GLUCOCORTICOID-INDUCED CLEFT PALATE IN THE MOUSE:
TWO MAJOR HISTOCOMPATIBILITY COMPLEX, H-2, LOCI
WITH DIFFERENT MECHANISMS

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ABSTRACT

Isolated cleft palate is induced in the progeny of pregnant mice that are given glucocorticoids. The incidence varies among inbred strains and with dose and stage of gestation when the drug is given. One chromosomal region responsible for strain-associated differences in sensitivity is the major histocompatibility complex, H-2. H-2<sup>a</sup> is associated with susceptibility, H-2<sup>b</sup> with resistance. There appear to be both maternal and embryonic genetic factors affecting the sensitivity to glucocorticoids. In experiments reported here congenic strains of mice with H-2<sup>a</sup>, H-2<sup>d</sup> and H-2<sup>u</sup> haplotypes on a C57BL/10 genomic background were used. This allowed the determination of the effect on sensitivity by two H-2 subregions; the subregions are H-2K to I-E and I-C to H-2D. Methods included dose-response analysis and reciprocal cross analysis using dexamethasone given on day 12 of pregnancy. Results show that each subregion affects the strain’s sensitivity to dexamethasone-induced cleft palate. The regression coefficients for B10.A-H-2<sup>a</sup> (45.4 ± 4.13) were different from those for B10.BR-H-2<sup>u</sup> (67.2 ± 10.8) and B10.D2-H-2<sup>d</sup> (70.5 ± 9.74). The estimated mean arcsine % cleft palate at 160 mg/kg was different for each strain: B10.A-H-2<sup>a</sup>, 53.1 ± 2.19; B10.BR-H-2<sup>u</sup>, 33.1 ± 2.27; B10.D2-H-2<sup>d</sup>, 25.0 ± 2.75. Different patterns of change in sensitivity were observed among the reciprocal crosses. In summary, the H-2K to I-E subregion seemed to influence both maternal and embryonic factors, whereas only embryonic factors were influenced by the I-C to H-2D subregion. These data suggest that the mechanisms affecting glucocorticoid sensitivity which are genetically encoded within each H-2 subregion are different, and there is an interaction between the alleles. The mode of interaction can be either complementation or epistasis.

GLUCOCORTICOID-induced cleft palate in the mouse was first described by FRASER and FAINESTAT in 1951. They found that the A strain was more susceptible to the teratogenic action of cortisone than the C57BL strain when 2.5 mg was given to each pregnant female on days 11 through 14 of gestation. With this treatment 100% of the A strain progeny had cleft palate compared with 19% of the C57BL strain. They also determined the frequency in reciprocal crosses between the strains. These observations showed that maternal and embryonic factors influenced the frequency (KALTER 1965; BIDDLE and FRASER 1976, 1978).

One of several chromosomal regions that influence cortisone-induced cleft palate sensitivity in these strains is associated with the major histocompatibility
complex, H-2. Bonner and Slavkin (1975) showed this by using congenic strains of mice with genetic differences restricted to the region on chromosome 17 which contains H-2. They found that the B10.A/SgSn strain, which carries the same H-2^a haplotype as the A strain, was more susceptible to the teratogenic action of cortisone than the C57BL/Sn strain, which carries the same H-2^b allele as the C57BL/6 strain. Cortisone-induced cleft palate frequency in the progeny of reciprocal crosses supported a conclusion that maternal and embryonic factors influenced the cleft palate frequency. A similar observation was recently reported by Melnick, Jaskoll and Slavkin (1981), and linkage between dexamethasone-induced cleft palate susceptibility and H-2^a was recently demonstrated by a backcross test (Bonner and Tyman 1982a). Mapping studies to date suggest that there are two loci associated with the H-2 complex that affect glucocorticoid-induced cleft palate (Tyman and Miller 1978; Bonner 1981; Gasser et al. 1981; Bonner and Tyman 1982b).

