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Genetic Influences on Mouse Growth at 23 and 32°C

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Abstract

Two inbred strains of mice and the reciprocal F_1 hybrids between them were reared in a temperate or in a hot humid environment. There was an age-related environmental effect on the rate of increase in body weight. Between 2 and 5 weeks the rate of weight gain was more rapid in the heat but at all other ages weight gain was more rapid in the temperate conditions. Periods of rapid growth were associated with low intra-strain variation in body weight.

The extent of the environmental effect varied primarily with maternal genotype, which was in part related to litter size effects, and secondarily with the genotype of the offspring. Hybrid luxuriance was greater at the lower temperature, being most apparent when inbreds had to compete directly with hybrids through being fostered together.

Weight at weaning was at least as great in the hot as in the control environment but adult weight was reduced in the heat. Post-weaning compensatory growth was greater in the cooler conditions, and in females than in males.

It is suggested that the rapid growth and lower weight variation of immature animals in the heat is related to temperature stress, there being problems of heat conservation in the temperate conditions, and that the lower weights of adults in the heat may also be related to temperature stress. In this case the problem is one of heat dissipation.

Introduction

Temperature variation, like many other environmental factors, has been shown to have a profound effect upon the patterns of mammalian growth and a number of experimental investigations have been made with rats and mice to analyse these effects (Sumner 1915; Sundstroem 1922; Ogle 1934; Ashoub *et al.* 1958; Nay 1961; Knudsen 1962; Harrison 1963; Pennycuik 1966; Reading 1966). Genetic factors also influence growth and an attempt has been made to analyse the genetic nature of quantitative variation in the adult size of the mouse (Grüneberg 1952). However, little attention has been given to the nature of the interaction between hereditary variation and systematic differences in environmental temperature. Barnett and his colleagues (Barnett and Manly 1959; Barnett and Neil 1972) have analysed the effects of strain differences in the growth response of mice in cold conditions and Harrison *et al.* (1959) have considered the effects of high temperatures on the post-weaning growth of inbred and hybrid animals. The extent of the influence of genotype on growth in a high environmental temperature during the early post-natal period does not appear to have been investigated. This paper is concerned with examining the nature of genetically determined differences in the pre-weaning growth response of mice to an elevated temperature and with investigating the long-term effects of such exposure on post-weaning growth and adult size.

Methods

Mice have been maintained under two régimes which differed only in their temperature and relative humidity. The environmental conditions have been designated 'hot' and 'control'. The hot room had a dry bulb temperature of 32·2±0·5°C and a wet bulb temperature of 26·7±0·5°C, while the temperatures of the control room were 22·8±1·6°C dry bulb and 17·8±1·0°C wet bulb. Lighting in both environments followed a 12-h dark/12-h light schedule.

Two inbred strains, C57BL and BALB/c, were reared and maintained by brother–sister matings. Reciprocal interstrain matings, referred to as F_1 matings with the maternal genotype indicated in a bracket, were established. 'F_1 (C57BL♀) offspring' is used to refer to the first generation hybrid offspring of a C57BL female and a BALB/c male.

Litters remained with their parents in breeding cages until they were 3 weeks old. Thereafter male and female offspring were separated and maintained in stock boxes which usually contained no more than 10 animals. The body weight of each animal was recorded to the nearest 0·5 g at birth, at weekly intervals for 6 weeks and then at 8 and 12 weeks of age. 'Birth weight' is the weight of an animal born within any 24-h period.

A fostering procedure was adopted for a number of litters in the control environment. Pairs of BALB/c and F_1 (BALB/c♀) litters, born on the same day, were divided into two groups as evenly as possible with respect to their birth weights and numbers of animals. The compounded inbred–hybrid litters, all with BALB/c mothers, remained together until weaning age. Individual body weights were recorded at 1, 2 and 3 weeks of age.

Results

The mean weight of male litter mates was calculated for each litter. From these means a mean weight for all males within a mating type was derived. Data for females were treated similarly. The growth curve characteristics for males and females were similar within the control environment, and also in the heat. The mean body weights of males from litters of all mating types in both environments are shown in Table 1. The rate of gain in body weight from birth to weaning was

Table 1. Mean body weights, with standard errors, of males in the control and hot environments

Breed	Control environment				Hot environment			
	Birth	3 weeks	6 weeks	12 weeks	Birth	3 weeks	6 weeks	12 weeks
C57BL inbred								
No. of animals	129	55	55	39	19	3	3	3
Mean body wt	1·3	7·1	15·0	22·3	1·3	7·3	14·4	18·3
Standard error	0·01	0·19	0·33	0·30	0·04	0·60	1·34	2·25
BALB/c inbred								
No. of animals	104	58	57	54	80	15	14	10
Mean body wt	1·4	8·8	17·6	22·7	1·3	8·1	16·4	20·9
Standard error	0·02	0·19	0·28	0·28	0·02	0·46	0·56	0·73
F_1 (C57BL♀)								
No. of animals	47	25	25	29	67	24	22	24
Mean body wt	1·4	7·4	18·3	25·9	1·4	8·1	17·2	24·2
Standard error	0·03	0·29	0·54	0·38	0·03	0·47	0·46	0·41
F_1 (BALB/c♀)								
No. of animals	49	34	35	36	27	20	20	18
Mean body wt	1·5	9·0	20·5	27·1	1·4	8·7	17·3	23·2
Standard error	0·02	0·25	0·35	0·36	0·04	0·32	0·54	0·49

regular but then increased for the next 2–3 weeks. This was followed by a gradual decline in growth rate. Males became progressively heavier than females from birth, although statistically significant differences in mean body weights were not present until after weaning, occurring first in the control environment at 4 weeks of age, and a week later in the heat.

