

Genetically Determined Transient Edema Found in the WB/ReJ Mouse Strain in a Teratogenic Survey with Acetazolamide

FRED G. BIDDLE

Departments of Pediatrics and Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada T2N 4N1

ABSTRACT A continuing survey of the genetic variability of different mouse strains to acetazolamide teratogenesis demonstrated the WB/ReJ strain expresses a high frequency of induced subcutaneous edema in day 15 fetuses. In treated WB/ReJ fetuses, the probability of expression of edema is independent of the expression of forelimb ectrodactyly and, with the dosage regime, there is no significant increase in acetazolamide-induced resorption. It was surprising to find a high frequency of spontaneous edema on day 15 in untreated WB/ReJ fetuses. The spontaneous edema is a transient trait with maximum expression (56%) on day 14, and it is resolved by day 17 without apparent consequence to the survival of previously affected fetuses. There is no sex dimorphism in the liability to express the transient edema. Preliminary genetic crosses to investigate the spontaneous edema were made between WB/ReJ and the C57BL/6J strain, which historically had not been observed to express spontaneous edema. A low frequency of spontaneous edema was observed on day 14 in both C57BL/6J and the reciprocal F_1 fetuses. The trait is not additive because there is dominance deviation of the BC_1 fetuses in the direction of the F_1 fetuses. The data were fitted to a threshold model suggesting that the developmental liability to express the difference in transient edema is determined by more than one gene, but the data can be interpreted by a minimum of two loci with duplicate epistasis. The observed differences in frequencies of edema suggest the genetic model can be tested with relatively few test crosses.

Acetazolamide is a well-known teratogen of the mouse (reviewed in Hirsch and Scott, '83). Most studies have focused on the postaxial ectrodactyly of the forelimbs with its right-sided predominance but there are multiple malformations that depend on time of administration and genetically determined reactivity (reviewed in Biddle, '88). An expanded strain survey of the genetic variation in malformation responses to acetazolamide, administered on days 9 and 10, produced variable but low frequencies of subcutaneous edema in day 15 fetuses (Biddle, '88). It was not known whether the edema, observed on day 15 in the treated fetuses, is a transient phenomenon because previous studies with acetazolamide, in which fetuses were examined at term, did not remark on it. The day 15 control fetuses of two strains in the survey,

DBA/2J and SWR/J, expressed a low frequency of edema, but no edema is found at term in the historical experience with these strains. Therefore, the edema, whether spontaneous or induced by acetazolamide in the day 15 fetuses, was difficult to interpret.

Inorganic salts of cadmium are teratogenic in the mouse (Keino and Yamamura, '74) and produce a primary malformation of postaxial forelimb ectrodactyly with right-sided predominance that is phenotypically similar to the acetazolamide response (Layton and Layton, '79). There is a genetic association between reactivity to cadmium-in-

Received May 15, 1990; accepted August 8, 1990.

Address reprint requests to Dr. Fred G. Biddle, Department of Pediatrics, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

duced ectrodactyly and the *cdm* gene for cadmium-induced testicular damage (Layton and Layton, '79). Resistance to cadmium-induced testicular damage is controlled by a recessive gene (*cdm*) (Taylor et al., '73) and, paradoxically, the strains that were homozygous for this testicular resistance trait (*cdm/cdm*) were more sensitive to cadmium-induced ectrodactyly than the ones that were homozygous (+/+) for the testicular sensitivity trait. In the genetic survey of cadmium-induced ectrodactyly, the fetuses were examined on day 14 and significant strain differences in induced edema were also noted that appeared to be independent of the ectrodactyly responses. The edema response to cadmium has not been studied further.

The genetic survey of the response to acetazolamide of the historical mouse strains was continued with the WB/ReJ strain and the same dosage regime used in the previous report (Biddle, '88). The expected forelimb ectrodactyly response was unremarkable in that it fell in the middle of the range of reactivities of the previously tested strains; however, there was a very high frequency (81%) of edema in the day 15 fetuses. Moreover, the untreated WB/ReJ control fetuses also exhibited a lower but still high frequency (39%) of edema on day 15 (Biddle, '89). The absence of edema at term in the WB/ReJ strain suggests that this strain may have a transient fetal edema.

The purpose of this report is to describe the acetazolamide treatment of WB/ReJ that brought the fetal edema to attention. The time course of expression of the spontaneous edema is documented. Preliminary genetic analysis of the edema trait of WB/ReJ was conducted with crosses to the C57BL/6J strain.

MATERIALS AND METHODS

The WB/ReJ (abbreviated WB) and C57BL/6J (abbreviated B6) strains were obtained originally from the Jackson Laboratory (Bar Harbor, Maine). The WB strain is WB/ReJ-*W*/+ and is maintained heterozygous for the dominant white spotting gene (*W*). Inbreeding has been continued in this laboratory by sister-brother mating. The strain is currently at the +F23 generation and is designated WB/ReJBid-*W*/+ in this laboratory. The WB mice used in this study were the +/+ (wild-type) progeny (without the *W* mutation) from the inbreeding strain

or the first generation progeny from +/+ × +/+ matings set up to produce +/+ animals. Inbreeding of the C57BL/6J strain has been continued by sister-brother mating. It is currently at the +F42 generation and is designated C57BL/6JBid.

The maintenance of the mice and methods of timed mating were described previously (Biddle, '88). The day of finding the copulation plug is defined as day 0 of gestation.

Acetazolamide was prepared as a suspension (100 mg/ml) in 0.3% carboxymethylcellulose-saline and administered orally (1,000 mg/kg) at each of three times (9 AM and 4 PM on day 9 and 9 AM on day 10) (Biddle, '88). The acetazolamide-treated litters and their controls were examined on day 15. The gravid uteri were dissected and examined under saline. Dead fetuses, regardless of malformations, were recorded as resorptions. External malformations in live fetuses were recorded.

The time of expression of the spontaneous edema of the WB strain was determined in untreated litters between days 12 and 18. Fetuses were sexed by examining the dissected and unstained gonads in a drop of saline with transmitted light (Whitten et al., '79). The presence of tubules defines the gonad as a testis (Fig. 1). Preliminary genetic analysis of the liability to express spontaneous edema was conducted with crosses between the WB and B6 strains.

