PRENATAL AND POSTNATAL INFLUENCES ON GROWTH, PROLIFICACY AND MATERNAL PERFORMANCE IN MICE¹

C. W. YOUNG², J. E. LEGATES, AND B. R. FARTHING³

North Carolina State University at Raleigh

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IN mammals the growth rate of the nursing young is influenced considerably by the lactational performance of the mother. The magnitude of this influence in mice has been investigated by BATEMAN (1954) and Cox, LEGATES and COCKERHAM (1959) through the use of cross-fostering experiments. These experiments permitted the variance for a trait to be partitioned so that the importance of prenatal (genetic and uterine) and postnatal maternal (lactational influences) could be estimated.

Among the traits studied was 12-day litter weight, a trait that FALCONER (1947) suggested as a convenient measure of milk production in mice. Results obtained by BATEMAN (1954) and by Cox *et al.* (1959) were quite different for this trait. The former found only 32% of the total variance in "performance" for litters of eight mice to be due to postnatal differences, while the latter estimated that postnatal effects were responsible for 71.5% of the variance in the 12-day weight of litters of six mice. BATEMAN's results lead to questioning the advisability of using weights of the nursing young to measure postnatal maternal performance, since so small a portion of the variance in these weights appeared to be conditioned by postnatal factors. On the other hand, the results of Cox *et al.* (1959) suggest that weights of the nursing young may be a satisfactory phenotypic measure.

This investigation was undertaken to assess the relative importance of prenatal and postnatal influences upon body weight and growth and to examine the genetic and phenotypic relationships between different growth and maternal characteristics. From this information, a further assessment was sought of the usefulness of the 12-day weight of litters standardized to six mice as a selection criterion for postnatal maternal performance. Results from other aspects of the investigations are presented in a companion paper (YOUNG and LEGATES 1965).

EXPERIMENTAL PROCEDURE

The data for this study were obtained from a mouse population formed by reciprocally crossing two F_1 stocks (CAF_1 , $AKD2F_1$) secured from the Jackson Memorial Laboratory at Bar Harbor, Maine. The mice used were from Generations 7, 8, 9, 13, 14, and 15 of a control popu-

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² Present Address: Department of Dairy Husbandry, University of Minnesota, St. Paul.

³ Present Address: Department of Experimental Statistics, Louisiana State University, Baton Rouge.

lation that had been randomly maintained following the cross of the two F_1 's. Computed inbreeding coefficients for the mice averaged just over 0.03 for Generation 7, and increased steadily to just under 0.08 for Generation 15.

The procedures followed were similar to those of Cox *et al.* (1959) in that three dams were placed in each cross-fostering group, and that each dam suckled from birth two of her own young and two young from each of the other two dams in her group. The requirements for a cross-fostering group were that the three litters were to be born within a 12-hour period, and the dams of the litters were not to be full or half sisters. Whenever possible, the sires of the three litters were not related to one another or to either of the three dams.

Three cross-fostering experiments were conducted. For these experiments 49 cross-fostering groups were formed from 147 litters that consisted of six or more young. The young in these litters were counted and the number born recorded. They were then sexed as accurately as possible, and six mice from each litter were identified, and placed in postnatal litters. In selecting mice for the postnatal litters, females were used first and the litters were completed with males chosen at random.

All mice in the cross-fostering groups were weighed individually at birth, 12, 21, 42 and 56 days of age to the nearest 0.1 gram. At 21 days the young were weaned and ear notched for permanent identification. Males and females were caged separately after weaning, four per cage, with postnatal litter mates ordinarily occupying the same cage. The cross-fostered mice were mated as soon as the youngest were at least 56 days of age. Since about two thirds of the mice in each of the experiments were females, it was necessary to mate each male with two females. Matings again were random except that litter mates were not paired. Data taken on the litters born to cross-fostered mice consisted of number born, number at five days, and 12-day weights of standardized litters of six mice.

Adjustment of data: Weights taken at 42 and 56 days of age required adjustment for differences due to sex. This adjustment was made by correcting all weights to a neutral point as suggested by FALCONER and KING (1953). Weights obtained in this study were similar to those given by Cox *et al.* (1959) to 42 days of age. The mean 56-day weights for males and females, which were not taken for the 1959 study, were 26.0 and 21.9 grams respectively.

The only other data requiring adjustment were the 12-day litter weights of the litters born to the cross-fostered females. These litters were not standardized to six until five days after birth, and regression analysis revealed the necessity for adjusting these litter weights for the number of young suckled during the first five days. This adjustment was made by taking into account both the linear and curvilinear regression of 12-day litter weight on number of mice in the litter immediately prior to standardization.

