THE INHERITANCE OF SUSCEPTIBILITY TO THE TERATOGENIC ACTION OF CORTISONE IN MICE[1]

HAROLD KALTER
Department of Genetics, McGill University, Montreal, Canada

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It has been found (FRASER and FAINSTAT 1951) that pregnant mice given cortisone during gestation will give birth to offspring a certain proportion of which have cleft palates. The frequency with which this deformity appears was observed to depend on: 1) the genotype of the treated animal, strain A being "susceptible," C57BL relatively "resistant," to this effect of cortisone; 2) the dose of the hormone administered, too small an amount being ineffective in producing cleft palates, too great an amount causing resorption of the entire litter; and 3) the time during the gestation period when the animal was treated.

The investigation reported here has provided further observations on the genetics of susceptibility to the teratogenic effects of cortisone.

MATERIALS AND METHODS

The animals used in this study were the A/Jax and C57BL/6Jax inbred strains of mice, F_1 females of reciprocal crosses between these strains, and females of the first backcross to strain A. Three or four females and one or two males were kept in each breeding cage. They were fed water and Purina Fox Chow *ad libitum*, supplemented twice a week by lettuce and white bread soaked in milk.

The breeding cages were examined periodically for females which were palpably pregnant and for females with vaginal plugs. All females which were palpably pregnant or had vaginal plugs were placed in separate cages where they were treated and remained for the duration of their pregnancies. After delivery the females were returned to their original breeding cages. The offspring were examined for gross abnormalities and then discarded or fixed in Bouin's solution for future histological study.

Pregnant females were given 2.5 mg of cortisone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate, Merck) for four successive days (called 4×2.5 mg cortisone) intramuscularly in the flank. It had previously been established that this dose of the hormone caused a relatively high incidence of cleft palate with a relatively low frequency of resorption of litters. The fluid in which the hormone is suspended has no gross effect on development (FRASER and FAINSTAT 1951).

[1] This paper is taken from a thesis submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE 1

Length of gestation in days measured from observation of vaginal plugs to parturition for females of strains A, C57BL (= B), and the F_1 and backcross (BC) generations.

Cross	Length of gestation in days									Total	Mean ± S.E.
	15	16	17	18	19	20	21	22	23		
A × A					5	6	1			12	19.67 ± 0.17
B × B	1			2	14	17	7	3	3	47	19.98 ± 0.19
A × B		1		2	7	5				15	19.00 ± 0.27
B × A		1		2	13	7	6	2		31	19.65 ± 0.22
F_1 × A		1	1	15	14	4				35	18.54 ± 0.15
BC × A				4	6	1				11	18.73 ± 0.18

None of 202 C57BL and 232 strain A offspring of untreated mothers was observed to have a cleft palate without cleft lip. The incidence of spontaneous cleft palate with cleft lip in the offspring of A mice in this laboratory is about five percent. Since this defect is quite different anatomically from that induced by cortisone, and since the treatment does not appear to increase the incidence of cleft palate with cleft lip, offspring affected in this fashion have been excluded from the data presented below.

Observations made in this laboratory have determined that the palatine processes fuse in the normal embryogeny of strain A/Jax early on the fifteenth day of gestation (WALKER unpublished).

RESULTS

The length of the gestation period. For a proper evaluation of the importance of the stage during gestation when the agent is administered, it is necessary that an accurate estimate of the age of the fetus at the time of treatment be known. This age can be calculated in a rough manner by counting back from the time of parturition. In order to do this, however, one must know the length of the gestation period. The commencement of the gestation period can be identified by means of the vaginal plug which, since it is the coagulated secretion of the seminal vesicles of the male (GRUNEBERG 1952), is an infallible sign of copulation. Observations of gestation length in females of various genotypes are presented in table 1. The combined data for the pure strains and for the hybrids are found in table 2. A test of significance shows that the mean gestation lengths for these two groups of animals are quite different ($t = 5.33$, d.f. = 149, $P << 0.001$). Since females were examined each morning

TABLE 2

Length of gestation in days measured from observation of vaginal plugs to parturition for pure strain and hybrid females (data from table 1).

Females	Length of gestation in days									Total	Mean ± S.E.
	15	16	17	18	19	20	21	22	23		
Pure strain	1	2		6	39	35	14	5	3	105	19.70 ± 0.13
Hybrid		1	1	19	20	5				46	18.59 ± 0.12

for several months for the presence of vaginal plugs, it is certain that they were formed not longer than twenty-four hours prior to the time of observation. Since mice tend to ovulate during the early morning (RUNNER and LADMAN 1950) and also tend to copulate then (SNELL *et al.* 1940) the majority of vaginal plugs seen were but six to ten hours old. This means that the values for the length of the gestation period in table 1 were probably slight underestimations. Taking this probable discrepancy into consideration, the mean gestation lengths of the treated animals have, therefore, been reckoned as 20 days for the pure strain animals and as 19 days for the F_1 and backcross (BC) animals.