Statistical comparisons were made by appropriate tests (Sokal and Rohlf, '81). Resorption, ectrodactyly, and edema in the acetazolamide-treated litters and their controls were analyzed by litter averages using the Freeman-Tukey arcsin transformation for small samples (Mosteller and Youtz, '61). Comparisons were made by t-tests. Tests for association between edema and sex in untreated litters were made by G-tests. Heterogeneity of the frequency of spontaneous resorption and edema was examined among different groups by the G-test. A posteriori tests for homogeneity were made with an experimentwise error rate of $\alpha = 0.05$.

RESULTS

Acetazolamide treatment

The response to the acetazolamide treatment of the WB strain and the control litters are listed in Table 1 in a form that can be compared with the previous survey of 11 strains (Table 1 in Biddle ['88]). There is no

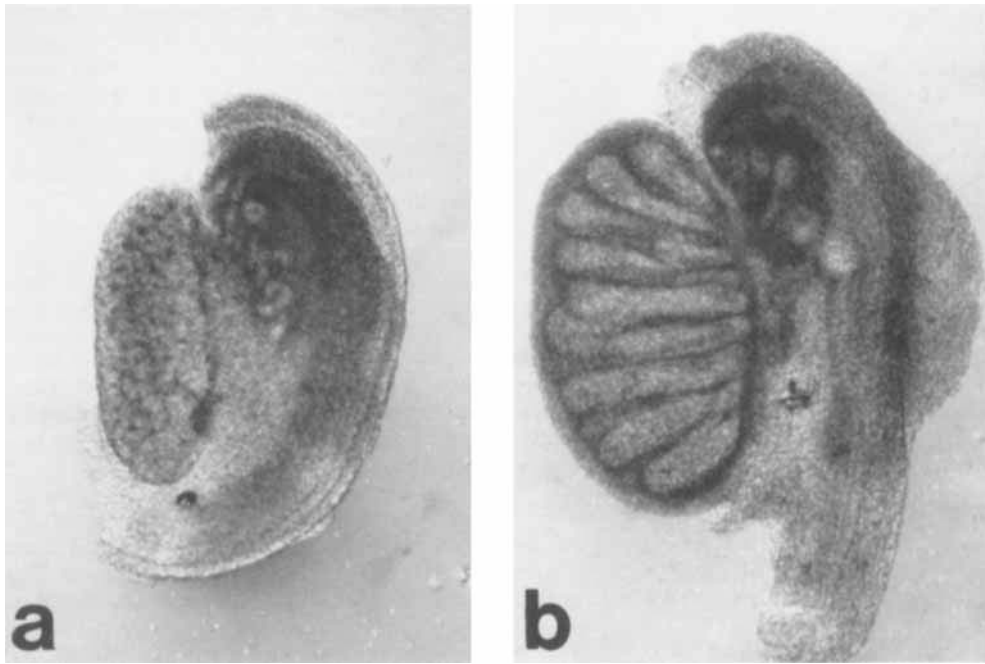


Fig. 1. Ventral view of an unstained left ovary (a) and testis (b) with attached mesonephros from day 14 mouse fetuses with transmitted light.

TABLE 1. Day 15 response of WB/Re to acetazolamide¹

	Control	Treated
No. litters	10	10
Resorption		
Total (%)	22/83 (26)	26/69 (38)
Litter average (arcsin \pm SE)	31.18 \pm 5.10	37.15 \pm 3.00
Ectrodactyly		
Total (%)	0/61	20/43 (46)
Litter average (arcsin \pm SE)	—	43.78 \pm 5.88
Edema		
Total (%)	24/61 (39)	35/48 (81)
Litter average (arcsin \pm SE)	39.46 \pm 5.00	64.14 \pm 4.83*

¹(1,000) mg/kg, p.o., 9 AM and 4 PM day 9 and 9 AM day 10.

*Significant increase in edema (2-tailed t = 3.548, 18 df, $P < 0.01$).

significant change in resorption after treatment with the dosage regime. Forelimb ectrodactyly of the acetazolamide type was observed in 46% of live fetuses (litter average of 43.78 arcsin units) and is similar to the response of the BALB/cByJ strain (43% of live fetuses or 43.33 \pm 5.12 arcsin units \pm SE from Table 1 in Biddle [88]). Ectrodactyly was only right-sided in the WB fetuses at this dosage.

A high frequency of subcutaneous edema (81%) was found in the treated WB fetuses. Unexpectedly, a high frequency of edema (39%) was also observed in the untreated day 15 fetuses. The increase in frequency of edema after acetazolamide is significant ($P < 0.01$ by t-test of the litter-averaged arcsin-transformed frequencies). Since resorption after acetazolamide treatment is not increased, the increase in frequency of WB fetuses with edema is a response to acetazolamide and is not due to a differential toxicity of acetazolamide in normal fetuses that did not express spontaneous edema. If the edema response to acetazolamide of WB fetuses is independent of the spontaneous edema, the frequency of edema in treated fetuses (81%) can be corrected for the natural frequency (39%) in day 15 fetuses so that the induced-edema response in day 15 fetuses is approximately 68% [(81-39)/(100-39)] (Abbott's formula in Finney [71]). In addition, among the live WB fetuses treated with acetazolamide, there is no association between expression of ectrodactyly and edema (G het = 0.04, 1 df, $P > 0.5$).

Previously, day 15 fetuses of C57BL/6 did not express spontaneous edema but they did

TABLE 2. Time of expression of spontaneous edema in WB/Re

Gestation day	No. litters	Resorption		Edema	
		N	%	N	%
12	5	4/39	10	0/35	0
13	10	11/78	14	1/67	1
14	20	28/155	18	71/127	56
15	20	40/164	24	44/124	35
16	11	10/88	11	3/78	4
17	4	8/34	24	0/26	0
18	4	3/21	14	0/18	0

respond to the acetazolamide regime with 12% edema (8/69 fetuses) (Table 5 in Biddle [88]). Also, no test for association between the ectrodactyly and edema responses was possible because most of the fetuses (68/69) expressed ectrodactyly.

Time of expression of spontaneous edema

Untreated WB litters were examined for obvious expression of the spontaneous edema between days 12 and 18 with emphasis on days 13 to 16 (Table 2). The spontaneous edema is transient with maximum expression on day 14. Therefore, the previous examination of the day 15 acetazolamide-treated fetuses and their controls (Table 1) was not at the time of maximum expression of the spontaneous trait. Figure 2 shows a typical affected and normal day 14 WB fetus.

