The results are consistent with model predictions that the animals that exhibit low seizure threshold pressures at slow and fast compression rates, and mice that show high seizure threshold pressures at both compression rates differ in two principal genetic elements.

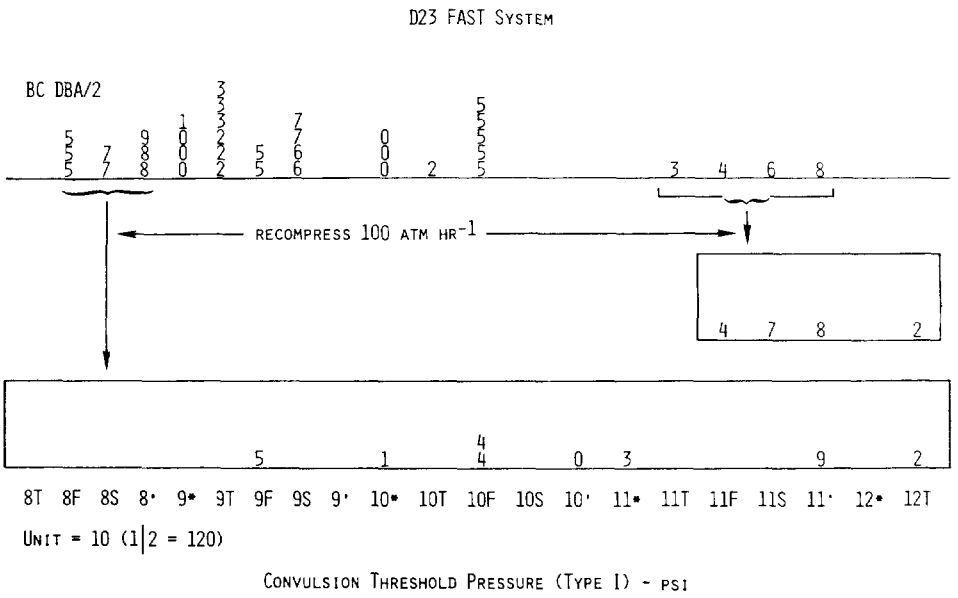

FIGURE 9.—D23 Fast system: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 3 among the BC DBA in the DBA/2J-BXD-23 breeding system (see Figure 6) and mice from the upper and lower extremes of the BC DBA distribution which were decompressed and then, three weeks later, were recompressed under the conditions described in Figure 2. The data are displayed as in Figure 3.

"Dissection" of the two-loci inheritance pattern

Evidence of major principal locus involvement in the D14 Slow system: The distributions of ${}^{100}_{I}P_c$ in the five generations of the D14 Slow system are presented in Figure 10. Bimodality in the BC DBA suggests predominant involvement of a single locus. The suggestion is strengthened by the results of maximum likelihood modeling presented in Table 3.

Major locus model C-OO, selected as the preferred model, has a higher likelihood than any other model, though the differences among the four C- models are trivial. Actually, C-OO and C-OC are equivalent because the value of the model parameter C in the latter converged, on iteration, to zero. The next most likely models cluster tightly at ΔL values still well below significance. The fact that this second group includes the three two-loci models, B-OO, B-OS and B-AO, as well as the one-locus model A-1, does not present a problem in model discrimination: the recombination values of B-OO ($r = 0.047 \pm 0.225$), B-OS ($r = 0.024 \pm 0.178$) and B-AO ($r = 0.0$) make them effectively equivalent to one-locus models and therefore very similar to the major locus C- models. There is associated with model A-2 (two equal, additive loci) a drop in likelihood of two $\log_e$ units, making it significantly less likely than the previous eight models. The multilocus A-LC and A-LO models show progressively lower likelihoods, the latter being the least likely model in all five breeding systems.