Statistical and analytical treatment: BATEMAN (1954) has given the form of analysis used in previous cross-fostering studies. The analysis given by Cox *et al.* (1959) is identical to the one required for this study, and is presented in Table 1 for a single cross-fostering group. The use of this analysis is based upon the model: $Y_{ijk} = \mu + a_i + p_j + (ap)_{ij} + e_{ijk}$, where Y_{ijk} is an observation on the k th mouse of the j th postnatal and i th prenatal litter, μ is the general mean for the group. The a_i and p_j are the effects of the i th prenatal and j th postnatal dams, respectively, while $(ap)_{ij}$ is the effect of interaction between the prenatal and postnatal effects. The

TABLE 1

Analysis of variance for a single fostering group showing the expectations of mean squares

Source of variation	Degrees of freedom	Expectation of mean square
Total	17	
Prenatal influences	2	$\sigma_e^2 + 2\sigma_{ap}^2 + 6\sigma_a^2$
Postnatal influences	2	$\sigma_e^2 + 2\sigma_{ap}^2 + 6\sigma_p^2$
Prenatal $\times$ Postnatal	4	$\sigma_e^2 + 2\sigma_{ap}^2$
Within full sibs	9	σ_e^2

e_{ijk} , results from random differences among full sibs born and reared alike. Assumptions made for the analysis are that the a_i , p_j , $(ap)_{ij}$ and e_{ijk} are independent random variables, each with mean zero, and with variances σ_a^2 , σ_p^2 , σ_{ap}^2 and σ_e^2 , respectively.

The components of variance of particular interest in this study were σ_a^2 and σ_p^2 . The former component includes variance due to all prenatal factors causing members of a litter to resemble one another to a greater extent than they resemble mice from unrelated litters. These factors are the hereditary differences between full-sib progenies and environmental differences in the uteri within which these full-sib progenies develop. Although little is known concerning the importance of uterine effects upon postnatally expressed traits in mice, BRUMBY (1960) found important uterine effects only for birth weights. In the present investigations, the prenatal effects generally have been attributed to genetic differences between the young for the trait measured.

The postnatal component of variance (σ_p^2) arises from differences in the direct effect of the dams upon the litters they nurse. Although other factors such as temperament and maternal instincts probably have some bearing upon these differences, there is little doubt that differences in lactational performance are the main source of this component. Accordingly, in the present investigations this component has been interpreted primarily as a measure of the influence of lactation on the traits studied.

RESULTS AND DISCUSSION

Prewaning growth of young as a measure of lactation: The initial effort was directed toward determining whether or not the weight of the suckling young provides an adequate measure of the lactational performance of the dam. The measurements needed for this purpose were the weaning and preweaning weights of the young. The analyses for these traits are given in Table 2. The same style

TABLE 2

Analyses of variance for individual weights at birth (X_1), 12 days (X_3), 21 days (X_4), and gain from birth to 21 days (X_9)

Source of variation	d.f.	X_1	X_3	X_4	X_9
Mean squares					
Prenatal	98	0.0551	0.6481	1.7233	1.4340
Postnatal	98*	0.0032	2.8147	7.1153	7.0068
Prenatal $\times$ Postnatal	196†	0.0065	0.1835	0.3844	0.3319
Within	441‡	0.0138	0.1753	0.4996	0.4352
Components of variance					
Prenatal (σ_a^2)	..	0.0086	0.0778	0.2282	0.1862
Postnatal (σ_p^2)	..	—0.0006	0.4483	1.1545	1.1453
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	—0.0036	0.0078	—0.0472	—0.0416
Within (σ_e^2)	..	0.0138	0.1753	0.4996	0.4352
Components of variance as percent of total§					
Prenatal (σ_a^2)	..	38	11	12	11
Postnatal (σ_p^2)	..	0	63	61	65
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	0	1	0	0
Within (σ_e^2)	..	62	25	27	24

* 95 for X_3 , 93 for X_4 and X_9 .

† 187 for X_3 , 182 for X_4 and X_9 .

‡ 415 for X_3 , 405 for X_4 and X_9 .

§ Negative estimates of these components assumed as zero

and numbering of variables have been followed as were used by Cox *et al.* (1959) to simplify comparison.

