CORTISONE-INDUCED CLEFT PALATE IN THE MOUSE. A SEARCH FOR THE GENETIC CONTROL OF THE EMBRYONIC RESPONSE TRAIT.[1]

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ABSTRACT

The cause of the difference in the mean tolerance (ED_{50}) to cortisone-induced cleft palate between the embryos of the A/J and C57BL/6J strains appears to be due to a small number of genes. A single major gene effect and a polygenic model, in the sense of many equal and additive genes, have been ruled out. The embryonic tolerance of C57BL/6J is greater than and dominant to that of A/J; two or three loci, possibly with independent effects, appear to explain the variability. A component of the variation in embryonic response may be associated with or linked to the major histocompatibility locus (*H-2*). No evidence was found to support the hypothesis of *X*-chromosome linked susceptibility to cortisone-induced cleft palate.

VARIATION in the frequency of cortisone-induced cleft palate between mouse strains has been known for a number of years (FRASER and FAINSTAT 1951; review by GREENE and KOCHHAR 1975). The A/J and C57BL/6J strains have been the most studied, with A/J more sensitive than C57BL/6J. The first attempt to investigate the genetic cause of this variation in response led to the conclusion that genetic differences in both the maternal and embryonic systems were involved and that the trait could be described as a "polyfactorial, quantitative character" (KALTER 1954).

Probit analysis of the cleft palate response to various dosages of cortisone with the A/J and C57BL/6J strains and their reciprocal crosses demonstrated that the kind of cleft palate response was the same in all genotypes (BIDDLE and FRASER 1976). The differences between genotypes were in mean dosage (ED_{50}) of cortisone required to induce cleft palate, that is, the mean embryonic tolerance. The C57BL/6J embryonic mean (ED_{50}) was greater than and dominant to that of A/J; the maternal effect of A/J relative to C57BL/6J dams caused a twofold reduction in embryonic tolerance.

A genetic analysis of the embryonic difference in tolerance to cortisone-induced cleft palate has been done, with a system of matings similar to that used by DAGG, SCHLAGER and DOERR (1966) and BIDDLE (1975) to investigate ter-

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atogenic response traits. Since the cortisone dose-response curves for different genotypes were parallel (BIDDLE and FRASER 1976), a single dose could be used to define response differences between genotypes. Males from backcrosses of F_1 animals to the A/J and C57BL/6J strains were expected to be segregants for genetic differences between the two parental strains. The genotypes of the backcross males were tested by mating them to A/J females, treating the females with cortisone, and measuring the frequency of cleft palate in the offspring.

MATERIALS AND METHODS

The A/J (A) and C57BL/6J (B6) strains of mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). The maintenance and breeding procedures were described previously (BIDDLE and FRASER 1976).

The crosses, and numbers of tested males, are presented in Table 1. By convention the maternal genotype is listed first. The males were timed-mated with virgin females of the A strain, which were subsequently treated with cortisone as described below. The cleft palate response of the progeny from the test matings is referred to as the sire score or the cleft palate score. As far as possible ten treated liters were collected per sire.

Because of problems with fertility of both the sires and A/J females, it was necessary to change the diets during the course of the study. Initially, Purina Laboratory Chow was used. Half way through the test matings of the BC_1 sires, the sires and A females were changed to Purina Mouse Chow. Following timed mating, the A females were switched to Laboratory Chow during pregnancy. The RBC_1 sires and their test-mated A females were maintained entirely on Mouse Chow.

The timed-mated A females were treated at noon on day 12 of gestation with cortisone acetate (*Cortone*, Merck-Frosst Laboratories) at 200 mg/kg, subcutaneously. Dosage was determined according to weight of the female at time of treatment. The dams were killed on day 18 and the products of conception were examined as described previously (BIDDLE and FRASER 1976). The cleft palate response (percent with cleft palate of total live fetuses without cleft lip) was recorded for all treated litters from each sire. The few litters with only one live fetus without cleft lip were discarded. A litter-averaged cleft palate score for each sire was determined with the Freeman-Tukey arcsin transformation for small sample sizes (MOSTELLER and YOUTZ 1961).

TABLE 1

Summary of test mating scores (arcsin) of the sires for cortisone-induced cleft palate

Sire	Number tested	Mean	Expected mean*	Variance
A	5	71.08 (89.5)†	——	10.6389
B6	5	23.10 (15.4)	——	20.4249
A.B6 F_1	5	49.53 (57.9)	47.09	20.0585
A.AB6 BC_1	38	58.86 (73.2)	59.10	73.0645
B6.AB6 RBC_1	19	42.56 (45.7)	35.10	68.2668

* Expected means based on an additive genetic model.
† Numbers in parentheses were obtained by transforming arcsin to percent.