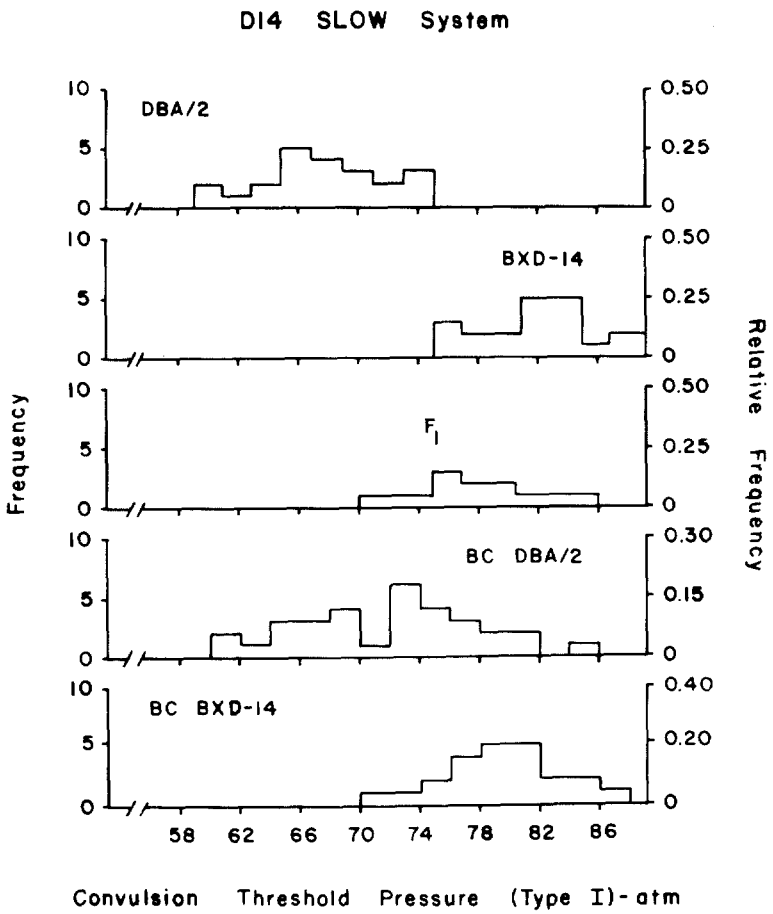

FIGURE 10.—D14 Slow system: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 2 among mouse strains DBA/2J and RI strain BXD-14, their F_1 hybrids and the backcross generations.

Breeding tests of major principal locus involvement in the D14 Slow system: Evidence of major locus involvement in the D14 Slow system was also sought in further breeding tests, the results of which are depicted in Figure 11. Mice selected from the low extreme of the BC DBA ${}^{100}_{I}P_c$ distribution produced, upon further backcrossing to DBA, an essentially DBA ${}^{100}_{I}P_c$ (70.6 ± 3.6) distribution among the offspring. Results of a second backcross to DBA of a sample from the high extreme of the BC DBA distribution were indicative of continued segregation, judging from the distribution in Figure 11 and the larger associated standard deviation (72.0 ± 6.5).

The possible presence of hidden genetic variability in ${}^{100}_{I}P_c$ was tested in another experiment by subjecting the D14 Slow generations to fast compression (Figure 12): parental strain DBA (62.5 ± 2.2) and the backcross to it (63.7 ± 3.5) form a relatively homogeneous assemblage; parental strain BXD-14 (66.3 ±