The experiments presented here used the congenic strains B10.BR/SgSn, B10.D2/nSn and B10.A/SgSn. The evolutionary history of the H-2 haplotypes carried by these strains is pertinent to this report. The H-2^k in B10.BR was originally derived from C57BR/cd. It is the same H-2 haplotype found in CBA, C3H and AKR strains. The H-2^d in B10.D2/nSn was originally derived from DBA/2, and it is found in BALB/c and C57BL/Ks. H-2^o in B10.A/SgSn was derived from A/WySn. It is important to note that the A strain H-2 haplotype is probably a recombinant of H-2^k and H-2^d (Klein 1975). Because of its historical order of discovery it was called H-2^o. The A strain was most likely produced via an initial outcross between mouse lines subsequently found to have H-2^k and H-2^d. During the outcross a recombination event probably occurred between the I-E and I-C regions of H-2 (see Table 1). The A line was inbred by Strong about 1922 (Altman and Katz 1979). Conclusions reached in this report are based on the fact that B10.A/SgSn and B10.D2 have heterologous regions of H-2 from H-2K to I-E; B10.A/SgSn and B10.BR/SgSn have heterologous regions from I-C to H-2D. Additionally, the strains that carry the C57BL/10Sn genomic background are congenic.

The method used to produce congenic strains with genetic differences restricted to chromosome 17, which contains H-2, was first developed by Snell and colleagues (Snell 1981). The purpose was to place a variety of the polymorphic alleles of H-2 on a constant genomic background so that the effects of the polymorphism could be ascertained. The probability of heterozygosity at loci not linked to H-2, that is, a 50 cM distance between H-2 and the uncontrolled locus within the congenic strain's genome, is less than 1% after eight backcross generations (Green 1981). B10.A/SgSn was backcrossed 11 times; B10.D2/nSn, 11 times; B10.BR/SgSn, nine times (Cherry 1979). For a locus that is 10 cM from H-2 it would take 45 backcross generations to reach a 1% probability of heterozygosity (Green 1981).

MATERIALS AND METHODS

Breeding stock of the following strains were obtained from The Jackson Laboratory, Bar Harbor, Maine: B10.A/SgSn, B10.BR/SgSn and B10.D2/nSn. All animals used in experiments were bred and
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TABLE 1

H-2 haplotype and the alleles for three congenic strains showing the restricted differences

<table>
<thead>
<tr>
<th>Strain</th>
<th>Haplotype</th>
<th>Allele*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K</td>
</tr>
<tr>
<td>B10.D2/nSn</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>B10.A/SgSn</td>
<td>a</td>
<td>k</td>
</tr>
<tr>
<td>B10.BR/SgSn</td>
<td>k</td>
<td>k</td>
</tr>
</tbody>
</table>

* Within the text the alleles of each haplotype will be abbreviated d = dd, a = kd, k = kk. The horizontal line highlights the strain genetic differences.

reared in our mouse breeding room. Breeding pairs (two females and one male) to produce stock for experiments were fed ad libitum Wayne Breeder Blox. Stock (maximum six per cage) and animals in experiments (maximum three per cage) were fed ad libitum Purina Laboratory Rodent Chow. All mice were given acidified tap water.

To produce timed matings two virgin females, 3 to 6 months old, were placed with singly caged males, and the day a copulation plug was detected was designated day 0 of pregnancy.

Dexamethasone (dexamethasone sodium phosphate, Decadron, a generous gift from MERCK, SHARPE and DOHME) at varying doses was given intraperitoneally on day 12 of pregnancy.

On day 18 the females were killed and weighed; the contents of each uterus was exposed. The number and position of implantation sites, resorptions, dead fetuses and live fetuses were recorded. All dead fetuses, regardless of condition, were treated statistically as resorptions. All fetuses were inspected for malformations.

The sampling unit in these experiments was defined as the litter. The percentage of cleft palate in each litter was transformed to an arcsine by using the Freeman-Tukey table of arcsine transformations for binomial distributions which was calculated by MOSTELLER and YOUTZ (1961). This transformation has a weighting factor for litter size. The percentages of cleft palate per litter were transformed so that the methods of parametric data analysis could be used. The degrees of freedom were calculated from the number of litters in each group.