GRUNEBERG (1952) has emphasized that, because of the great variability of the gestation period and the fairly constant degree of maturity at birth, there must be differences in the rate of development which make the chronological age of an embryo an unreliable indicator of its developmental age. In view of this variability and because the vaginal plug was not observed in some treated females, a precise estimation of the developmental stage treated was not possible for this material. To introduce some degree of uniformity it was decided to estimate the ages of fetuses from pure strain and hybrid females at the time of treatment by counting back from 20 and 19 days, respectively, for all litters.

The unusual occurrence of gestation lengths of as short as 15 and as long as 22 and 23 days have been recorded in table 1. In the case of the shortest period, two live offspring were born, both normal in outward appearance. It is possible that an observational oversight was the cause of imputing so short a gestation length to this pregnancy. Omitting it from the calculation would, of course, raise the figure for the mean gestation length toward 20 days and would not, therefore, invalidate the assigned mean. Concerning the longer pregnancies, normal variation cannot be excluded as their cause.

GENETIC ANALYSIS

Strain differences. The results of administering 4×2.5 mg cortisone to pregnant A and C57BL females confirmed the original impression that these strains respond quite differently to this treatment (table 3). Treatments begun on the eleventh day of gestation produced the highest incidence of cleft palate in both strains. The χ^2 values for days 11, 12 and 13 reveal the differences between these strains in response to the teratogenic effects of the hormone to be real. (All χ^2 tests have been corrected by YATES' method, where appropriate.)

Reciprocal crosses between strains. As the first step in a genetic analysis of this situation, the A and C57BL strains were crossed to each other reciprocally. (Following the usual convention, in all crosses to be mentioned, the female parent is written first.) The results obtained (table 4) show that when strain A (susceptible) animals were used as the female parent ($A \times B$) the frequencies of cleft palate were higher than when the C57BL (resistant) animals were the female parent ($B \times A$). The former cross gave incidences

TABLE 3

Incidence of cleft palate (C.P.) in progeny of pregnant A and C57BL (= B) ♀♀ treated with 4 × 2.5 mg cortisone, begun on various days of gestation.[1]

Gestation day	A × A				B × B				χ^2	P
	Number ♀♀ treated[2]	Number born[3]	Number with C.P.	Percent C.P.	Number ♀♀ treated[2]	Number born[3]	Number with C.P.	Percent C.P.		
8	1	1	0	....	....	....	....	....		
9	....	....	....	....	8	18	3	16.67		
10	1	2	1	....	13	59	8	13.56		
11	10	36	36	100.00	22	75	14	18.67	61.72	$\ll 0.001$
12	4	14	11	78.57	15	69	5	7.25	30.46	$\ll 0.001$
13	4	19	9	47.37	22	100	5	5.00	20.57	$\ll 0.001$
14	8	38	0	0	13	46	1	2.17		
15	5	18	10	55.56	6	25	0	0		
16	1	3	0	....	5	25	0	0		
17[4]	....	....	....	....	6	29	2	6.90		
18[5]	1	6	0	....	6	32	0	0		
19[6]	....	....	....	....	5	21	0	0		
Total	35	137	67	....	121	499	38	....		

[1]This table includes results presented in FRASER and FAINSTAT (1951).
[2]Excluding 10 A and 26 B ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[3]Excluding 4 A × A and 18 B × B offspring whose palates were unexaminable due to cannibalization by mothers.
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.
[6]Treatments begun on this day consisted of 1 × 2.5 mg cortisone.

TABLE 4

Incidence of cleft palate (C.P.) in progeny of crosses A × B and B × A; treatments 4 × 2.5 mg cortisone, begun on various days of gestation (B = C57BL).

Gestation day	A × B				B × A				χ^2	P
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.		
8	....	....	....	....	3	5	1	....		
9	....	....	....	....	7	15	3	20.00		
10	2	12	4	33.33	9	39	0	0	....	0.002[3]
11	11	46	20	43.48	12	82	3	3.66	28.30	≪0.001
12	14	49	9	18.37	14	75	3	4.00	5.44	0.02
13	4	7	1	....	14	93	0	0	....	0.07[3]
14	5	35	0	0	8	47	0	0		
15	2	6	2	....	6	34	0	0		
16	2	9	0	....	2	3	0	....		
17[4]	2	8	0	....	3	23	0	0		
18[5]	....	....	....	....	4	27	0	0		
Total	42	172	36	....	82	443	10	....		