The edema disappears, apparently without consequence to the previously affected fetuses because there is no increase in resorption. The resorption rate among litters examined on days 12–18 does not differ from an average of 18.0% (G het = 10.66, 6 df, $P = 0.10$). Also, there is no difference in expression of edema in day 15 fetuses in Table 2 and the independent set of day 15 control fetuses in Table 1 (G het = 0.26 1 df, $P > 0.50$).

Association between fetal sex and edema

Gonadal sex was determined in some of the WB litters examined on days 14–16 (Table 3). There is no association between sex and edema on either day 14 (G het = 0.85, 1 df, $P > 0.30$) or day 15 (G het = 1.76, 1 df, $P > 0.10$). Day 16 results were not tested because there were few affected fetuses.

Spontaneous edema in crosses between WB/Re and C57BL/6

Spontaneous edema has not been recorded in the historical experience with the C57BL/

6 (B6) strain. A small sample of B6, the F_1 embryos from the reciprocal crosses between WB and B6, and the four types of backcrosses of the F_1 to WB were examined on day 14 (Table 4) and compared with the observations for WB (see Table 2).

Unexpectedly, edema was observed in low frequency in the day 14 B6 fetuses (Table 4). The single affected fetus in each type of F_1 was male but, from the later examination of the backcross fetuses, there is no edema association with sex that is due either to a sex dimorphism or to X-chromosome-linked genetic factors. The low and similar frequencies of edema in B6 and the reciprocal F_1 embryos compared with the WB strain indicate that spontaneous edema of WB is a recessive trait.

As expected there is an increase in frequency of edema in the four types of BC_1 fetuses from the crosses to the WB strain when compared with the F_1 fetuses. Within each of the four types of BC_1 , there is no association of edema with sex (Table 4). Nevertheless, among the four types of BC_1 there is apparent heterogeneity in the frequency of edema in total live fetuses (Table 5). The BC_1 fetuses from the reciprocal WB.B6 F_1 and B6.WB F_1 males crossed to WB females are significantly different.

The apparent heterogeneity in the frequency of edema in total live fetuses was tested in a replicate sample of the four types of backcross matings. If the observed difference between 3% and 12% of edema from the backcrosses of WB.B6 F_1 and B6.WB F_1 males, respectively, is true ($P < 0.05$) (Table 5), a new sample of 154 fetuses from each type of F_1 male would allow us to be 80% certain of detecting this difference (Sokal and Rohlf, '81). The results of these replicate backcross matings are shown in Table 6. Again, there is no association of edema with sex, and there is no heterogeneity in the frequency of edema among the four types of BC_1 fetuses. These replicate backcrosses are used in further analysis, and the first sample is discarded.

For a genetic interpretation of the spontaneous edema trait, the F_1 and replicate BC_1 generations were pooled separately and compared with the frequencies of edema in WB and B6 (Table 7). The first backcross generation in the direction of the WB strain shows the expected increased in expression of edema above the F_1 frequency but, relative to the difference between the WB strain

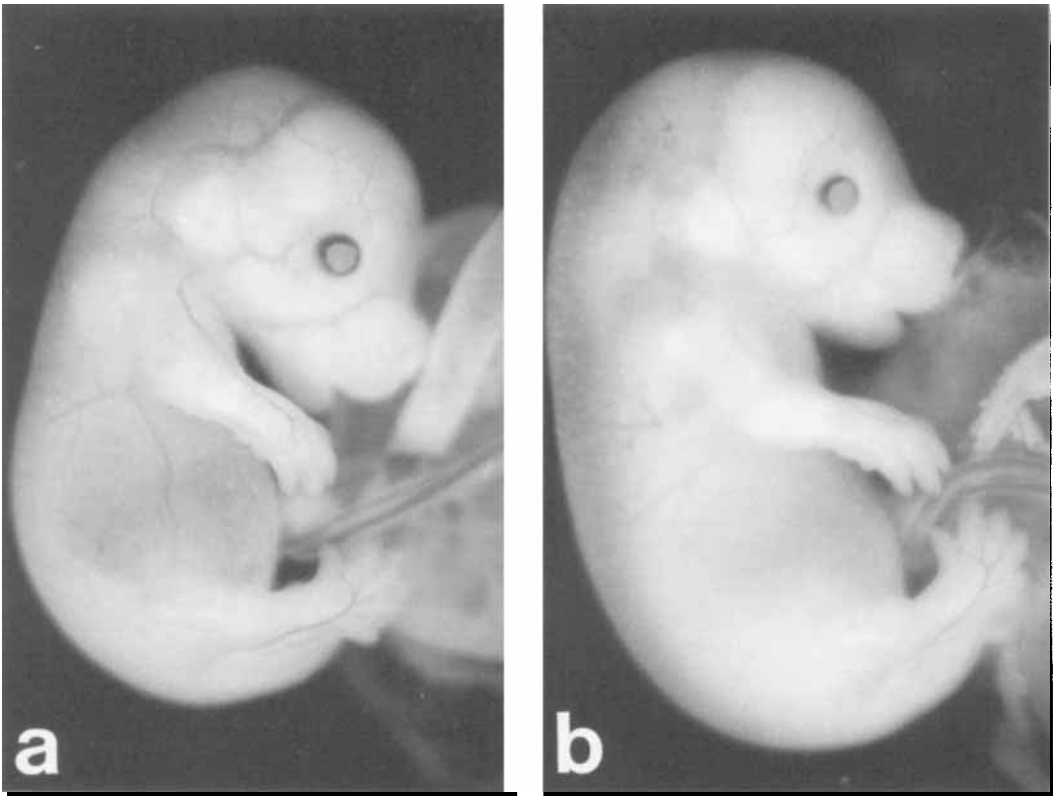


Fig. 2. Normal (a) and edematous (b) day 14 WB/Re fetuses. In side view, edema is evident from the head caudally along the dorsal spine.