Data were analyzed by methods described by SOKOL and ROHLP (1981). Methods included (1) computation of regression with more than one value of Y (the arcsine for each litter) with an unequal sample size of litters for each group Y; (2) tests for the equality of regression coefficients of several regression lines: (3) estimation of Y from X to determine an estimate of the mean arcsine and standard error of the mean cleft palate occurrence for each strain at specified doses of dexamethasone; (4) calculation of the mean arcsine and standard error of the mean for each reciprocal cross that was given a single dose of dexamethasone; (5) single classification analysis of variance with unequal sample sizes; and (6) the Tukey-Kramer method for multiple comparisons among pairs of means based on unequal sample sizes. In these computations all litters were used regardless of litter size.

RESULTS

Shown in Table 2 is the percentage of cleft palate and the mean arcsine for each strain, B10.A, B10.D2 and B10.BR, each given different distributions of doses of dexamethasone. The lowest dose was 20 mg/kg given to B10.A; the highest was 460 mg/kg given to B10.D2. One dose of dexamethasone was excluded from the regression analysis: 80 mg/kg, given to B10.D2, did not induce cleft palate.
The resorption frequency is also shown in Table 2 for each group of litters. Even though there is a considerable amount of variation among the groups there do appear to be strain-associated differences. BIO.A and B10.D2 are equivalent but B10.BR has a considerably greater frequency. This suggests that genes associated with the I-C to H-2D region may modulate the resorption frequency, but confirmation must await statistical analysis.

Finally, Table 2 shows the increase in resorption at the highest dose of dexamethasone given to B10.D2, 460 mg/kg. Dexamethasone must become toxic to the embryo at this high dose, but there is no significant deviation of this group's arcsine from the regression, so we do not believe that the drug selectively killed embryos with cleft palate. If that were the case a lower frequency than predicted from the regression would have been observed.

Results of regression analysis on the three congenic strains are shown in Table 3 and graphically in Figure 1. The regression of arcsine % cleft palate on log dosage for each strain was significant, and there was no significant deviation of any group from linearity. The test for equality among the regression coefficients showed that B10.D2 and B10.BR were equal (P > 0.75), 67.2 and 70.5, respectively, and that the mean regression coefficient from their pooled regression, 6 = 68.2, was significantly greater than the regression coefficient for BIO.A which was 45.4 (P < 0.025).

Estimates of the mean arcsine at two doses of dexamethasone, 160 and 226 mg/kg, were computed from the regressions and are shown on Table 3. Results of statistical tests for significant differences between these estimated means will be shown in Table 5 later in the text.
TABLE 3

Results of regression analysis on three congenic strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Regression coefficient (a) (s.e.)</th>
<th>(\hat{y}_{160}) (s.e.)</th>
<th>(\hat{y}_{226}) (s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.A/SgSn</td>
<td>kd</td>
<td>45.4 (4.13)</td>
<td>53.1 (2.19)</td>
</tr>
<tr>
<td>B10.BR/SgSn</td>
<td>kk</td>
<td>67.2 (10.8)</td>
<td>33.1 (2.27)</td>
</tr>
<tr>
<td>B10.D2/nSn</td>
<td>dd</td>
<td>70.5 (9.74)</td>
<td>25.0 (2.75)</td>
</tr>
</tbody>
</table>

Each strain had a significant regression: B10.A, \(F_a = 120.77^{***}\); B10.BR, \(F_a = 38.58^{**}\); B10.D2, \(F_a = 52.36^*\). Regression coefficients for B10.D2 and B10.BR were equal, \(F = 0.03\), \(P > 0.75\); pooled regressions for B10.D2 and B10.BR were significantly different from B10.A, \(F = 7.37^*\). Asterisks denote confidence level: * \(P < 0.05\), ** \(P < 0.01\), *** \(P < 0.001\). S.E. = standard error.

Estimated mean arcsine at the dose of 160 mg/kg.

Estimated mean arcsine at the dose of 226 mg/kg.