[1]Excluding 9 A and 28 B ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[2]Excluding 27 A × B and 27 B × A offspring whose palates were unexaminable due to cannibalization by mothers.
[3]Probability derived by use of FISHER's exact treatment of the 2 × 2 table (FISHER 1950).
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

TABLE 5

Incidence of cleft palate (C.P.) in progeny of crosses AB × A and BA × A; treatments 4 × 2.5 mg cortisone, begun on various days of gestation.

Gestation day	AB × A				BA × A				χ^2	P
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.		
6	2	4	0	....	....	....	....	....		
7	2	5	0	....	....	....	....	....		
8	4	20	0	0	4	13	2	15.38	....	0.15[3]
9	5	40	4	10.00	9	24	6	25.00	1.41	0.24
10	13	83	20	24.10	11	44	10	22.73	0.002	0.93
11	15	116	26	22.41	11	71	18	25.35	0.08	0.78
12	15	86	10	11.63	22	158	28	17.72	1.16	0.28
13	5	24	0	0	6	43	1	2.33	....	0.64[3]
14	2	17	0	0	4	36	0	0		
15	4	37	0	0	1	11	0	0		
16	2	15	0	0	2	14	1	7.14		
17[4]	2	20	0	0	2	21	0	0		
18[5]	1	12	0	0	....	....	....	....		
Total	72	479	60	....	72	435	66	....		

[1]Excluding 12 AB and 13 BA ♀♀ whose litters were resorbed, aborted, or eaten before examination.
[2]Excluding 42 AB × A and 30 BA × A offspring whose palates were unexaminable due to cannibalization by mothers.
[3]Probability derived by use of FISHER's exact treatment of the 2 × 2 table (FISHER 1950).
[4]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.
[5]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

of the defect intermediate between those for the A × A and B × B crosses. The incidences from the B × A cross, however, were lower than those for the resistant (C57BL) strain mice (for day 11, $\chi^2 = 7.71$, $P = 0.005$).

A difference involving a maternal influence, in a situation such as this, may be due to several causes: it may result from inherent differences in the maternal and/or fetal response to the agent, transmitted through the cytoplasm; it may stem from innate differences in response of the mothers, transmitted through the nucleus; it may be due to short-acting cytoplasmically inherited differences in the fetal response. On *a priori* grounds the last is an unlikely explanation for the phenomenon.

First backcross. To distinguish between the former possibilities, F_1 females from reciprocal crosses of A and C57BL animals (AB ♀ ♀, from the cross A × B; BA ♀ ♀, from the cross B × A) were mated to A males. These crosses are designated as AB × A and BA × A, respectively. Table 5 reveals that both types of F_1 females had similar frequencies of affected offspring, a result which would not be expected on the basis of a hereditary cytoplasmic influence. It does indicate that genetic factors in the mother, inherited through the nucleus, are responsible for the maternal effect.

Second backcross. The female offspring of the cross F_1 × A were mated to strain A males. (This cross is denoted BC × A.) The data pertaining to the frequency of cleft palate in this cross are contained in table 6, which shows an incidence of 56 percent for treatments begun on day 11. The significance of these data will be discussed below.

TABLE 6

Incidence of cleft palate (C.P.) in progeny of cross BC × A; treatments 4 × 2.5 mg cortisone begun on various days of gestation (BC = backcross).

Gestation day	BC × A			
	Number ♀♀ treated[1]	Number born[2]	Number with C.P.	Percent C.P.
8	5	17	10	58.82
9	10	52	17	32.69
10	21	128	71	55.47
11	14	91	51	56.04
12	13	71	15	21.13
13	4	25	0	0
14	2	4	0	....
15	5	41	0	0
16[3]	1	7	0	....
17[4]	7	42	0	0
18[5]	3	15	0	0
Total	85	493	164	....

[1]Excluding 16 ♀♀ whose litters were resorbed, aborted, or eaten before examination.

[2]Excluding 24 offspring whose palates were unexaminable due to cannibalization by mothers.

[3]Treatments begun on this day consisted of 3 × 2.5 mg cortisone.

[4]Treatments begun on this day consisted of 2 × 2.5 mg cortisone.

[5]Treatments begun on this day consisted of 1 × 2.5 mg cortisone.