The breeding data for the cortisone-induced cleft palate response were analyzed by procedures similar to those of BIDDLE (1975). Some of the methods described by STEWART (1969) and STEWART and ELSTON (1973) were also used. The major part of the test mating program is outlined schematically in Figure 1; superimposed on it is a hypothetical "single gene" model which was used to begin the genetic analysis of the difference in embryonic tolerance to cortisone-induced cleft palate.

In addition to the main breeding study, three other types of genetic tests were carried out. First, preliminary breeding data (BIDDLE and FRASER 1974) suggested bimodality of the cleft palate scores of the BC_1 sires and thus the possibility of a single major gene difference. This was tested with the test-mating scores from a minimum of five BC_2 males that were derived from each of seven BC_1 sires and untreated A females. An average of five cortisone-treated litters were obtained from each BC_2 sire test mated with A females. The BC_1 male parents were picked with prior knowledge of their preliminary cleft palate scores to be similar either to A.B6 F_1 or to A sires. The restriction that this places on the interpretation of the data from the BC_2 sires will be considered in the discussion.

Second, the suggestion of *X*-linked embryonic sensitivity factors to cortisone-induced cleft palate (FRANCIS 1973) was tested with five B6.A F_1 sires each test mated with ten A females. The female and male embryonic responses from the B6.A F_1 sires were compared with those from A.B6 F_1 sires. The hypothesis of *X*-linked embryonic sensitivity predicts that female progeny from A.B6 F_1 sires will have a higher frequency of cortisone-induced cleft palate than female progeny from B6.A F_1 sires, and that male progeny from the two reciprocal F_1 sires will have identical responses.

Third, association tests were made to search for possible linkage between the embryonic cortisone response trait and other genetic variants that were segregating in the BC_1 and RBC_1 sires. Of the known single-gene differences between the A and B6 strains, only albinism (*c*), brown (*b*) and the major histocompatibility locus (*H-2*) were scored. The A strain is albino brown

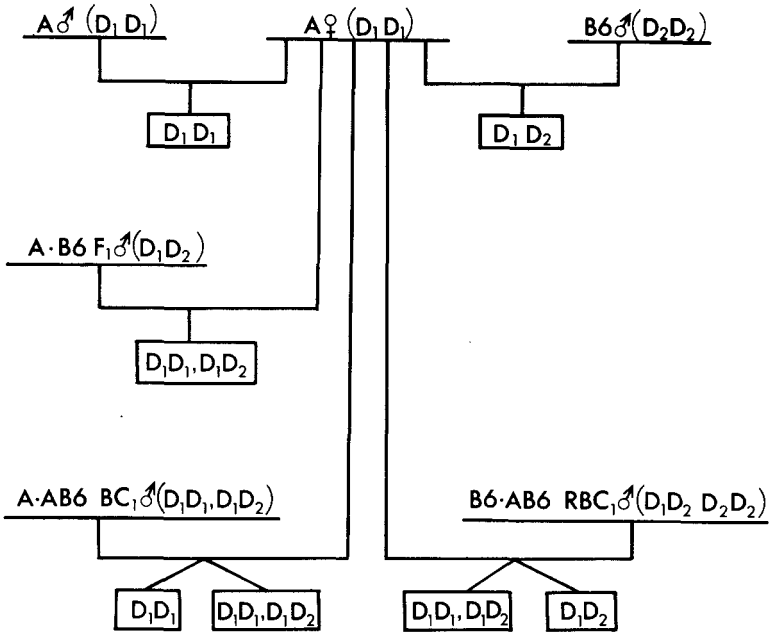

FIGURE 1.—Test mating scheme used to investigate the genetic control of the embryonic response difference to cortisone-induced cleft palate. The segregation of hypothetical alleles D_1 (A strain) and D_2 (B6 strain) at a single autosomal locus is superimposed on the mating scheme.

(*cc, bb*) and defined here as homozygous for *H-2*^{a}; the B6 strain is pigmented black (++, *BB*) and defined here as homozygous for *H-2*^{b}. The pigment genotypes of the BC_1 sires were inferred from their coat colors but were determined for the RBC_1 sires, that are phenotypically black, from the eye color of their progeny from the test matings with A females. *H-2* differences were typed in the BC_1 sires by a microdroplet lymphocyte cytotoxicity assay with anti-B6 serum (MITTAL *et al.* 1968) and in the RBC_1 sires by a hemagglutination assay with antiserum D-4 (N.I.H. Transplantation Immunology Branch) against antigenic specificity H-2D-4 of the A strain (STIMPFLING 1964). Some of the BC_1 and RBC_1 sires died before they could be typed for *H-2*.