The results presented in Table 2 indicate that postnatal effects account for over 60% of the total variance in 12-day weight, 21-day (weaning) weight, and gain from birth to 21 days. Prenatal influences were important for birth weight, accounting for 38% of the variance in this trait, but they were responsible for only 11 or 12% of the variance in X_3 , X_4 and X_9 . Interactions between prenatal and postnatal factors were apparently unimportant, as three of the four estimates of this component were negative and the fourth accounted for only 1% of the total variance. Except for birth weight, the unanalyzed portion of the variance accounted for approximately one fourth of the total.

The results presented to this point agree closely with those reported by Cox *et al.* (1959). They indicate that the postnatal maternal performance of the dam is by far the most important factor involved in determining the growth of the young during the suckling period. They further indicate that either the genes of the young or a carryover effect from the uterine environment of the dam, or both, have a less important, but probably real influence upon the growth of the young during this period. In the data of Cox *et al.* (1959), prenatal differences accounted for less variance in all traits than was the case in this study, but the differences in the percentages of the variances represented were all small. These differences probably were due in part to the larger estimates obtained for the interaction variances in the earlier study. The estimates of the interaction component of variance tended to be negative in the present investigation. Although some of the estimates seem unreasonably large to be attributed to sampling, no other explanation can be advanced.

An assessment of the effectiveness of early postnatal growth as a measure of lactation can be made from the results presented in Table 2. Routinely, the mice are not identified at birth; hence, in evaluating the importance of lactational differences among dams the mean 12-day weight of individuals nursed by the dam is the variable that is analyzed, rather than the weights of the individual mice. The phenotypic variance for the mean 12-day weight of litters of six mice raised prenatally and postnatally by the same dam can be expressed as:

$$\sigma_{\bar{X}_3}^2 = \sigma_a^2 + \sigma_p^2 + \sigma_{ap}^2 + \sigma_e^2/6$$

where $\bar{X}_3$ represents the mean 12-day weight of a mouse from a litter of six as contrast to X_3 , which represents the individual mouse weight in the model employed for the fostering studies. Where the dam suckles the young she bears, the only interaction effect is that between the prenatal and postnatal effects of the same dam. In cross-fostering experiments, the interaction variance includes any effects from transferring young from one dam to another, and any effect of different combinations of prenatal and postnatal environments. If it were not for these differences in the expectations of the interaction variances, the phenotypic variance could be obtained directly from the variance components estimated in the cross-fostering experiments.

In order to investigate the importance of these effects, mean squares were com-

TABLE 3

Orthogonal comparisons for individual degrees of freedom from prenatal by postnatal interaction for 12-day weight of 48 cross-nursing groups with no missing observations

Comparison	Prenatal and postnatal combination of dams*									Mean square
	AA	AB	AC	BA	BB	BC	CA	CB	CC	
1	0	-1	+1	+1	0	-1	-1	+1	0	0.1888
2	+1	-1	0	-1	0	+1	0	+1	-1	0.1480
3	+2	-1	-1	-1	+2	-1	-1	-1	+2	0.1508
4	-1	-1	+2	-1	+2	-1	+2	-1	-1	0.1336

* The first letter indicates the prenatal dam and the second the postnatal dam.

puted for the single degrees of freedom comprising the interaction variance. The data used for this purpose were those from 48 cross-fostering groups where all mice were alive when 12-day weights were taken. The orthogonal comparisons used and the mean squares obtained are presented in Table 3.

The mean squares in Table 3 are all representative of 48 degrees of freedom, and each has an expectation, $\sigma_e^2 + 2\sigma_{ap}^2$. In this table the first comparison is a contrast of the combination of prenatal A with postnatal B *vs.* prenatal B with postnatal A. The second comparison represents dam A plus the combining effect of B with C versus dam C plus the combining effect of A with B. Comparison 3, between the fostered and the nonfostered mice is of major interest. If transferring young to a different dam at birth, or a foreign milk source is important, a large mean square for this comparison would be expected. The fourth comparison to complete this orthogonal set is also complex. It is a comparison of B and combinations of A and C versus A, C, and all combinations involving B. Like comparison 2, it is difficult to interpret.

It can be seen from comparison 3 that there is no suggestion of an influence due to an antagonism between the young and their foster mother or her milk. Hence, it was assumed that the contribution due to such potential interactions is the same regardless of whether or not the young are cross-fostered. With this assumption the phenotypic variance ($\sigma_{\bar{X}_3}^2$) can be estimated directly from the components of variance given in Table 2. The estimate of $\sigma_{\bar{X}_3}^2$ for 12-day weight is: $\sigma_{\bar{X}_3}^2 = 0.0778 + 0.4483 + 0.0078 + 0.1753/6 = 0.5631$. The postnatal component of variance σ_p^2 accounts for 79.6% of the total variance of $\bar{X}_3$.