FIGURE 1.—Dose-response analysis on three congenic strains, B10.A (○), B10.BR (■) and B10.D2 (△). The cleft palate frequency expressed as an arcsine is the Y coordinate and the dose of dexamethasone expressed as logs is the X coordinate.

The frequency of cleft palate and the mean arcsine, induced by a single dose of dexamethasone in all possible reciprocal crosses between the three congenic strains, is shown in Table 4. This was a test for maternal and embryonic genetic factors. The dose of 160 mg/kg was given to crosses between B10.A and B10.BR and between B10.A and B10.D2; 226 mg/kg was given to crosses between B10.D2 and B10.BR. Single classification analysis of variance showed a high amount of variability within these groups for cleft palate frequency (\(F_a = 51.1\), \(P \ll 0.001\)).

The results of statistical tests for significant differences between all possible comparisons of observed and estimated mean arcsines at 160 mg/kg are shown...
TABLE 4

Frequency of cleft palate in reciprocal crosses given a single dose of dexamethasone

<table>
<thead>
<tr>
<th>Cross</th>
<th>Dose (mg/kg)</th>
<th>Litters</th>
<th>Resorptions/implants</th>
<th>Mean</th>
<th>Cleft palate fetuses/total</th>
<th>Mean arcsine</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B10.A x B10.BR</td>
<td>160</td>
<td>14</td>
<td>13/104</td>
<td>12.5</td>
<td>47/91</td>
<td>51.6</td>
<td>47.36</td>
</tr>
<tr>
<td>B10.BR x B10.A</td>
<td>160</td>
<td>30</td>
<td>51/232</td>
<td>22.0</td>
<td>102/181</td>
<td>56.3</td>
<td>51.75</td>
</tr>
<tr>
<td>B10.A x B10.D2</td>
<td>160</td>
<td>18</td>
<td>22/141</td>
<td>15.6</td>
<td>52/119</td>
<td>43.7</td>
<td>39.80</td>
</tr>
<tr>
<td>B10.D2 x B10.A</td>
<td>160</td>
<td>16</td>
<td>19/132</td>
<td>14.4</td>
<td>29/113</td>
<td>25.7</td>
<td>29.69</td>
</tr>
<tr>
<td>B10.BR x B10.D2</td>
<td>226</td>
<td>18</td>
<td>25/133</td>
<td>18.8</td>
<td>38/108</td>
<td>35.2</td>
<td>36.08</td>
</tr>
<tr>
<td>B10.D2 x B10.BR</td>
<td>226</td>
<td>13</td>
<td>11/90</td>
<td>12.2</td>
<td>29/79</td>
<td>36.7</td>
<td>38.08</td>
</tr>
</tbody>
</table>

in Table 5. The convention used for writing the embryonic haplotypes is to indicate the source of each H-2 allele carried by the embryo, i.e., maternal/paternal. For example, kk/kk compared with kk/kd represents the cross \( B10.BR \times B10.BR \) compared with \( B10.A \times B10.A \). The Tukey-Kramer method for multiple comparisons among pairs of means was used since there were considerable differences between the variances and between the sample sizes for the means. Absolute observed differences (AOD) between each of two means, which are being compared, are the values below the diagonal in Table 5, and the minimum significant differences (MSD) are above the diagonal. Differences larger in AOD than their MSD value are above the diagonal. Differences larger in AOD than their MSD value are significant at the level marked by the asterisk. The results in Table 5 are also shown graphically in Figure 2, a and b.

The first difference to note is the strain difference; \( kd/kd \) has a mean arcsine significantly higher than either \( kk/kk \) or \( dd/dd \), \( P < 0.01 \). The difference between \( kk/kk \) and \( dd/dd \) was significant (AOD, 8.06; MSD, 7.66; \( P < 0.05 \)).

Differences can also be seen in the reciprocal crosses between the strains B10.A and B10.BR. There was a significant difference between the means for the \( kk/kd \) and \( kk/kk \) embryos within the same \( kk \) maternal intrauterine environment (AOD, 18.7; MSD, 9.84; \( P < 0.01 \)). There was no significant difference between any of the other crosses between these two strains (see Figure 2a).