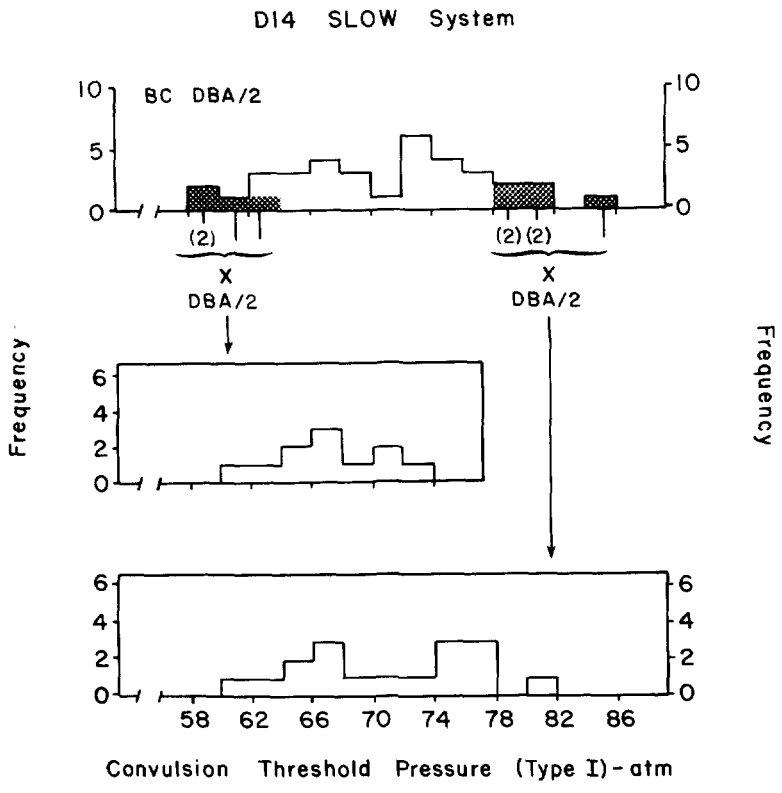

FIGURE 11.—D14 Slow system: HPNS Type 1 seizure threshold pressure frequency distributions produced under the conditions described in Figure 2 among the BC DBA/2 in the DBA/2J-BXD-14 breeding system (see Figure 10) and the offspring of crosses between mice of the lower extreme of the BC DBA/2 distribution and DBA/2, and mice of the upper extreme of the BC DBA/2 distribution and DBA/2.

3.7), its F_1 hybrid with DBA (66.1 ± 3.9), and backcross to BXD-14 (65.0 ± 3.3) also converge, upon fast compression, to a homogeneous ${}^{1000}_{I}P_c$ distribution and are also similar to the former assemblage in mean ${}^{1000}_{I}P_c$. These data are consistent with the model prediction that most of the mean difference in ${}^{100}_{I}P_c$ between the DBA and BXD-14 strains is associated with a single gene difference at the "slow compression" locus.

It remains to demonstrate segregation at the other hypothesized minor principal locus independent of the major locus. Following the effect of the minor locus on seizure threshold is more problematic due to the apparently relatively minor influence of the *B* allele upon raising ${}_{I}P_c$ in the absence of the major locus dominant allele. That is, presumed genotypes *aa BB* and *aa bb* are not differentiable on the basis of either ${}^{100}_{I}P_c$ or ${}^{1000}_{I}P_c$ among the BXD strains.

The essence of breeding tests devised to expose the action of the minor locus is to try to reconstitute, from BXD RI strains with ${}^{1000}_{I}P_c$ means in the "DBA-like" range and genotypes thought to be contrasting heterozygotes *AA bb* and *aa BB*, a