RESULTS

The results of the main breeding study are summarized in Table 1 and Figure 2. The means of the test mating scores (arcsine) for cortisone-induced cleft palate from the five types of sires are plotted against sire genotype as frequency of A strain genes (Figure 2). The cleft palate scores for individual sires from each generation are also indicated. There is a good fit of the means of the A.B6 F_1 and BC_1 sire scores to the regression line between the A and B6 inbred sires. The cortisone-induced cleft palate trait, when assessed by sire scores, behaves additively. Since the B6 embryonic tolerance (ED_{50}) is dominant to that of A, there is no indication of interaction (epistasis) if many loci are involved.

The mean of the RBC_1 sires (Table 1 and Figure 2) is higher than expected on the basis of either a single gene model or no interaction if more than one locus is

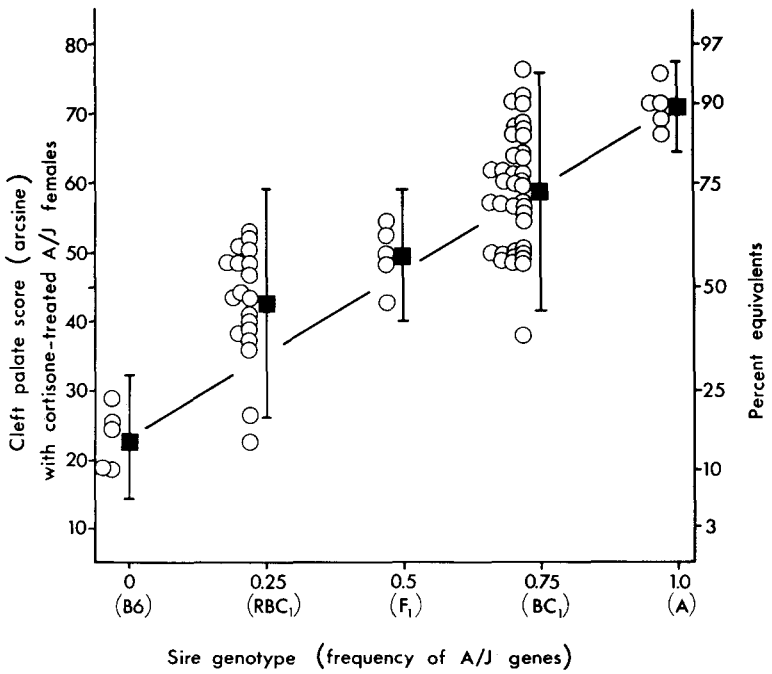

FIGURE 2.—Regression of mean sire scores (■, arcsin, ± 2 s.d.) for cortisone-cleft palate on sire genotype as frequency of A strain genes. Individual sire scores in each generation are plotted as open circles. Percent scale, equivalent to arcsin transformation, is also shown.

TABLE 2

*Effect of the two diets on cortisone-induced cleft palate in the A/J and C57BL/6J strains**

Diet	A/J (100 mg/kg)		C57BL/6J (600 mg/kg)	
	No. treated litters	Cleft palate† (arcsin)	No. treated litters	Cleft palate (arcsin)
Mouse chow	8	43.35	10	35.30
Laboratory chow	12	35.51	9	29.91
Difference		7.84		5.39

* Cortisone was administered s.c. on day 12 to the two strains at the indicated dosages.
† Cleft palate is the litter-averaged response using the Freeman-Tukey arcsin transformation. Average change in cleft palate response with diet for the two strains is 6.62 arcsin units.

involved. However, the test-mated and cortisone-treated A females for these sires were maintained on Mouse Chow instead of Laboratory Chow during pregnancy. Table 2 summarizes a comparison of the effects of the two diets on cortisone-induced cleft palate in the A and B6 strains from a study done concurrently with the test matings of the RBC_1 sires. Changing from Laboratory Chow to Mouse Chow was known to decrease the A strain tolerance (ED_{50}) to cortisone-induced cleft palate and the diet effect is additive, that is, the cleft palate frequency is