TABLE 3. Association between sex and edema in WB/Re fetuses

Gestation day	No. litters	Edema		Normal	
		Female	Male	Female	Male
14	10	22	16	12	14
15	16	18	19	21	39
16	10	2	1	29	37

and F_1 generation, the observed BC_1 frequency cannot be interpreted in terms of what is expected for a single-gene cause of the difference in frequency of the spontaneous trait. When the frequency of edema among the four genotypes in Table 7 was tested for homogeneity ($P=0.05$), the result is indicated in Table 7. When the BC_1 was compared separately with the F_1 , there is a significant increase in frequency in the BC_1 ($G\text{ het} = 5.87, 1\text{ df}, P<0.025$). These preliminary genetic observation will be explored further in the Discussion.

DISCUSSION

The WB/Re strain was tested for response to acetazolamide in order to expand a previously reported genetic survey of the common and historical strains of the mouse (Biddle, '88). The ectrodactyly response of WB is not unique because it is not significantly different from the previously tested BALB/cByJ strain. Similarly, WB does not exhibit an increase in acetazolamide-induced resorption at the dosage used.

The high frequency of edema induced by acetazolamide in day 15 fetuses of WB is unique, so far, among the strains that have been assayed with the same dosage and time of treatment (Biddle, '88). Other tested strains either did not express edema or expressed only a low frequency and, therefore, it was felt the generalized edema could be an induced malformation or the reflection of malformations in other organ systems. The high frequency of spontaneous edema in day

TABLE 4. Edema in day 14 fetuses in C57BL/6 (B6), reciprocal crosses between C57BL/6 and WB, and backcrosses to WB

Mating (female × male)	No. litters	Resorption		Edema		Normal	
		N	%	Female	Male	Female	Male
B6 × B6	10	14/87	16	1	3	33	36
WB × B6	10	8/74	10	0	1	30	35
B6 × WB	11	8/101	8	0	1	46	46
WB × WB.B6F ₁	18	26/133	20	1	2	50	54
WB × B6.WBF ₁	23	38/183	20	9	8	66	62
WB.B6F ₁ × WB	7	10/64	16	1	1	24	28
B6.WBF ₁ × WB	8	8/83	10	2	4	36	33

TABLE 5. Edema in day 14 fetuses from backcross matings

Mating (female × male)	Edema/live fetuses	
	N	%*
WB × WB.B6F ₁	3/107	3 ¹
WB × B6.WBF ₁	17/145	12 ²
WB.B6F ₁ × WB	2/54	4 ^{1,2}
B6.WBF ₁ × WB	6/75	8 ^{1,2}

*Frequencies with the same superscript are not significantly different ($P = 0.05$ and critical $G = 7.82$).

15 fetuses of WB along with the high frequency of acetazolamide-induced edema presents an opportunity to determine the genetic cause of the spontaneous trait, its relationship to low frequencies of edema observed in other strains, and the effect of the teratogenic treatment which results in a phenotype grossly indistinguishable from the spontaneous trait.

Subcutaneous edema is a transient and normal characteristic of WB fetuses that appears to have gone unnoticed or, at least, unreported. The frequency of expression is maximum on day 14 (see Table 2) and, in the sample size examined, it is resolved by day 17 with no apparent developmental consequence. WB fetuses appear normal at term.

Preliminary genetic crosses were made between WB and the C57BL/6 strain, in which spontaneous fetal edema had not been observed, but in which a low frequency (12%) was found on day 15 in response to acetazolamide (Table 5 in Biddle [88]). Also, the WB strain is ancestrally related to C57BL/6 (Russell and Lawson, '59). A few animals that were $W/+$ were crossed to the C57BL/6 strain and sister-brother matings were carried out to select different genetic backgrounds that promoted survival of the W/W homozygote. Four strains were selected, designated WB/Re, WC/Re, WH/Re, and WK/Re. The WB and WC are closely related and, in the light of the present ob-

servation, it will be important to analyze the WC/Re strain for expression of the spontaneous edema trait and its response to acetazolamide.

Untreated B6 and hybrid fetuses were examined on day 14 assuming that, if edema were expressed, it would be maximal on day 14. The transient edema is considered to be a threshold trait. The liability to express edema is normally distributed on a probability scale and a developmental threshold causes edema to be expressed, albeit transiently, because the condition regresses as fetal development proceeds in WB.

B6 fetuses expressed a low frequency of edema (5%) on day 14. This was unexpected because no edema was found in a previous examination of day 15 fetuses. Edema was found in low frequency (1–2%) in the reciprocal F_1 fetuses and, therefore, the high frequency of transient edema in the WB strain is a recessive trait. In order to assess the genetic control of the trait, the reciprocal F_1 males and females were backcrossed to WB to recover genes for the expression of the trait. The pooled BC_1 fetuses exhibit a significant increase in frequency of edema compared with the F_1 fetuses.

A preliminary interpretation of the genetic data can be made to establish a working hypothesis that will be tested in further genetic analysis. Figure 3 is a genetic diagram of the correspondence between the edema phenotype (frequency of edema on a probability scale) and fetal genotype as a proportion of WB autosomal genes. The dashed regression line predicts the expected frequencies of edema for the F_1 and BC_1 fetuses for an additive genetic model. The low frequency of edema of B6 is dominant to the high frequency of WB. The solid regression line between the F_1 and WB fetuses predicts the expected frequency of edema for the BC_1 fetuses for a one-locus genetic model or for multiple loci with additivity between loci.

TABLE 6. Day 14 examination of replicate backcross matings

Mating (female \times male)	No. litters	Resorption		Edema		Normal		Edema/live
		N	%	Female	Male	Female	Male	%
WB \times WB.B6F ₁	23	29/186	16	5	1	63	88	4
WB \times B6.WBF ₁	25	37/192	19	3	9	65	78	8
WB.B6F ₁ \times WB	20	14/181	8	6	1	85	75	4
B6.WBF ₁ \times WB	21	15/182	8	3	5	84	75	4

TABLE 7. Pooled genetic crosses for spontaneous edema

Generation	Edema/live fetuses	
	N	%*
WB/Re (WB)	71/127	56 ¹
C57BL/6 (B6)	4/73	5 ²
F ₁ (pooled)	2/159	1 ²
BC ₁ (pooled replicate)	33/646	5 ²

*Frequencies with the same superscript are not significantly different ($P = 0.05$ and critical $G = 7.82$).

The average of the BC₁ fetuses shows the expected increase in frequency of edema but the frequency is less than expected from a one-locus model or multiple loci with additivity between loci.