As almost 80% of the variation in the 12-day weights of litters of six young is due to differences in the postnatal maternal influence of the dams nursing the young, it is concluded that this trait is a satisfactory phenotypic measure of postnatal maternal effects. Likewise, from results of other analyses given in Table 2 it is apparent that either weaning weight or gain from birth to weaning would serve this purpose equally well for this population.

Postweaning weights and gains. The lactational performance of the dam would be expected to have considerably less influence on weights and gains taken subsequent to weaning than on those taken prior to or at the time of the physical

TABLE 4

Analysis of variance for weights at 42 days (X_5), 56 days (X_6) and gains from 21 to 42 days (X_{10}), and from 42 to 56 days (X_{11})

Source of variation	d.f.	X_5	X_6	X_{10}	X_{11}
Mean squares					
Prenatal	98	5.8349	6.7947	2.9970	1.4871
Postnatal	90	6.9533	6.5579	2.0638	1.3071
Prenatal $\times$ Postnatal	171	2.1282	2.6314	1.5791	1.0463
Within	357	2.3940	2.8977	1.6603	0.8454
Components of variance					
Prenatal (σ_a^2)	..	0.6955	0.7885	0.2709	0.0826
Postnatal (σ_p^2)	..	0.8657	0.7024	0.0834	0.0449
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	—0.1133	—0.1155	—0.0372	0.1120
Within (σ_e^2)	..	2.3940	2.8977	1.6603	0.8454
Components of variance as percent of total*					
Prenatal (σ_a^2)	..	18	18	13	8
Postnatal (σ_p^2)	..	22	16	4	4
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	0	0	0	10
Within (σ_e^2)	..	60	66	82	78

* Negative estimates of these components assumed as zero.

separation of the dam and her young. However, since later weights are not independent of weaning weight, and since weaning weight is largely a function of the lactational performance of the dam, later weights are expected to be influenced to some degree by differences in postnatal maternal performance. In the case of postweaning gains, there is no direct dependency on weaning weight; hence, postweaning gains should be less affected by the postnatal maternal performance of the dam. However, it is likely that the postnatal maternal influence of the dam might be carried over to some extent and affect gains made during the postweaning period.

Results from analyses of postweaning weights and gains are given in Table 4. Postnatal effects accounted for 22% and 16% of the total variance in 42 and 56-day weights, respectively, while prenatal effects accounted for 18% of the variance in both instances. These results suggest that the postnatal influence of the dam has an important impact on the weights of the young mice until they near their mature size. Even at 56 days of age, the postnatal maternal influence of the dam was nearly as important a source of variability in the weights of the young as were the genetic and possible uterine effects (prenatal) and the genetic effects common to full sibs.

In the case of postweaning gains, only 4% of the total variance was attributable to postnatal effects. This percentage was the same for gain from 21 to 42 days as for gain from 42 to 56 days. It seems logical that the postnatal maternal influence of the dam would have had more effect upon gains made immediately subsequent to weaning.

If a negative genetic correlation exists between lactational performance and

fat deposition as has been suggested by DICKERSON (1947), and if the 42 to 56-day gain is largely a matter of fat deposition, as can be speculated on the basis of work reported by FOWLER (1958), then the postnatal variability in 42 to 56-day gain is not unexpected. In this event, the dams having genotypes for high milk production would tend to be genetically below average for the ability to deposit fat. Their offspring would tend to have poor gains from 42 to 56 days owing to their receiving a poorer than average gene complement for fat deposition. On the contrary, mice having heavier weaning weights owing to having been nursed by dams with superior lactational performance might be expected to gain less after weaning than would young mice that received less milk during the suckling period. In either case these influences on the 42 to 56-day gains would be reflected as a postnatal component in the analysis of variance.

The percentage of total variation attributed to prenatal influences was less for postweaning gains than for postweaning weights. In this study only 13% of the total variance in gain from 21 to 42 days was found to be due to prenatal effects. This percentage declined to 8% for gain from 42 to 56 days. This reduction may have been due in part to the emergence of a rather large interaction component, accounting for 10% of the total variance in gain from 42 to 56 days.

Cox *et al.* (1959) did not analyze the gain from 21 to 41 days, but the data from their study were analyzed in conjunction with the work reported here. The agreement between the two studies was less precise for postweaning traits than it was for preweaning traits and weaning weight. Twenty-one percent of the total variance in gain from 21 to 42 days in their data was accounted for by prenatal effects and 10% was attributed to postnatal factors. When the two analyses were pooled, prenatal and postnatal effects accounted for 16 and 6%, respectively, of the total variance in gain from 21 to 42 days.