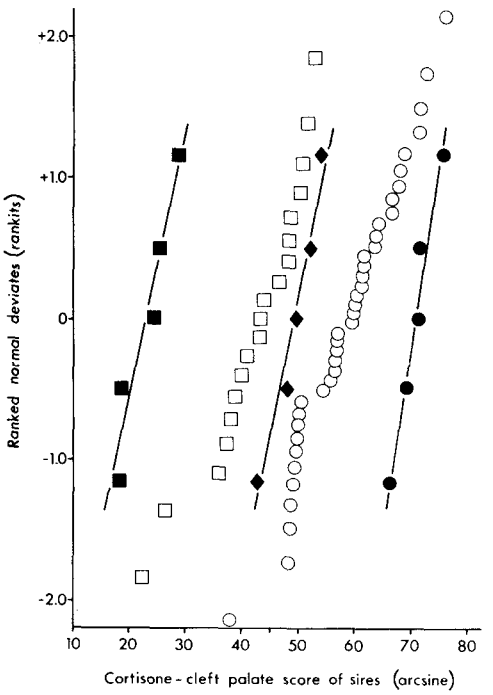

FIGURE 3.—Rank-ordered distributions (rankits) of the cortisone-cleft palate scores from the sires of each generation plotted against sire score (arcsin). The symbols are: ■, B6; ◆, A.B6 F_1; ●, A; □, B6.AB6 RBC_1; ○, A.AB6 BC_1. The straight lines were fitted by least squares.

increased by a constant relative amount for a given dose of cortisone (BIDDLE and FRASER, unpublished probit analysis). If the diet effect in B6 is assumed also to be additive and equal to the effect in A, The average change of 6.62 arcsin units in the cleft palate response in the A and B6 strains (Table 2) may be used as an approximation to the difference between the effects of the two diets. If the observed mean of the RBC_1 sires (42.56, Table 1) is reduced by this amount, the result of 35.94 agrees well with the expected mean (35.10) from the additive model.

The rank-ordered distributions of the A, B6, and A.B6 F_1 sire scores are compared in Figure 3 by a procedure using rankits (SOKAL and ROHLF 1969, pp. 121–125) and the between-sire variances for the three crosses can be considered to be normally distributed. Although the variance of the A sires is approximately half that of the other two sires (see Table 1), no further transformation of the scale for the cleft palate response was attempted to make the variances of the three generations equivalent.

The distributions of the BC_1 and RBC_1 sires are shown in Figure 3 and they do not appear to be normal by inspection. The Kolmogorov-Smirnov one-sample test (SIEGEL 1956) suggests that each of the two backcrosses is composed of more than one distribution. The deviation from one distribution was tested with the cumulative numbers of sires in equal but arbitrary class intervals across the range of sire scores in each backcross. For the BC_1 sires, $D_{max} = 0.244$, $P = 0.04$; for the RBC_1 sires, $D_{max} = 0.323$, $P = 0.04$.

It seems reasonable to reject a polygenic model with loci of equal and additive effect. The distributions of both the BC_1 and RBC_1 sires deviate from one distribution. Also, the means of the inbred sires (A and B6) have been recovered in the BC_1 and RBC_1 sires. Since there is no clear evidence of bimodality in either the BC_1 or RBC_1 sire distributions with modes corresponding to the A.B6 F_1 sires and inbred sires, the single gene model must be rejected also.

To search for possible modes within the BC_1 and RBC_1 sire distributions, the rank-ordered distributions of the sire scores were differentiated with the midpoint formula technique (STEWART 1969; STEWART and ELSTON 1973). The results are shown in Figure 4, and the means (± 1 and 2 standard deviations) for the A, B6, and A.B6 F_1 sires are indicated. At least three and possibly four modes are indicated in the BC_1 sires and two in the RBC_1 sires. (Keep in mind the previous comments about the diet change that will lower the position of the RBC_1 sire distribution.)

The test mating data from the BC_2 sires are summarized in Table 3 by families of BC_2 sires from the seven BC_1 males. On the basis of a single gene difference, the BC_1 sires should be either heterozygous or homozygous for the A genotype, the sons of the "high" BC_1 males should all test "high" and the sons of the "low" BC_1 males should test either "high" or "low." The differentiated rank-ordered distributions of the BC_2 sires from each of the two selected groups of BC_1 sires are presented in Figure 5. Bimodality of the cleft palate scores is suggested from the "low BC_1"-derived BC_2 families demonstrating that they are segregating for the cortisone response trait. A single mode is suggested from the "high BC_1"-