Figure 4 is a further interpretation of the observed genetic data and is a visual explanation of Figure 3. The calculations for Figure 4 are listed in Table 8. If the development of the lymphatic system is under genetic control, there will be random differences in development among the fetuses of any one generation. The distribution of liability relative to a threshold to express edema in WB fetuses is situated to allow 56% to express edema, while 44% are visibly normal. The B6 strain and F₁ distributions of liability are genetically located on the normal side of the threshold so that only a low frequency of fetuses by chance fall across the threshold and express edema.

The genetically informative region is between the phenotype of the WB and the F₁ fetuses. If the difference in liability to express edema is controlled by a single gene, the expected mean frequency of edema in the first backcross generation can be predicted from the average of the F₁ and WB frequencies transformed to a probability scale. In this case, the probit-transformed frequencies (Table 8) give an expected mean of $\frac{1}{2}(F_1) + \frac{1}{2}(WB) = 3.98$ or $\approx 15\%$. The average of the four observed BC₁ probit-transformed frequencies ($\pm 95\%$ limits) is 3.34 ± 0.27 or 4.8% (2.6–8.2%) and excludes the expected the BC₁ frequency. Therefore, a single-gene model can be rejected. Simi-

larly, a multiple-locus model with additivity between loci can be rejected by the same results.

If multiple genetic loci control the difference in liability to express edema between WB and B6, there is interaction or dominance between loci. The next simple genetic model is a two-locus model, with independent segregation between loci but with duplicate epistasis (dominance between loci). In symbolic terms, WB is proposed to be genetically (a/a, b/b) and B6 is (+/+, +/+). The F₁ is therefore (a/+, b/+). In the first backcross generation, there are four predicted genotypes in equal frequency. The duplicate epistatic model predicts only (a/a, b/b) genotype will be phenotypically like the WB strain and the rest will be indistinguishable from the F₁ fetuses. Therefore, the probit transformed frequencies (Table 8) predict the BC₁ frequency of edema will be $\frac{3}{4}(F_1) + \frac{1}{4}(WB)$ equal to 3.40 or approximately 5.5%, and this is observed.

In Figure 4 the expected distribution of liability of the BC₁ is shown as a dotted distribution for the one-locus model and as a solid distribution for the two-locus, duplicate epistatic model. The expected frequencies of fetuses with edema are the indicated areas under the two curves.

The working hypothesis to account for the transient edema of WB and the net genetic difference in this trait between the WB and B6 strains is essential for the design of its test by genetic segregation analysis. Previous genetic studies of threshold traits of development in the mouse, e.g., acetazolamide-induced ectrodactyly (Biddle, '75) or cortisone-induced cleft palate (Biddle and Fraser, '77) or spontaneous cleft lip (Juriloff, '80), have performed a segregation analysis with test mating and then fitted different genetic models to the data. These studies have demonstrated dominance in the traits and some degree of multiple-locus interaction.

The suggested two-locus genetic model with duplicate epistasis is the direction in

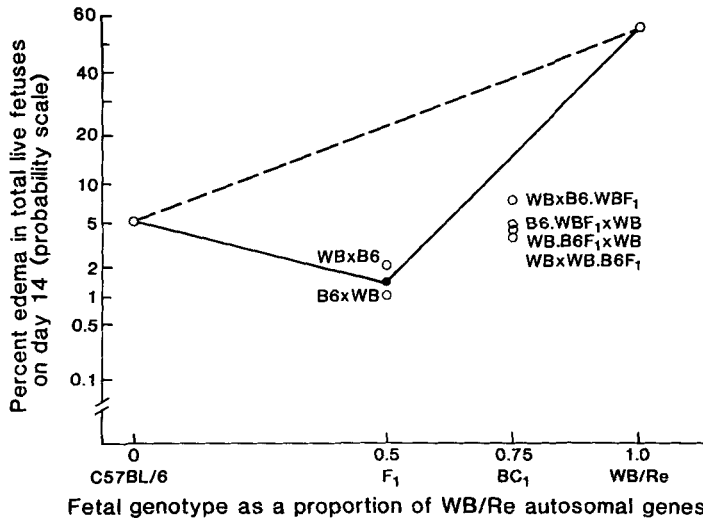


Fig. 3. Genetic diagram of the correspondence between phenotype or frequency of edema (on a probability scale) and fetal genotype (as a proportion of WB/Re autosomal genes). The dashed regression line predicts the expected frequencies of edema for the F_1 and BC_1 fetuses for an additive genetic model. There is domi-

nance deviation of low frequency of edema. The solid regression line between the F_1 and WB fetuses predicts the expected frequency of edema for the BC_1 fetuses for a one-locus genetic model or multiple-locus models with additivity between loci.

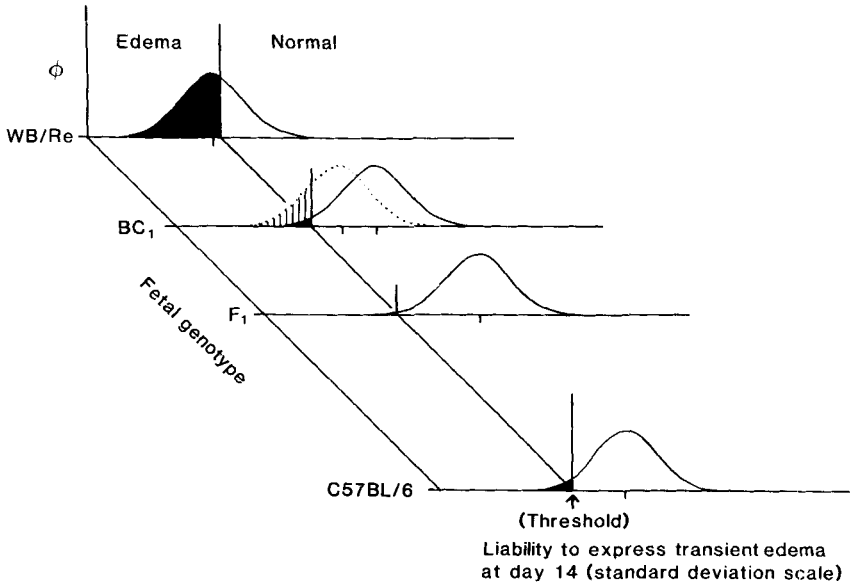


Fig. 4. Developmental liability to express transient edema in day 14 WB/Re, F_1 , and C57BL/6 fetuses are plotted as unit normal distributions relative to a threshold. The predicted distributions for the BC_1 fetuses are

shown as a dotted distribution for a one-locus model and a solid distribution for a two-locus model with duplicate epistasis. The frequencies of edema are the areas under the distributions to the left of the threshold.