Prolificacy and lactational performance: The present investigation differed from earlier ones in that the mice in each fostering group were mated and the females allowed to raise their own litters. The number of mice born to the cross-fostered females, and the 12-day litter weights of their standardized litters were analyzed in the same manner as were the other traits. In this way it was possible to detect any direct effect of the postnatal maternal influence of the dam of a cross-fostered female on the lactational performance and prolificacy of that cross-fostered female. Any direct effect would show up as a contribution to the postnatal component of variance. It was also possible to detect any transmitted effect for prolificacy and lactational performance, since genes for these traits would cause variation that would contribute to the prenatal component of variance. Since only cells in the two-way table represented by cross-fostered females were observable for these traits, the degrees of freedom for the analyses were substantially reduced. Nevertheless, approximately 100 degrees of freedom remained for each source of variation in the analysis of number born and 12-day litter weight. These results of the analyses are given in Table 5.

The postnatal component of variance for number born was a small negative value and it was assumed to be zero. The comparable component for 12-day litter weight accounted for only 1% of the total variance in this trait. These results in-

TABLE 5

Analyses of variance for number born (X_7) and 12 day litter weight (X_8)

Source of variation	d.f.	X_7	X_8
Mean squares			
Prenatal	96*	6.2708	23.7137
Postnatal	89	3.9663	14.5166
Prenatal $\times$ Postnatal	100†	4.0700	11.6049
Within	125‡	4.2880	15.1966
Components of variance			
Prenatal (σ_a^2)	..	0.7017	3.8335
Postnatal (σ_p^2)	..	— .1518	0.1760
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	— .0470	— 1.8116
Within (σ_e^2)	..	4.2880	15.1966
Components of variance as percent of total§			
Prenatal (σ_a^2)	..	14	20
Postnatal (σ_p^2)	..	0	1
Prenatal $\times$ Postnatal (σ_{ap}^2)	..	0	0
Within (σ_e^2)	..	86	79

* 95 for X_8 .† 91 for X_8 .‡ 111 for X_8 .

§ Negative estimates of these components assumed as zero.

dicate that the lactational performance of the dam has little direct effect upon the prolificacy or lactational performance of the young she nurses.

The prenatal components of variance given in Table 5 accounted for 14% of the total variance in number born, and for 20% of the total variance in 12-day litter weight. These results indicate that for both traits the transmission of genes from parent to offspring is the major cause of analyzed variation in this study. An estimate of heritability based on full sister relationships can be obtained by multiplying the percentages due to prenatal effects by two. This gives estimates for the heritability of number born of 0.28 and for the heritability of 12-day litter weight (a measure of lactational performance) of 0.40. These estimates are somewhat higher than most estimates for number born and lactational performance, and they may reflect either an important contribution from non-additive genetic effects, or a positive increment from sampling.

Two investigations with these stocks have suggested that preweaning growth of young mice is largely a function of the lactational performance of the dam, and that the genes of the young mice appear to have a relatively minor influence on their growth prior to weaning. These results are in contrast to those of BATEMAN (1954) and BRUMBY (1960) who reported that prenatal influences were more or as important as postnatal influences as sources of variation in 12-day weights or performance. BATEMAN's material should be reasonably comparable to the stocks used in the current investigations, but the paucity of data from his fostering studies might well account for the disparity in results. BRUMBY's stocks involved lines genetically differentiated for size as a result of long time selection.

Correlated effects on maternal performance were identified as an apparent result of selection for size. Hence, the relative importance of prenatal and postnatal effects might not be expected to be similar to those in unselected stocks. OKSH and SUTHERLAND (1964) have reported results that corroborate the findings of the present investigation.

SUMMARY

The magnitude of prenatal and postnatal influences on growth, prolificacy, and lactational performance were investigated by a cross-fostering studies involving 1,326 mice from an unselected population arising from a cross of four inbred lines. Postnatal influences accounted for approximately 80% of the variance in the 12-day weight of litters of six mice. Twelve-day weight, weaning weight, or gain from birth to weaning should provide satisfactory measures of the dam's lactational performance. Postnatal influences were more important than were prenatal influences in determining weight at 42 days, and were nearly as important as prenatal influences in determining 56-day weight. Postweaning gains were affected more by prenatal than by postnatal influences, but some effect from the latter was found. Direct postnatal maternal effects of the dam on the number of young born to her daughters or their nursing ability could not be detected. In contrast, substantial genetic effects for both the number of young born and the 12-day litter weight were indicated.

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