Pattern of inheritance. The investigation of the mode of inheritance of the character susceptibility to the teratogenic effects of cortisone and that of the character relative fertility (which subject will be discussed later) was complicated by the fact that one cannot usually collect enough data from any one female to classify her for either of these characteristics with any degree of confidence. This fact precluded the application of rigorous mathematical techniques for the analysis of the number of genes involved. However, the data that were obtained have been studied to gain certain indications concerning the situation.

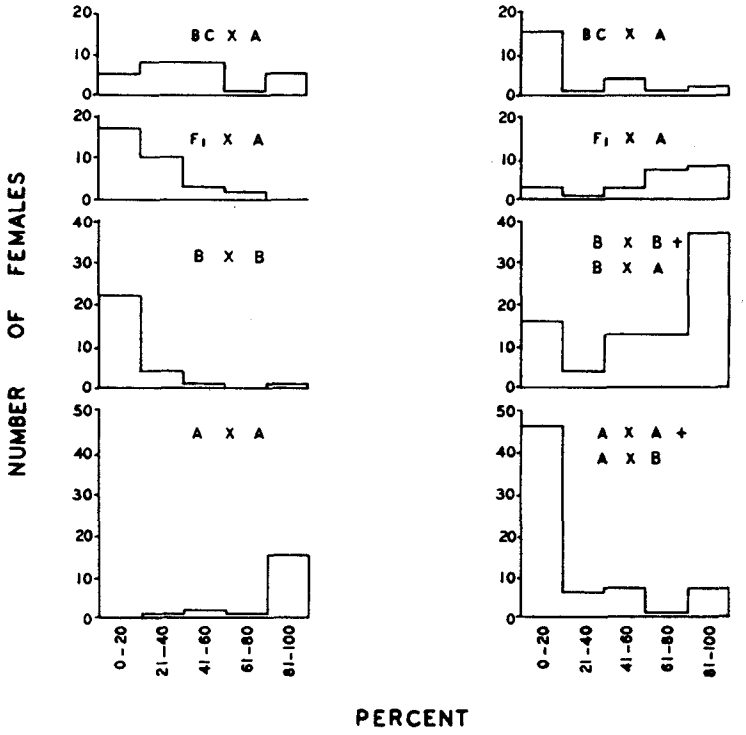

FIGURE 1.—Percentage of total progeny of females of different genotypes with cleft palate (left) and percentage of vaginal plugs which were followed by conception (right) in females of different genotypes.

Histograms constructed from the data concerning the performances of individual females of different genotypes regarding incidence of cleft palate per litter and the frequency with which copulations were followed by an established pregnancy are found in figure 1. The variability of the incidence of cleft palate in the offspring of the backcross females which is illustrated in figure 1 (top left) appears greater than that for females of other genotypes. This is indicative of a segregation of the genetic factors determining susceptibility to the teratogenic effects of cortisone. This increase in variability is consonant with a polyfactorial mode of inheritance of the character. On the

TABLE 7

Frequency of occurrence of pregnancy following isolation with vaginal plugs (V.P.) of females in different crosses (B = C57BL, BC = backcross).

Cross	Number ♀♀ isolated with V.P.	Number pregnant	Percent conceived
1 A × A	65	13	20.00
2 B × B	99	58	58.59
3 A × B	83	17	20.48
4 B × A	65	41	63.08
5 F_1 × A	56	37	66.07
6 BC × A	49	13	26.53

other hand, the portion of figure 1 illustrating the character relative sterility (right half) shows no such difference in degree of variability. The distribution for the backcross females is very similar to that for the strain A females.

RELATIVE STERILITY

One index of the fertility of female mice is the proportion of copulations (i.e., vaginal plugs) that result in palpable pregnancy. This proportion has been shown (FAINSTAT 1951) to be low for A and higher for C57BL females. The results of this investigation confirmed this finding and also indicated that such was the case regardless of the males to which these females were mated (tables 7 and 8). The females employed were roughly equivalent as to age; however, since the strain A females were much less fertile than the C57BL females, the latter became palpably pregnant, and were, therefore, treated, more often than were the A animals.

The fertility of the F_1 females (66%) was not significantly different from that of the C57BL females (59%), which suggests that the genetic basis for fertility may be dominant to that for sterility (table 7, lines 2 and 5; table 8, line 6). The backcross females, however, did not differ significantly from the A animals (26% and 20%; table 7, lines 1 and 6; table 8, line 7).

These values of fertility are in no way to be construed as characterizing any but cortisone-treated females, and may, indeed, be considered the outcome of such treatment, since work proceeding in our laboratory (WALKER personal

TABLE 8

Comparisons of fertility of females of various crosses (B = C57BL, BC = backcross).