which further genetic analysis is proceeding. If the BC_1 males are test-mated with WB-strain females, the four predicted BC_2

genotypes in the two-locus model will produce specific distributions of genotypes in the BC_2 fetuses. With duplicate epistasis

TABLE 8. Probit-transformed frequencies of edema from the genetic crosses and location of mean liabilities relative to the threshold

Fetal generation	Observed edema		Threshold—mean (probit—5.00)
	%	Probit	
WB/Re (B6)	56	5.15	+0.15
C57BL/6 (B6)	5	3.36	-1.64
F_1			
WB × B6	2		
		2.81	-2.19
B6 × WB	1		
BC_1			
WB × WB.B6F ₁	4		
WB × B6.WBF ₁	8		
		3.34 ± 0.27	-1.66
WB.B6F ₁ × WB	4		
B6.WBF ₁ × WB	4		

and averaging the probit-transformed frequencies of edema that have been observed for the F_1 and WB-strain fetuses, the four types of BC_1 males will produce the following expected frequencies of edema in BC_2 fetuses:

BC_1 genotype	Expected frequency of edema in BC_2 fetuses
a/a, b/b	56%
a/+, b/b	15%
a/a, b/+	15%
a/+, b/+	5%

The generation of minimum sample sizes to distinguish between 56% and 15% edema is very practical. A minimum sample of 25 fetuses from each test mating will discriminate between 56% and 15% edema with 95% confidence and 80% certainty (see, e.g., pp. 776–778 in Sokal and Rohlf [81]). However, a much larger sample of approximately 203 BC_2 fetuses would be required to discriminate between expected frequencies of edema of 5% and 15% and perhaps exceeds the reproductive capacity of the mouse. Also, with the two-locus duplicate-epistatic model, the putative a/a, b/b genotype of BC_1 males, which is expected to produce 56% edema in BC_2 fetuses, will be expected in one-fourth of the BC_1 males. Sixteen BC_1 males would need to be test mated in order to reduce the probability of missing this class to less than 1%. Therefore, this is a feasible study to do.

The use of anti-Rh immunoglobulin therapy has reduced Rh fetal-maternal isoimmunization as the major cause of human fetal edema. Attention has become focused on the nonimmunological factors in the remain-

ing cases. Four principal pathophysiological factors predominate in various discussions (Giacoa, '80; Im et al., '84): intravascular hydrostatic pressure, decreased plasma oncotic pressure, increased capillary permeability, and obstruction of lymph flow. The causes of these factors are heterogeneous (Machin, '89; Villaespesa et al., '90). Added to this variability and heterogeneity are the cases that are sonographically identified in utero but resolve spontaneously during follow-up (Mueller-Heubach and Mazer, '83; Platt et al., '78; Robertson et al., '85; Shapiro and Sharf, '85).

The best described and most striking pathology of fetal hydrops and cystic hygroma is associated with human 45,X (Turner) syndrome (van der Putte, '77; Byrne et al., '84). The jugular lymphatic sacs do not communicate with the jugular veins. The lymphatic obstruction may produce a pathway of pathogenesis leading to compression of left-sided blood flow and coarctation of the aorta in this syndrome (Clarke, '84). Some support for this pathway is found in abortuses selected with nuchal webbing, fetal hydrops and female genitalia; there is an association but not a 1:1 relationship (Lacro et al., '88). The Noonan syndrome of multiple congenital malformations shares many similarities with the 45,X syndrome and the lymphedema has been suggested to play a causative role in the pathogenesis of the clinical phenotype (Witt et al., '87). Nevertheless, coarctation of the aorta and left heart obstruction are not a common feature.

The genetic cause of the 45,X syndrome is straightforward but the spectrum of malformations and their relationship may be the result of interaction of the chromosomal aneuploidy with genetic background. The Noonan syndrome with its multiple malformations is more insidious. Its genetic control has defied interpretation by single-gene models of inheritance and it may be genetically heterogeneous.

In the mouse, early development of the lymphatic system has been described (van der Putte, '75), but no systematic collation has been made of the causes of fetal edema and any pathologies that may result from or may be associated with the edema. A survey of the descriptions of mouse mutants (Lyon and Searle, '89) reveals a limited number of examples in which fetal edema has been noted and that are not simply epidermal blisters or hematomaous blebs (Table 9).

TABLE 9. Autosomal genes of the mouse with associated fetal edema¹

Gene symbol	Name	Genetics	Chromosome
<i>oed</i>	Edematous	Recessive	—
<i>Ph</i>	Patch	Semidominant	5
<i>Ps</i>	Polysyndactyly	Semidominant	4
<i>Ra</i>	Ragged	Semidominant	2
<i>Ra^{op}</i>	Opposum	Semidominant	2
<i>T^{hp}</i>	Hairpin tail	Dominant (edema expressed when transmitted by female)	17
<i>Xt</i>	Extra toes	Semidominant	13
<i>Xt^{bp}</i>	Brachyphalangy	Semidominant	13

¹From Lyon and Searle ('89).

The best characterized pathology associated with edema has been reported for the maternally derived hairpin tail (*T^{hp}*) mutant in *T^{hp}/+* fetuses (Babiarz et al., '88). The edema and associated malformations are expressed when chromosome 17 marked by the *T^{hp}* deletion is transmitted to the fetus by the mother. The fetuses are therefore partially monosomic and have the wild-type chromosome 17 transmitted by the male. The epigenetic effects leading to the pathologies and the developmental interactions caused by the absence of an intact maternally derived chromosome 17 are unknown. To the list in Table 9 can be added some of the tertiary trisomies (partial duplications/deficiencies) that are generated by meiotic numerical nondisjunction from heterozygotes for reciprocal translocations. In some cases the appearance of severely edematous fetuses at day 15 has been used as a criterion to screen further for cardiac anomalies (Kirk and Searle, '88). For the primary trisomies (whole chromosomal trisomies), edema appears to be characteristic of all the trisomies that survive into the last third of gestation (Gropp, '84). Generalized growth retardation and hypoplasia may be characteristics of the trisomies (Gearhart et al., '86), but genetic background appears to play an important role in the expression of edema with specific trisomies. Edema is found in association with trisomy-19 (Ts-19) in some reports (Gropp, '84), but edema is conspicuous by its absence in others (Pexieder et al., '81). For other malformations, such as cleft palate, the interaction between trisomy and genetic background has been well documented. The different frequencies of cleft palate associated with Ts-19 are due

to genetic differences in the developmental liability of different strains to express cleft palate (Vekemans and Trasler, '86).