There appeared to be differences in the means in crosses between B10.A and B10.D2, and the pattern of distribution of mean arcsines was different from the pattern of crosses between B10.A and B10.BR (see Figure 2b). For example, the mean for \( kd/kd \) was significantly different from the mean for \( kd/dd \) (AOD, 13.3; MSD, 12.5; \( P < 0.05 \)), which is a change in embryonic haplotype within the same \( kd \) maternal intrauterine environment. There appeared to be a change in the mean when the embryonic haplotypes were the same (\( kd/dd \); \( dd/kd \), the reciprocal cross) and the maternal intrauterine environment was changed from \( kd \) to \( dd \), but the difference between the means (AOD, 10.1; MSD, 16.3) was not statistically significant. There was a statistically significant difference when there was a concomitant change in both the embryonic and maternal haplotypes. This can be seen by comparing \( kd/kd \) with \( dd/kd \) (\( P < 0.01 \)) or by comparing \( dd/dd \) with \( kd/kk \) (\( P < 0.05 \)).
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TABLE 5

Results of reciprocal cross test with all possible comparisons between the haplotypes (maternal/paternal) (160 mg/kg)

<table>
<thead>
<tr>
<th>Ranked haplotypes</th>
<th>kd/kd</th>
<th>kk/kd</th>
<th>kd/kk</th>
<th>kd/dd</th>
<th>kk/kk</th>
<th>dd/kd</th>
<th>dd/dd</th>
</tr>
</thead>
<tbody>
<tr>
<td>kk/kd</td>
<td>1.36</td>
<td>—</td>
<td>15.37</td>
<td>14.70</td>
<td>9.84</td>
<td>14.16</td>
<td>10.59</td>
</tr>
<tr>
<td>kd/kk</td>
<td>5.75</td>
<td>4.39</td>
<td>—</td>
<td>17.38</td>
<td>13.52</td>
<td>16.92</td>
<td>14.07</td>
</tr>
<tr>
<td>kd/dd</td>
<td>13.31*</td>
<td>11.95</td>
<td>7.56</td>
<td>—</td>
<td>12.75</td>
<td>16.31</td>
<td>13.34</td>
</tr>
<tr>
<td>kk/kk</td>
<td>20.06**</td>
<td>18.70**</td>
<td>14.31*</td>
<td>6.75</td>
<td>—</td>
<td>12.12</td>
<td>7.66</td>
</tr>
<tr>
<td>dd/kd</td>
<td>23.42**</td>
<td>22.06**</td>
<td>17.76**</td>
<td>10.11</td>
<td>3.36</td>
<td>—</td>
<td>12.74</td>
</tr>
<tr>
<td>dd/dd</td>
<td>28.12**</td>
<td>26.76**</td>
<td>22.37**</td>
<td>14.81*</td>
<td>8.06*</td>
<td>4.70</td>
<td>—</td>
</tr>
</tbody>
</table>

Values below the diagonal are the AODs between the mean arcsine for each haplotype. Values above the diagonal are the MSDs at a 95% confidence level. The asterisks indicate significant values: *P < 0.05, **P < 0.01.

A retrospective analysis of the data revealed that the maternal effect could be seen another, rather unconventional, way. The absolute observed difference in the comparison between kd/kk and kd/dd is not significantly different (AOD 7.56, MSD, 17.4). The maternal H-2 haplotypes are the same kd, but the embryonic haplotypes are different. Nevertheless, in the comparison between embryonic haplotypes kk/kd and dd/kd, which form the same embryonic haplotype pair as in the previous comparison except that the maternal haplotypes are different, kk and dd, respectively, a significant difference can be seen in their cleft palate frequencies (AOD, 22.1; MSD, 14.2; P < 0.01).

The final set of comparisons to be made is the cross between B10.D2 and B10.BR which were given a single dose of 226 mg/kg. The data are in Table 6 and are shown graphically in Figure 2c. No significant difference was found in the percentage of cleft palate between any of the crosses except for the difference between B10.BR and B10.D2 (P < 0.05).