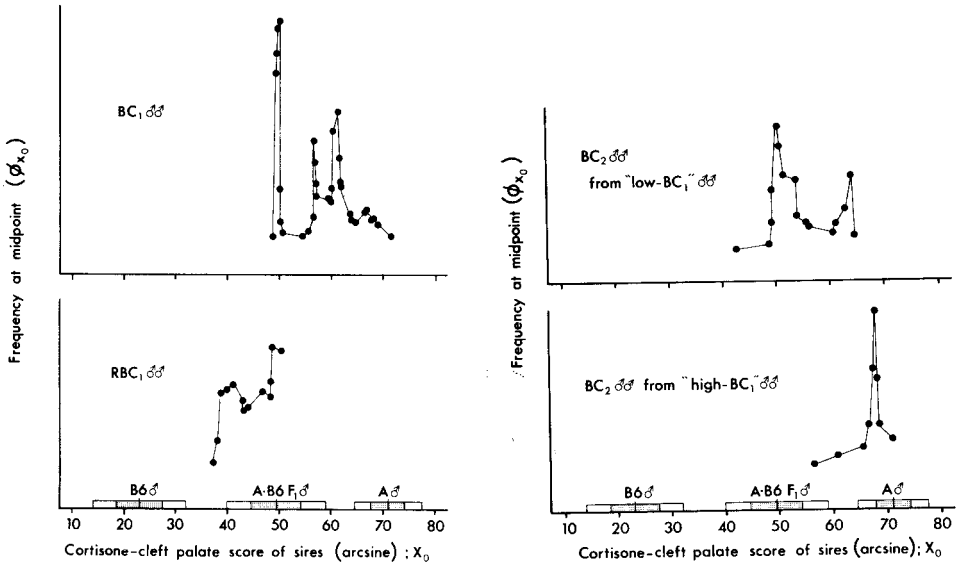

FIGURE 4 (left).—Frequency distributions of cortisone-cleft palate scores in the BC_1 and RBC_1 sires. Ordinate gives frequencies at X_0 on abscissa calculated from midpoint formula (STEWART 1969). The means of the parental and F_1 sires are shown on the abscissa with ± 1 s.d. (hatched area) and ± 2 s.d. (open area).

FIGURE 5. (right)—Frequency distributions of cortisone-cleft palate scores in the BC_2 sires from "low BC_1" males and from "high BC_1" males. Figure constructed similar to FIGURE 4.

TABLE 3

Summary of cortisone-induced cleft palate scores from families of BC_2 sires

BC_1 sires		BC_2 families		
Sire No.	Cleft palate score (arcsin)	No. RBC_2 sires	Mean cleft palate score (arcsin)	Variance
"low BC_1" sires				
58	48.43	5	58.81	63.0358
47	48.48	6	58.72	98.0128
60	49.23	5	46.54	103.3523
66	49.90	6	53.96	73.1571
"high BC_1" sires				
61	64.50	5	66.88	97.6729
68	68.97	5	58.37	182.0713*
48	71.61	5	69.36	53.3719

* A mean of 68.63 and variance of 15.5451 are obtained from the A/J sires when the response data from their first 5 litters only are considered. *F* ratio test indicates that the sampling variance of the BC_2 family from ♂ 68 BC_1 ("high" group) is too large to be considered as not to be segregating for response factors ($P<0.025$).

derived BC_2 families, but the tail of the distribution extends into the range of the A.B6 F_1 sires, which argues against homogeneity.

The BC_2 sire data cannot be used to support a single gene model (see discussion); however, they can be used to reject it. The variances (Table 3) of the BC_2 family means do not exhibit significant heterogeneity (Bartlett's test for homogeneity of variances), although the variance of the BC_2 family from male 68 BC_1 (Table 3) in the "high BC_1"-derived group is suspiciously large. There is no criterion by which to distinguish segregating from nonsegregating BC_2 families among themselves. If the variances of the BC_2 families can be compared with the sampling variance of the A sires (Table 1), or better with the sampling variance obtained by examining only the first five litters from each of the five A sires so that sample sizes are approximately equal (mean = 68.63, s^2 = 15.5451, and see footnote to Table 3), the variance of the BC_2 family from the male 68 BC_1 is significantly too large ($P < 0.025$) and this BC_2 family may be considered to be still segregating. Therefore the preliminary suggestion of a single-gene mode of inheritance for the cortisone-cleft palate trait (BIDDLE and FRASER 1974) must be rejected.

The breeding data from the A.B6 F_1 and B6.A F_1 sires (Table 4) were tested for the presence of *X*-linked embryonic response factors. The cleft palate responses of the female and male progeny are listed separately as the litter-averaged arcsin. The cleft palate response in the total offspring of each sex from each sire is included for comparison. The mean cleft palate response (arcsin) in female progeny is significantly greater from A.B6 F_1 sires than from B6.A F_1 sires (t = 1.93, 8 df, $P < 0.05$, one-tailed probability) and supports *X*-linkage. However,

TABLE 4

Cleft palate responses (arcsin) of female and male progeny from reciprocal F_1 sires test mated with A/J females