Interest in the transient edema of the WB strain arises from the threshold nature of the trait. The inbred strains of the laboratory mouse have many examples of low-frequency malformation traits that are threshold traits of development. For these traits, not all individuals with the same genetic liability do express the trait. For example, lateral cleft lip, open eyelids, and atrial septal defects are found in the A family of strains (Kalter, '79; Fraser and Rosen, '75); microphthalmia/anophthalmia, micrognathia/agnathia, and ventricular septal defects are found in the C57BL/6 family of strains (Kalter, '68; Nora et al., '68). These are variously called spontaneous malformations but the reality is that all individuals of a specific inbred strain have the same genetic liability to express the traits whereas only a few in fact do express them.

The cleft lip trait is becoming amenable to genetic interpretation because there is a concerted and committed effort to analyze it. Genetic segregation analysis demonstrates liability to express cleft lip of the A family is determined by a single recessive gene (Juriloff, '86). Furthermore, maternal genetic differences control the frequency of cleft-lip expression (Juriloff and Fraser, '80; Juriloff, '82) and epigenetic factors differ between cleft-lip expressing and normal lip fetuses, which have the same a priori genetic liability to express cleft lip, so that cleft-lip fetuses have an increased sensitivity to thyroxine-induced death (Juriloff and Harris, '85). For other traits of the A family, open eyelids is genetically independent of cleft lip (Biddle and Fraser, '86), but the atrial septal defect has not been studied genetically.

Threshold models have formed the conceptual framework for the so-called multifactorial, threshold models of development (which are not polygenic models of inheritance) (Fraser, '76). Computer simulations of putative single-gene models of malformations in man and mouse, using a model for endocardial cushion defects, show clearly how chance or stochastic events in development can have a major and predictable role in the final phenotypic expression (Kurnit et al., '87). We have to constantly remind ourselves that major events of induction that determine embryonic structures are threshold models (Jacobson and Sater, '88).

The transient edema of the WB strain appears amenable to genetic analysis. The apparent genetic heterogeneity in expression of edema response to teratogens such as cadmium (Layton and Layton, '79) and acetazolamide (Biddle, '88; also this report) and edema response to chromosomal aneuploidy provides an impetus to define the genetic control and embryological basis of the transient edema of the WB strain. A known genetic liability to express edema would provide the framework for systematic analysis of the effects of specific teratogens, single mutant genes, and different chromosomal aneuploidies.

ACKNOWLEDGMENTS

This work was supported by grant MT-6736 from the Medical Research Council of Canada. Salary support was provided by the Alberta Children's Hospital Foundation. I thank L.M. Oland for assistance with the acetazolamide treatments and F. Yang for help in preparation of the manuscript. The replicate analysis of the BC₁ fetuses was conducted by L.R. Mulholland as part of an independent studies project for Biology 528 at the University of Calgary.

LITERATURE CITED

- Babiarz, B.S., M.J. Donovan, and H.J. Hathaway (1988) The developmental pathology of maternally derived *T^{ho}* fetuses. *Teratology*, 37:353-364.
- Biddle, F.G. (1975) Teratogenesis of acetazolamide in the CBA/J and SWV strains of mice. II. Genetic control of the teratogenic response. *Teratology*, 11:37-46.
- Biddle, F.G. (1988) Genetic differences in the frequency of acetazolamide-induced ectrodactyly in the mouse exhibit directional dominance of relative embryonic resistance. *Teratology*, 37:375-388.
- Biddle, F.G. (1989) Transient fetal edema: A new genetically-determined trait of fetal development in the mouse. *Bull. Genet. Soc. Can.*, 20(suppl.1):46 (abst.)
- Biddle, F.G., and F.C. Fraser (1977) Cortisone-induced cleft palate in the mouse. A search for the genetic control of the embryonic response trait. *Genetics*, 85:289-302.
- Biddle, F.G., and F.C. Fraser (1986) Major gene determination of liability to spontaneous cleft lip in the mouse. *J. Craniofac. Genet. Dev. Biol.*, 2(Suppl.):67-88.
- Byrne, J., W.A. Blanc, D. Warburton, and J. Wigger (1984) The significance of cystic hygroma in fetuses. *Hum. Pathol.*, 15:61-67.
- Clarke, E.B. (1984) Neck web and congenital heart defects: A pathogenic association in 45 X-O Turner syndrome? *Teratology*, 29:355-361.
- Finney, D.J. (1971) *Probit Analysis*. 3rd Ed. Cambridge University Press, Cambridge.
- Fraser, F.C. (1976) The multifactorial/threshold concept—Uses and misuses. *Teratology*, 14:267-280.
- Fraser, F.C., and J. Rosen (1975) Association of cleft lip and atrial septal defect in mice: A preliminary report. *Teratology*, 11:321-324.
- Gearhart, J.D., M.T. Davison, and M.L. Oster-Granite (1986) Autosomal aneuploidy in mice: Generation and developmental consequences. *Brain Res. Bull.*, 16:789-801.
- Giacoia, G.P. (1980) Hydrops fetalis (fetal edema). *Clin. Pediatr.*, 19:334-339.
- Gropp, A. (1984) Fetal hydrops in chromosome disorders as principle of damage in developmental pathology; clinical observations in man and experimental studies in the mouse. In: *One Medicine*. A.O. Ryder and M.L. Byrd, eds. Springer-Verlag, Berlin, pp. 84-95.
- Hirsch, K.S., and W.J. Scott (1983) Searching for the mechanism of acetazolamide teratogenesis. In: *Issues and Reviews in Teratology*. H. Kalter, ed. Plenum Press, New York, Vol. 1, pp. 309-347.
- Im, S.S., N. Rizos, P. Joutsij, J. Shime, and R.J. Benzie (1984) Nonimmunologic hydrops fetalis. *Am. J. Obstet. Gynecol.*, 148:566-569.
- Jacobson, A.G., and A.K. Sater (1988) Features of embryonic induction. *Development*, 104:341-359.
- Juriloff, D.M. (1980) The genetics of clefting in the mouse. In: *Etiology of Cleft Lip and Cleft Palate*. M. Melnick, D. Bixler, and E.D. Shields, eds. Alan R. Liss, Inc., New York, pp. 39-71.
- Juriloff, D.M. (1982) Differences in frequency of cleft lip among the A strains of mice. *Teratology*, 25:361-368.
- Juriloff, D.M. (1986) Major genes that cause cleft lip in mice: Progress in the construction of a congenic strain and in linkage mapping. *J. Craniofac. Genet. Dev. Biol.*, Suppl. 2(Suppl.):55-66.
- Juriloff, D.M., and F.C. Fraser (1980) Genetic maternal effects on cleft lip frequency in A/J and CL/Fr mice. *Teratology*, 21:167-175.
- Juriloff, D.M., and M.J. Harris (1985) Thyroxine-induced differential mortality of cleft lip mouse embryos: Dose- and time-response studies of the A/WySn strain. *Teratology*, 31:319-329.
- Kalter, H. (1968) Sporadic congenital malformations of newborn inbred mice. *Teratology*, 1:193-200.
- Kalter, H. (1979) The history of the A family of inbred mice and the biology of its congenital malformations. *Teratology*, 20:213-232.
- Keino, H., and H. Yamamura (1974) Effects of cadmium salt administered to pregnant mice on postnatal development of the offspring. *Teratology*, 10:87 (abst.).
- Kirk, K.M., and A.G. Searle (1988) Phenotypic consequences of chromosome imbalance in the mouse. In: *The Cytogenetics of Mammalian Autosomal Rearrangements*. A. Daniel, ed. Alan R. Liss, Inc., New York, pp. 739-768.
- Kurnit, D.M., W.M. Layton, and S. Matthyse (1987) Genetics, chance, and morphogenesis. *Am. J. Hum. Genet.*, 41:979-995.
- Lacro, R.V., K.L. Jones, and K. Benirschke (1988) Coarctation of the aorta in Turner syndrome: A pathologic study of fetuses with nuchal cystic hygromas, hydrops fetalis, and female genitalia. *Pediatrics*, 85:445-451.
- Layton, W.M., and M.W. Layton (1979) Cadmium induced limb defects in mice: Strain associated differences in sensitivity. *Teratology*, 19:229-236.
- Lyon, M.F., and A.G. Searle (eds.) (1989) *Genetic Variants and Strains of the Laboratory Mouse*. 2nd Ed. Oxford University Press, Oxford.
- Machin, G.A. (1989) Hydrops revisited: Literature review of 1,414 cases published in the 1980s. *Am. J. Med. Genet.*, 34:366-390.