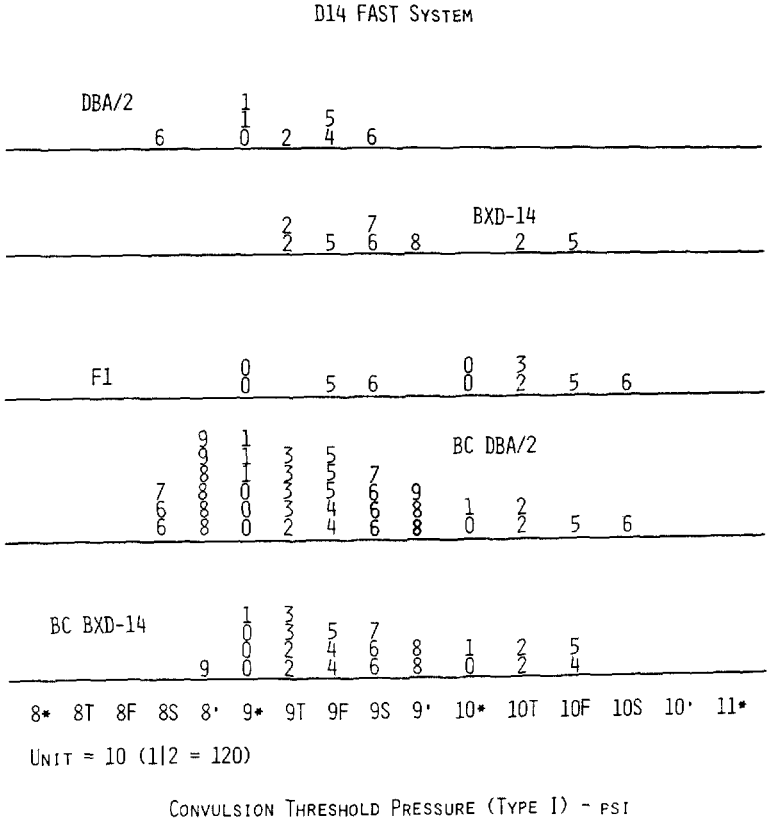

FIGURE 12.—D14 Fast system: HPNS Type I seizure threshold pressure frequency distributions produced under the conditions described in Figure 3 among mouse strains DBA/2J and RI strain BXD-14, their F_1 hybrids and the backcross generations. The data are displayed as in Figure 3.

"C57-like" ${}^{1000}_{I}P_c$ phenotype with genotype *Aa Bb*. Based on the results of the previous section, BXD-14 satisfies the requirements of the *Aa bb* genotype. Strain VB was selected as a candidate for the *aa BB* genotype on the basis of its ${}^{100}_{I}P_c$ and ${}^{1000}_{I}P_c$ means (72.2 ± 5.0 and 69.0 ± 3.8, respectively), *i.e.*, the smallest compression rate effect of the "DBA-like" strains (see Figure 4), though the magnitude of the compression rate effect may be of limited utility in identifying the *aa BB* genotype.

"Dissection" of the two-loci pattern: Evidence for a minor principal locus: The mean ${}^{1000}_{I}P_c$ of the F_1 hybrids of the VB and BXD-14 strains, 76.6 ± 3.8 (N=8), approximates a fast compression "C57-like" phenotype. This result, while contrasting sharply with the ${}^{1000}_{I}P_c$ "DBA-like" phenotypic status of the parents, is consistent with the expectation derived from other results of this study that a "C57-like" phenotype at fast compression requires the presence of the dominant alleles at both loci. We conclude that strain VB has HPNS Type I seizure principal loci genotype *aa BB*.

DISCUSSION

The results reported in this study strongly suggest that a significant proportion (> 60%) of the mean difference in susceptibility to a high-pressure-induced seizure (HPNS Type I) between the C57BL/6J and DBA/2J mouse strains is associated with two unlinked autosomal loci, one of major and the other of minor effect. The remaining difference in mean susceptibility probably results from segregation of an unknown number of loci, each of small effect on ${}_{I}P_{c}$. Considering the BXD strain distribution patterns of ${}_{I}P_{c}$ in Figure 4, these "polygenes" may segregate independently of the major locus.

The major locus exhibits conditional dominance characteristics depending upon compression rate and minor locus genotype. At slow compression, it manifests strong, though incomplete, dominance apparently independent of minor locus genotype. Its expression is, however, highly sensitive to compression rate, losing its dominance altogether at fast compression. The major locus interacts with the weakly dominant and relatively compression-rate-insensitive minor locus to retain dominance at fast compression only when the dominant alleles of both loci are present. This behavior of the major locus doubtless accounts for the increase in resolution among the eleven genetic models of variability in ${}_{I}^{1000}P_{c}$ compared to that of ${}_{I}^{100}P_{c}$ for the breeding systems involving the same strains. That is, the greater resolving power of the former, manifested in the larger spread of joint $\log_e$ likelihoods of the model parameter estimates, results from the sharp discrimination of genotypes designated *AA BB* and *AA bb*, which at slow compression are undifferentiable by our methods.

A principal result of this study is the demonstration that the outcome of genetic analysis of HPNS Type I seizure susceptibility importantly depends upon manipulation of the test conditions. If we had employed the maximum likelihood modeling procedure involving the C57BL/6 and DBA/2 strains, their first and second generation hybrids and the BXD strains in a test of the "preferred" model on data collected only at 100 atm hr^{-1}, the result reported for the seizure susceptibility difference would have been of one major locus inheritance with inconclusive evidence of linkage (five discordant strains out of 23 tested) to the *H-2* locus on chromosome *17*. If only the 1,000 atm hr^{-1} compression rate had been used, the modeling outcome and the BXD SDP taken together would have suggested more strongly one major locus inheritance with high probability of linkage to *H-2* on chromosome *17* (two discordant strains of 22). We have shown that additional genetic variability was present in the "DBA-like" group of BXD strains rapidly compressed that was not expressed among the "DBA-like" groups compressed at 100 atm hr^{-1}.