A.B6 F_1 sires					B6.A F_1 sires				
Sire No.	Cleft palate/females		Cleft palate/males		Sire No.	Cleft palate/females		Cleft palate/males	
5	52.30 (62.6)*	(26/45)†	47.32 (54.0)	(21/35)	8	48.77 (56.6)	(25/43)	53.80 (65.1)	(25/38)
6	52.67 (63.2)	(23/35)	51.01 (60.4)	(24/37)	9	38.94 (39.5)	(16/30)	55.13 (67.3)	(28/43)
14	46.07 (51.9)	(18/37)	49.25 (57.4)	(24/35)	10	44.89 (49.8)	(20/44)	54.42 (66.1)	(19/30)
24	41.85 (44.5)	(18/39)	47.40 (54.2)	(20/40)	19	45.33 (50.6)	(20/40)	51.81 (61.8)	(24/39)
25	57.99 (71.9)	(26/38)	53.23 (64.2)	(24/39)	20	41.16 (43.3)	(14/32)	51.32 (60.9)	(30/46)
Means	50.18 (59.0)		49.65 (58.1)			43.81 (47.9)		53.30 (64.3)	

* Number in parentheses is percent from the transformed arcsin scores.

† Ratio in parentheses is the proportion of cleft palate in each sex from the total progeny in each sire.

the mean cleft palate response (arcsin) in male progeny is significantly less from A.B6 F_1 sires than from B6.A F_1 sires ($t = 2.70$, 8 *df*, $P < 0.05$, two-tailed probability) and is inconsistent with the hypothesis of *X*-linkage. A hypothesis of solely *X*-linked effects can be ruled out. An alternate approach may be to compare the cleft palate response in the total progeny of each sex from the reciprocal F_1 sires rather than the litter-averaged arcsin values. The teratology literature has been uncertain as to what constitutes the sampling unit—the litter or the embryo (HASEMAN and HOGAN 1975). However, in this example, litter effects are not expected. If a nonparametric ranking test (Mann-Whitney U-test, SIEGEL 1956) is used with the total frequency of cleft palate in each sex from each sire, there is no evidence for *X*-linkage. In this case, the probability that the cleft palate response of the female progenies are the same from the two F_1 sires is 0.17 (one-tailed) and, for the male progenies, it is 0.40 (two-tailed).

Table 5 is a summary of the association tests between the *c*, *b*, and *H-2* marker loci and the embryonic cortisone-cleft palate trait measured by arcsin scores from the BC_1 and RBC_1 sires. The distributions of sire scores for the two backcrosses arranged by marker genotype are presented in Figure 6. There is no association with albinism (*c*). With brown (*b*), there is no association in the RBC_1 sires but the association found in the BC_1 sires is in a direction inconsistent with genetic linkage. With *H-2*, the association in both RBC_1 and BC_1 sires is significant and is in the direction consistent with genetic linkage; the combined probability (Fisher's test of combining probabilities; SOKAL and ROHLF 1969) for the hypothesis of no association is less than 0.025. The *H-2* association with the cortisone-cleft palate trait was also examined by a different U-statistic procedure (SMITH 1975). In this case the sires from the two backcrosses were divided into families according to their F_1 sires (four families for BC_1 sires and three families

TABLE 5

Association between marker genes and embryonic cortisone-cleft palate trait

Marker locus	Sire	Genotype	No. of sires	Mean arcsin cleft palate score	P*
albino (*c*)	RBC_1	*c*/+	8	45.76	0.11
		+/+	12	40.24	
	BC_1	*c*/*c*	16	57.94	0.37
		c/+	22	59.54	
brown (*b*)	RBC_1	*b*/+	9	45.24	0.14
		+/+	10	40.16	
	BC_1	*b*/*b*	8	54.96	<0.02
		b/+	14	62.16	(inconsistent)
H-2†	RBC_1	*H-2ab*	8	47.06	0.05
		H-2bb	11	41.54	
	BC_1	*H-2aa*	14	61.12	0.04
		H-2ab	12	54.87	

* Probability of no association, one-tailed, Mann-Whitney U-test.
† Combined probability of no association between *H-2* marker and the embryonic cortisone-cleft palate trait is less than 0.025.