Crosses compared	χ^2	P
1 A × A : B × B	17.48	$<<0.001$
2 A × B : B × A	25.87	$<<0.001$
3 A × A : A × B	0.007	0.96
4 B × B : B × A	0.22	0.64
5 F_1 × A : A × A	24.60	$<<0.001$
6 F_1 × A : B × B	0.52	0.47
7 BC × A : A × A	0.34	0.56
8 BC × A : B × B	12.26	<0.001

communication) indicates that entirely untreated strain A females are much more fertile than the treated strain A females, the data for which appear in tables 7 and 8. Untreated C57BL females, however, are approximately as fertile as their treated strainmates (TRASLER personal communication).

CLEFT PALATE AND SEX

No difference between the sexes in proportion of affected animals was detected in the offspring of the cross $F_1 \times A$, the cross from which the largest number of offspring was obtained (table 9).

TABLE 9

Sex distribution of normal and affected offspring of cross $F_1 \times A$ (C.P. = cleft palate).

	Normal	C.P.	Total
♀♀	379	58	437
♂♂	357	54	411
Total	736	112	848

$\chi^2 = 0.002$ $P = 0.93$

DISCUSSION

The inheritance of the character "susceptibility to the teratogenic effects of cortisone" has been shown to be independent of any transmissible cytoplasmic influence and to be wholly transmitted through the nuclear genotype (at least in so far as the differences between strains A and C57BL are concerned). The expression of the character, however, is dependent on an interaction between the genotypes of the mother and fetus. The F_1 hybrids of the cross $A \times B$ had a much lower incidence of cleft palates than did strain A embryos in the same uterine environment. That some portion of the increased resistance was due to hybrid vigor was shown in the cross of resistant strain females to susceptible strain males ($B \times A$), where the incidence of the abnormality in the offspring was significantly lower than that for the cross of resistant strain females to resistant strain males ($B \times B$).

It would appear from figure 1 that a segregation of the genetic factors responsible for susceptibility to the teratogenic effects of cortisone has appeared in the backcross females, as evidenced by the comparative flatness (i.e., increased variability) of the distribution in the histogram representing the cross $BC \times A$. As has been stated above, however, the paucity of results from individual females precluded any attempt to analyze the data statistically. All that can be said, therefore, is that the patterns illustrated in figure 1 (left half) are consistent with the types of distribution found in the transmission of a polyfactorial, quantitative character.

It has been pointed out (FAINSTAT 1951) that there is an apparent inverse relationship in the A and C57BL strains between the two characters susceptibility and fertility. The data for the pure strains and F_1 hybrids are consistent with this interpretation. The backcross animals, however, did not show such

an inverse relationship. They showed a distribution indicative of the occurrence of segregation for the character susceptibility but no such segregation for sterility. This suggests that the two characters are not causally related. The evidence points to the fact that there is no difference in the degree of fertility of treated and untreated C57BL females. On the contrary, however, the difference in this phenomenon between treated and untreated strain A females is marked, their fertility being decreased by more than half by the treatments. This situation may be the result of failure of conception or of preimplantational or early post-implantational destruction of the embryos. The latter possibility would seem to be the more likely since it would be consistent with the greater susceptibility of strain A females to cortisone effects, some of which may be the production of a systemic debility. This interpretation falls down, however, in the consideration of the backcross females, whose offspring had a significantly lower incidence of cleft palates than strain A offspring had (for day 11, $\chi^2 = 21.30$, $P \ll 0.001$), but which were not statistically different in fertility from the A females (table 8). One way out of this dilemma would be to assume that the backcross and A females are susceptible in different degrees to different effects of cortisone. If this were so it would definitely indicate the causal nonrelatedness of the characters.

SUMMARY

Administration of cortisone to pregnant female mice induced the appearance of cleft palates in the offspring. The incidence of the defect was high in strain A (susceptible) animals and comparatively low in C57BL (resistant) animals. Reciprocal crosses between these strains gave incidences of the defect intermediate between those of the pure strains when the female parent was of the susceptible strain, and incidences significantly lower than those for the resistant strain when the female parent was of the resistant strain.

Similar incidences of the defect resulted from matings of the reciprocal F_1 females to susceptible strain males. Thus the factors controlling susceptibility are not cytoplasmically inherited and involve both the maternal and fetal genotypes.

Strain A females were significantly more sterile than C57BL females when mated to males of either their own or the other strain. The F_1 females were as fertile as the C57BL females; the backcross females were as sterile as the A females. The data suggest that susceptibility to the teratogenic effects of cortisone is not causally related to relative sterility.

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