The outcome of the modeling procedure might have been still different if some intermediate compression rate had been employed. The effect on ${}_{I}P_{c}$ of linear compression rates between about 20 atm hr^{-1} and 1,000 atm hr^{-1} can be described adequately by the equation ${}^{a}P_{c} = {}^{b}P_{c} + K \log {}^{b}P/a_{p}$, where ${}^{n}P_{c}$ is the seizure threshold pressure at compression rate ${}^{n}P$ (BRAUER *et al.* 1979). Application of the modeling procedure using a compression rate of, say, 500 atm hr^{-1} would probably have resulted in a "preferred" model specifying a much more complex

(multilocus) inheritance pattern. Support for the complex model would have been provided by the probable absence of discernible groups in the ${}^{500}_{I}P_c$ BXD SDP resulting from the intermediate positions occupied by the five "change-of-status" BXD strains. Employing two compression rates has permitted, by exposing involvement of a rate-sensitive physiological process and interaction with a relatively minor, but less rate-sensitive, one, more nearly adequate characterization of the heritable basis of murine high-pressure seizure susceptibility differences than could be achieved by use of a single compression rate.

The analytical orientation of this study amounted to a biological iterative procedure in which the "noise" resulting from segregation of polygenes and from one or the other of the two principal loci was dampened so that the effects of the major elements could be considered separately, as well as together. In practice, the parameter estimates derived initially from application of the maximum likelihood procedure became the basis for decisions to repeat the modeling procedure for other breeding systems.

ELSTON's and STEWART's (1973) genetic modeling procedure was intended to produce a first-order approximation to the inheritance modes of strain differences in continuously distributed biological variables as a guide to further experimentation. Its performance in the present case was impressive, even though in our data a substantial number of tied values prevented use of the associated goodness-of-fit tests. In particular, the weak discrimination (small ΔL) between the major locus C- models and the two interacting loci B- models in the CD Slow and D23 Slow breeding systems, far from being a weakness of the method, appear, based upon further tests, to have accurately represented the genetic situation. Likewise, the modeling results of the CD Fast and D23 Fast systems, by revealing the relative superiority of models B-OO and B-OS and augmented discriminatory power, establish the success of the procedure from the point of view of orienting further attempts at genetic analysis of ${}_{I}P_c$ differences between inbred mouse strains. As powerful a tool as the modeling procedure is, its capabilities are greatly extended when used in conjunction with recombinant inbred strains. Further gain in genetic analysis of ${}_{I}P_c$ past the CD Fast stage without benefit of the BXD strains would have been much more difficult due to problems of resolution stemming from the variability inherent in the test parameter.

The approach used in this study has made possible the development of a genetic model of a high-pressure-induced convulsive seizure in which contributions to differences in the behavior arising from genetic causes can be distinguished with some precision from those environmentally caused. In demonstrating that variability between inbred strains in ${}_{I}P_c$ is predominantly associated with the segregation of two loci, we show the possibility of critically identifying the physiological basis of the variability, which in turn opens the way to exploring the effects of the principal loci on others involved in seizure etiology. Recent demonstration of an association of the Type I seizure with localized subcortical brain centers (MANSFIELD, GILLEN and BRAUER 1979) may lead to a unifying concept of high-pressure seizure susceptibility in which a type of behavior whose genetic basis can largely be specified has anatomical correlates in the brain.

In addition to the inherent interest of the high-pressure phenomena in relation to genetic models of convulsive disorders, our results may be applicable to problems of human performance under high hydrostatic pressure: identification of divers at low risk of developing the life-threatening seizures would have the effect of significantly increasing the safe working depths at no additional cost and requiring no new technology.

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