- Mosteller, F., and C. Youtz (1961) Tables of the Freeman-Tukey transformations for the binomial and Poisson distributions. *Biometrika*, 48:433-440.
- Mueller-Heubach, E., and J. Mazer (1983) Sonographically documented disappearance of fetal ascites. *Obstet. Gynecol.*, 61:253-257.
- Nora, J.J., R.J. Sommerville, and F.C. Fraser (1968) Homologies for congenital heart diseases: Murine models, influenced by dextroamphetamine. *Teratology*, 1:413-416.
- Pexieder, T., S. Miyabara, and A. Gropp (1981) Congenital heart disease in experimental (fetal) mouse trisomies: Incidence. In: *Mechanisms of Cardiac Morphogenesis and Teratogenesis*. T. Pexieder, ed. Raven Press, New York, pp. 389-399.
- Platt, L.D., J.V. Collea and D.M. Joseph (1978) Transitory fetal ascites: An ultrasound diagnosis. *Am. J. Obstet. Gynecol.*, 132:906-908.
- Robertson, L., A. Ott, L. Mack and Z.A. Brown (1985) Sonographically documented disappearance of nonimmune hydrops fetalis associated with maternal hypertension. *West. J. Med.*, 143:382-383.
- Russell, E.S., and F.A. Lawson (1959) Selection and inbreeding for longevity of a lethal type. *J. Hered.*, 50:19-25.
- Shapiro, I., and M. Sharf (1985) Spontaneous intrauterine remission of hydrops fetalis in one identical twin: Sonographic diagnosis. *J. Clin. Ultrasound*, 13:427-430.
- Sokal, R.R., and F.J. Rohlf (1981) *Biometry. The Principles and Practice of Statistics in Biological Research*. 2nd Ed. W.H. Freeman and Co., San Francisco.
- Taylor, B.A., H.J. Heiniger and H. Meier (1973) Genetic analysis of resistance of cadmium-induced testicular damage. *Proc. Soc. Exp. Biol. Med.*, 143:629-633.
- van der Putte, S.C.J. (1975) The early development of the lymphatic system in mouse embryos. *Acta Morphol. Neerl.-Scand.*, 13:245-286.
- van der Putte, S.C.J. (1977) Lymphatic malformation in human fetuses. A study of fetuses with Turner's syndrome or Status Bonnevie-Ullrich. *Virchows Arch. A Path. Anat. and Histol.* 376:233-246.
- Vekemans, M., and T. Trasler (1986) Liability to cleft palate in trisomy 19 mouse embryos. *J. Craniofac. Genet. Dev. Biol.*, 2(Suppl):235-240.
- Villaespesa, A.R., M.P.S. Mier, P.L. Ferrer, I.A. Baleriola, and J.I.R. Gonzalez (1990) Nonimmunologic hydrops fetalis: An etiopathogenetic approach through the postmortem study of 59 patients. *Am. J. Med. Genet.*, 35:274-279.
- Whitten, W.K., W.G. Beamer, and A.G. Byskov (1979) The morphology of fetal gonads of spontaneous mouse hermaphrodites. *J. Embryol. Exp. Morphol.*, 52:63-78.
- Witt, D.R., H.E. Hoyme, J. Zonana, D.K. Manchester, J.P. Fryns, J.G. Stevenson, C.J.R. Curry, and J.G. Hall (1987) Lymphedema in Noonan syndrome: Clues to pathogenesis and prenatal diagnosis and review of the literature. *Am. J. Med. Genet.*, 27:841-856.