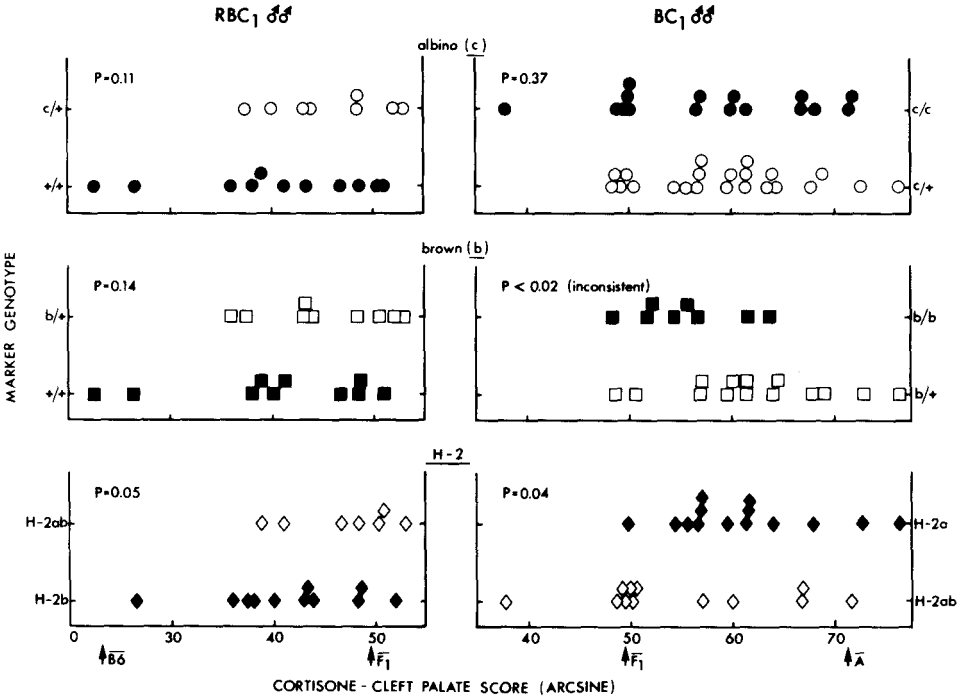

FIGURE 6.—Association tests between the albino (*c*), brown (*b*), and *H-2* marker genes and the cortisone-cleft palate trait. The distributions of RBC_1 and BC_1 sire scores (arcsin) are arranged by marker genotype; the solid and open symbols denote homozygosity and heterozygosity, respectively, for the marker. The means of the A, A.B6 F_1, and B6 sires are indicated by arrows below the abscissa. The probabilities from the hypothesis of no association were derived from the Mann-Whitney U-test.

for RBC_1 sires). The procedure permits the use of all backcross sires tested for cortisone-cleft palate; the test for association is between the marker (in this case *H-2* phenotype) in the sires tested for it and the positive and negative deviations of the sire scores from the family means. The probabilities of no association between *H-2* and the cortisone-cleft palate trait were 0.038 for BC_1 sires and 0.075 for the RBC_1 sires.

DISCUSSION

A specific genetic model cannot be determined from the breeding data for the cause of the difference in mean embryonic tolerance to cortisone-induced cleft palate between the A and B6 strains. A single major gene can be rejected because of the absence of distinct bimodality in the test-mating scores of the BC_1 and RBC_1 sires. The test-mating data of the BC_2 sires also permit rejection of the single gene model. Although there was apparent bimodality in the "segregating" BC_2 families, the sampling variance of at least one BC_2 family, derived from a BC_1 male that was similar in mean score to A sires, was significantly too large and indicated that this family was still segregating for response factors.

A polygenic model, in the sense of many equal and additive loci, can also be rejected. When the range of either the BC_1 or RBC_1 sires is examined by cumulative numbers in arbitrary class intervals and compared to that expected for one distribution, there is a significant difference. Also, when the sampling variance of the BC_1 and RBC_1 sires is compared to the phenotypic range between the F_1 and A sires and the B6 and F_1 sires, respectively, it is too large to be due to the segregration of many factors. If the assumption of equal and additive loci that are independently segregating is met and the A and B6 strains have accumulated all the "plus" and "minus" acting loci, respectively, the Wright-Castle estimate (WRIGHT 1968) of the number of segregating factors is 2.0 with the BC_1 sires and 3.6 with the RBC_1 sires. (An average of the variance of the F_1 and the appropriate inbred sires was used to estimate the environmental variance.)

Since the embryonic tolerance of B6 is greater than and dominant to that of A (BIDDLE and FRASER 1976), speculation about several simple genetic models may be considered with the observed distributions of backcross sires beyond that of a single major gene. There is no evidence for epistasis, such as found with the embryonic response difference to ectrodactyly induction by acetazolamide (BIDDLE 1975), because the sire scores behaved additively. Therefore, if more than one locus is involved in the difference between ED_{50} means for cleft palate induction, their effects must be either equal or independent. A two-locus, equal and additive model would result in a 1:2:1 distribution of sire scores in the backcrosses; a two-locus, independent effect model would result in a 1:1:1:1 distribution in each. The possibility of 4 clusters in the BC_1 sires, although not significant, is suggested. A three-locus, equal and additive model would result in a 1:3:3:1 distribution; however, the size of the major peak in the BC_1 sires that corresponds to the F_1 sire mean argues against this. Further speculation is unwarranted with these limited data. The dearth of information on the extent of genetic variability in the embryonic cortisone-cleft palate trait in the many other inbred strains of mice (STAATS 1972) also calls for caution.