DISCUSSION

Two major conclusions can be made from these data. First, two H-2-associated loci influence the degree of sensitivity to dexamethasone-induced cleft palate. One locus maps in the region on chromosome 17 containing H-2K to I-E and another maps in the region with I-C to H-2D. This is supported by the highly significant difference between the strains in the estimated mean arcsines at 160 mg/kg. Mapping of this steroid hormone sensitivity trait is a conclusion based on assumptions discussed in the introduction of this paper. The assumption is that the most probable genetic difference between B10.A and B10.D2 is the region that contains H-2K to I-E and the genetic difference between B10.A and B10.BR is the region that contains I-C to H-2D. It is possible, but highly improbable, that the difference between these strains is the result of mutations that could have occurred elsewhere in the genomic background since the strains were formed, or that the strains were never truly congenic, but that they have
C57BL/10Sn genomic backgrounds that are still contaminated with genes whose origin was the H-2 haplotype donor from the initial outcross which was made during congenic strain formation.

The second major conclusion is that the mechanism through which the hormone sensitivity is influenced appears to be different for each H-2-associated locus. This is supported by the results from different analyses. These are the dose-response analysis and the reciprocal cross analysis.

The conventional interpretation of differences between regression coefficients derived from dose-response analysis is that variables causing the regression coefficient differences (in our case the variables are the gene loci associated
with the H-2 haplotypes) have different functional relationships between themselves, the sources of the stimulation (the dexamethasone), and the response (the cleft palate frequency). In other words, the difference between the regression coefficients suggests that the mechanism through which the hormone causes the birth defect is different in the different congenic strains. A theoretical example is pertinent to this discussion. Let us assume that normal palate growth requires glucocorticoids. The hormone must be endogenously synthesized and growth which is induced by the hormone requires the presence of an intracytoplasmic receptor in the target cells. Hormone synthesis in one organ and hormone induction at the target cell are independent processes, and dexamethasone can influence both processes. It can act through a feedback inhibition mechanism to stop the synthesis of glucocorticoids, and it can inactivate hormone induction by irreversibly binding to the intracytoplasmic receptor. Interference by dexamethasone of either theoretical mechanism would have the same result, the inhibition of growth, but the effectiveness of dexamethasone's interference would vary between the mechanisms because of the different kinetic or binding characteristics within each mechanism. A difference in mechanisms of this type could cause changes in dose-response regression coefficients. It is a plausible hypothesis that key enzymes for glucocorticoid synthesis are encoded in one of the H-2-associated loci (Pollack et al. 1979) and that glucocorticoid receptor characteristics are associated with another (Bonner 1975; Goldman et al. 1977; Gupta and Goldman 1982).

The reciprocal cross analysis also suggests that the mechanisms in each of the H-2-associated loci are different. This is supported by the different phenotypic characteristics that are observed in each cross. The cross between B10.BR and B10.A and the significant difference between it and the B10.BR parental strain, and the lack of any other significant difference in the crosses between these two strains, indicate that only an embryonic factor maps in the I-C to H-2D region. The crosses between B10.A and B10.D2 suggest the presence of both maternal and embryonic genetic factors in the H-2K to I-C region, but confirmation must await a dose-response analysis to obtain statistically significant differences between the reciprocal crosses. Nevertheless, it appears that B10.A and B10.BR have the same maternal intrauterine environmental factor, but B10.D2 has a different, less sensitive maternal factor.
To summarize, we conclude there are three factors. A maternal factor appears to be encoded in the region H-2K to I-E. The H-2K\textsuperscript{k} to I-E\textsuperscript{k} allele carries a maternal factor with a high sensitivity phenotype (susceptible); allele H-2K\textsuperscript{d} to I-E\textsuperscript{d} carries a low sensitivity phenotype (resistant). There are two embryonic factors. One maps in the region H-2K to I-E, and a second one maps in I-C to H-2D. The H-2K\textsuperscript{d} to I-E\textsuperscript{d} allele carries a low sensitivity phenotype (resistant); H-2K\textsuperscript{k} to I-E\textsuperscript{k} has a high sensitivity phenotype (susceptible). The I-C\textsuperscript{k} to H-2D\textsuperscript{k} allele carries an embryonic factor with a low (resistant) sensitivity to dexamethasone; I-C\textsuperscript{d} to H-2D\textsuperscript{d} has a high (susceptible) sensitivity. The organization of these alleles in each strain is shown in Table 7. It appears that the mechanisms through which these factors mediate these effects are different.