The BC_2 sire data cannot be used other than to reject the single gene hypothesis. The BC_2 sires (or familes of sires) were selected with prior knowledge of the cortisone-cleft palate scores of their BC_1 male parents. Therefore, if a single gene model was suggested, apparent bimodality of the BC_2 sires and the occurrence of segregating and nonsegregating BC_2 families would only support the hypothesis but would not constitute an independent test of the model (see discussion in BLOOM and FALCONER 1964). The limited BC_2 sire data were not examined further to speculate about other genetic models.

There appears to be no evidence for differences between *X*-linked response factors with the A and B6 strains (Table 4). The hypothesis that a component of the maternal effect on cortisone-induced cleft palate is due to *X*-linked genes (FRANCIS 1973) is not supported in the present study.

Other approaches to the analysis of the embryonic cortisone-cleft palate trait might be taken. The methods presented by STEWART and ELSTON (1973) and ELSTON and STEWART (1973) to test observed backcross distributions by maximum likelihood against specified genetic models might be examined. In turn the

most likely models could be tested with appropriate breeding designs. Less critical evaluations, such as the nonparametric test between observed and expected distributions, suggested by MODE and GASSER (1972) and which appears similar to the procedure of COLLINS (1967, 1968), are inappropriate in the present study because of the small sample of parental and F_1 sires that were used.

The association between *H-2* and the embryonic cleft palate trait (Table 5) is intriguing. Although it is in a direction in both backcrosses, consistent with genetic linkage, distinction is still required between genetic linkage to a component of the embryonic response trait and a simple pleiotropic effect of a gene in the *H-2* complex (see discussion in SMITH 1975). Studies of the A/J, C57BL/10 (which is reported to be similar to C57BL/6J though differences have been described, DOSTAL and JELINEK 1973) and the congenic line B10.A, that has the A-strain *H-2* specificities, suggest there is linkage between the maternal effect on cortisone-cleft palate and *H-2* (BONNER and SLAVKIN 1975). If linkage is confirmed and identity can be established for both the maternal and embryonic traits, an interesting hypothesis exists: a gene acting on development in the embryo may have an effect later on the same ontogenetic system but, this time, acting through the mother.

The possible assignment of a gene in the *H-2* complex to a role in determining the cortisone-cleft palate tolerance trait should be regarded with caution until the trait can be measured in a more fundamental way. Variants in androgen-dependent traits have been found to be associated with the *H-2* complex, such as strain differences in plasma testosterone concentration and plasma testosterone-binding capacity for which a specific locus, androgenic hormone-1 (*Hom-1*), has been suggested (IVANYI *et al.* 1972) and also in concentration of the Ss-Slp antigens in genotypes that allow their expression (HANSEN, KRASTEFF and SHREFFLER 1974). With an increasing awareness and use of the range of normal variation in the murine endocrine system and of the many levels on which these traits are operationally determined (review by SHIRE 1974), discretion is required in the assignment of separate gene functions on the basis of phenotypic variation.

A recent note by GOLDMAN *et al.* (1976) reported that embryos of strains A/J and CBA/J (a strain that is resistant to glucocorticoid-induced cleft palate) differ quantitatively and qualitatively in electrophoretically resolvable cortisol-binding proteins isolated from embryonic palatal shelves. If this is related to the difference between the embryos in mean tolerance (ED_{50}) to glucocorticoid-induced cleft palate, an interesting possibility arises in the light of the variation in androgen-dependent traits discussed above. A general steroid-binding transport or metabolizing system may be linked with the *H-2* complex and manifested on a number of different levels: concentration of plasma testosterone, variation in weight of androgen-dependent organs, concentrations of Ss-Slp antigens in genotypes capable of their expression, differences in mean tolerance (ED_{50}) of embryos to glucocorticoid-induced cleft palate, and so forth.

Many resources are available for unravelling the genetic complexity of the trait of cortisone- and other glucocorticoid-induced cleft palate. These include

the many inbred strains and their congenic sublines (STAATS 1972) and the recently developed recombinant inbred strains with their strain distribution patterns of marker genes (BAILEY 1971). Most of these remain unexploited in teratological studies.

Further studies of the cortisone-induced cleft palate trait are essential if the cause of the genetic variation in embryonic tolerance is to be defined. It appears that a small number of loci are involved in the difference between the A/J and C57BL/6J strains and that one of these may be associated with the *H-2* complex. If the cortisone-cleft palate trait has a relatively simple cause, there will be a greater chance for a meaningful investigation of its ontogeny.

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