The greatest incidence of cleft palate is noted when both susceptible loci are present on both chromosomes. The absence of one susceptible locus from one chromosome results in a reduction in the frequency of cleft palate. And as seen in the crosses between B10.BR and B10.D2, two susceptible loci in the trans chromosomal arrangement have a resistant phenotype. This suggests that the susceptible loci must be on the same chromosome to produce the susceptible phenotype.

A model of interpretation of these observations is that the dexamethasone sensitivity loci in H-2K to I-E and I-C to H-2D interact. One type of interaction is epistasis, in which the phenotype of one locus hides the phenotype of another locus in a different chromosomal region. This can be seen upon comparison of the similar cleft palate frequencies in B10.D2 and B10.BR, which carry one pair of susceptible loci and one pair of resistant loci each, and B10.A which carries both pairs of susceptible loci. Since B10.D2 and B10.BR have resistant phenotypes, it is possible that each resistant locus superimposes its trait on the susceptible locus in a nonallelic interaction when resistant and susceptible loci are in a cis chromosomal arrangement (see Table 7, numbers 1, 2 and 4).

The interaction can also be allelic. This can be seen in the difference in cleft palate frequency between F\textsubscript{1} embryonic haplotypes and the parental strains. For example, an interaction between the embryonic factors in the resistant H-2K\textsuperscript{d} to I-E\textsuperscript{d} allele and the susceptible H-2K\textsuperscript{k} to I-E\textsuperscript{k} alleles can explain the reduction in cleft palate frequency seen in the change in embryonic genotype from \textit{kd/kd} to \textit{kd/dd} (Table 7, number 6).

Implicit in the notion that one allele superimposes its trait over another is the idea that one allele is "stronger" than another; they can be ranked according to "strength" (Table 8). The stronger allele is the one whose genotype more closely resembles the phenotype observed. For example, the strongest allele appears to be the resistant allele in H-2K\textsuperscript{d} to I-E\textsuperscript{d}. It superimposes its trait over the susceptible allele in I-C\textsuperscript{d} to H-2D\textsuperscript{d} as when both alleles are in the B10.D2 strain. The next allele in the ranking appears to be the susceptible allele in I-C\textsuperscript{d} to H-2D\textsuperscript{d}. The effect of this allele is stronger than the resistant allele in I-C\textsuperscript{k} to H-2D\textsuperscript{k} which can be seen in the B10.A and B10.BR reciprocal crosses. So the change in embryonic genotype from \textit{kk/kk} to \textit{kk/kd} caused the frequency to increase significantly, but the change in genotype from \textit{kd/kd} to \textit{kd/kk} caused no change in frequency. In other words, the change from \textit{k} to \textit{d} was more effective than the change from \textit{d} to \textit{k}, indicating that the effect mediated by \textit{d} is stronger.
The final pair to rank is \( H-2K^k \) to \( I-E^k \) and \( I-C^k \) to \( H-2D^k \). The answer is that the resistant allele in \( I-C^k \) to \( H-2D^k \) is stronger than the susceptible allele in \( H-2K^k \) to \( I-E^k \). This can be seen in the B10.BR strain in which these two alleles are in the cis chromosomal arrangement but the resistant phenotype is expressed.

Within the framework of this model, the resistant phenotype observed in the B10.D2 and B10.BR crosses, in which both susceptible alleles were in the trans chromosomal arrangement, can be explained. The strongest allele, \( H-2K^d \) to \( I-E^d \), which has a resistant phenotype, could have superimposed its trait over the susceptible allele \( I-C^d \) to \( H-2D^d \). Also \( I-C^k \) to \( H-2D^k \) could have superimposed its resistant phenotype over the susceptible phenotype carried by \( H-2K^k \) to \( I-E^k \). The net result of these interactions would be the observed resistance (Table 7, number 3).

A second model that appears compatible with the experimental data is based on positive gene interaction. The data suggest there is complementation between the two genes controlling the embryonic factor, and it appears they must be on


the same chromosome if interaction is to take place. In addition, there is evidence of gene dose effects. The evidence for this follows: (1) B10.A (H-2 kd/kd) is sensitive; (2) B10.BR (H-2 kk/kk) and B10.D2 (H-2 dd/dd) which each contain one set of susceptibility loci (H-2K'k to I-E'k or I-C'd to H-2D'd, respectively) are resistant, demonstrating that both loci must be present to produce a sensitive embryo; (3) the embryos from the reciprocal crosses B10.BR × B10.D2 which contain both susceptibility loci on separate chromosomes are resistant, suggesting that the two loci must be on the same chromosome if complementation is to occur; and (4) the absence of one locus from one chromosome as in B10.A × B10.D2 F1 vs. B10.A × B10.A results in a significant reduction in the frequency of cleft palate.

The observation that both embryonic loci must be on the same chromosome if complementation is to occur suggests that the genes interact at the level of transcription (DNA or nuclear RNA) rather than at a later stage as demonstrated in the two-gene control of the expression of a murine antigen (Jones, Murphy and McDevitt 1978), in which it was found that (1) the genes could be either cis or trans, (2) each gene produced a product independent of the other, and (3) only when both products were present did the phenotype appear.

Although these data show that H-2 genes are a major modifier of susceptibility, they are not the only ones involved. Reports by Biddle and Fraser (1976, 1977), Kalter (1965), Bonner and Slavkin (1975), Tyran and Miller (1978), Gasser et al. (1981) and Veckmans, Taylor and Fraser (1981) disclosed that other gene loci probably exist. An example is in the report by Kalter (1965) that showed the cortisone-induced cleft palate frequency in CBA and C3H strains. CBA has 12% cleft palate and C3H had 68%. Both of these strains carry the same H-2k haplotype as B10.BR in this report. Certainly, genes other than H-2 affect the frequency in CBA and C3H.

Another interesting observation made by Kalter (1965) was the outcome of reciprocal crosses between A strain and CBA strain. There was no maternal effect observed between the strains, just as in the reciprocal crosses between B10.A and B10.BR in this report. All of these strains, A, CBA, B10.A and B10.BR, carry H-2Kk to I-Ek, which we believe to carry the maternal factor. Since none of these strains differ in this allele, it is interesting to see that they also do not differ in a maternal factor.

The data give us no clue about the biochemical characteristics of the factors or gene products, except that one has a maternal origin and the other two are

### TABLE 8

<table>
<thead>
<tr>
<th>H-2 allele</th>
<th>Glucocorticoid-induced cleft palate phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-2K'd to I-E'd</td>
<td>Resistant</td>
</tr>
<tr>
<td>I-C'd to H-2D'd</td>
<td>Susceptible</td>
</tr>
<tr>
<td>I-Ck to H-2D'k</td>
<td>Resistant</td>
</tr>
<tr>
<td>H-2Kk to I-Ek</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

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embryonic. The H-2 complex is a very large region of chromosome 17 and has room to code for a thousand proteins. The number of gene products identified so far is small in comparison (VITETTA and CAPRA 1978). Nevertheless, the idea that the known H-2 gene products are functional in development is an intriguing one, especially when considering the H-2K and H-2D products. They are cell surface glycoproteins with an amino acid sequence similar to some sequences found in immunoglobulin (STROMINGER et al. 1978). Do these molecules have a self-recognition function on the cell surface and an integral part in the morphogenetic process (BONNER 1979)?

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LITERATURE CITED


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