There was a clear strain difference in mean body weights among both males and females of the two inbred lines in the control situation. The absolute weight difference was maximal at 5 weeks ($P < 0{\cdot}001$ in males and females) but then decreased, more rapidly in males than in females. By 12 weeks the mean body weights of males of the two strains were indistinguishable, though the difference between the females was still significant at the 0·1% probability level.

The differences between the mean body weights of animals of each strain when reared in the hot, as opposed to the control, environment are plotted against age in Fig. 1. The mean weights of litters of both mating types showed little effect of environmental temperature. This small effect varied according to age and genotype

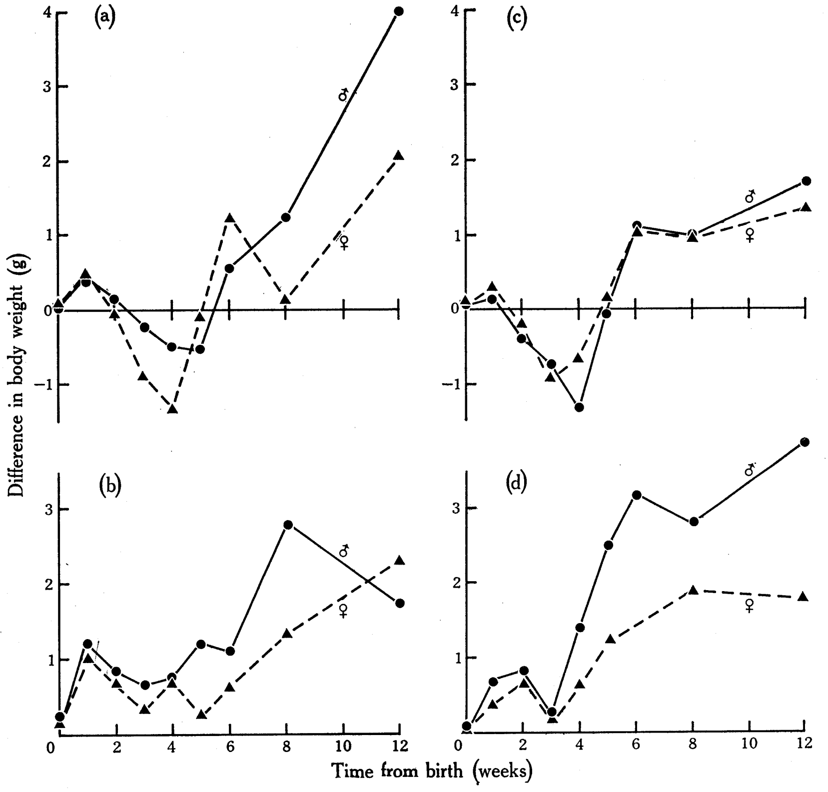

Fig. 1. Environmental differences in the mean body weights of mice (mean in control minus mean in hot environment). (*a*) C57BL inbreds. (*b*) BALB/c inbreds. (*c*) F_1 (C57BL♀) hybrids. (*d*) F_1 (BALB/c♀) hybrids.

of the animals. At each age the weight differences of F_1 (C57BL♀) offspring were similar in extent and timing to those of inbred C57BL litters, and the weight responses of the F_1 litters of BALB/c mothers were very like those of the inbred litters of mothers of this strain.

During the period of lactation and in the immediate post-weaning period there was no evidence for greater growth in hybrids than in inbreds. Later, the weight increase of hybrids was greater than that of inbreds, the difference being apparent earlier in the control than in the hot environment and in hybrids with a BALB/c mother than in those with a C57BL mother.

The effects of environmental and genetic differences and the interaction between them, on developmental lability, as expressed in variability in body weight, was examined. Within-litter variation in mean body weights during the pre-weaning period tended to be greater in BALB/c than in C57BL inbreds in both environments and in F_1 (BALB/c♀) than in F_1 (C57BL♀) offspring in the heat. There was not, however, any evidence for significant, systematic differences in within-litter variation between inbreds and hybrids in either environment. In addition to these genotypic effects there was also an environmental effect. Among litters with BALB/c mothers variation was higher among those reared in the heat than among controls and the effect was more marked among inbred than among hybrid animals.

During the pre-weaning period variation in weight among litters of the same genotype tended to be low during periods of rapid growth. Thus mean body weights of litters of BALB/c mothers were, at all ages, less variable when the litters were reared in the control environment than when reared in the heat. Conversely, between 1 and 3 weeks of age the mean body weights of litters of C57BL mothers were less variable when the animals had been reared in the heat than when they had been reared in the control environment.

A linear regression analysis was undertaken to determine the extent to which the aforementioned effects of genotype and environmental temperature on body weight were due to differences in the sizes of litters. For this purpose the mean weights of litters at birth and at 3 weeks of age were regressed on the sizes of litters at these two times (see Fig. 2). The determinants of litter size have been analysed elsewhere (Garrard *et al.* 1974). Because of the difficulty of breeding C57BL in the heat the data from this strain are inadequate for a regression analysis. Both at birth and at 3 weeks, body weight was inversely related to litter size in the control and hot environments. For most of these regressions the coefficients are statistically highly significant. The relationship was similar in all litters with BALB/c mothers, although at any particular litter size animals reared in the control environment were heavier than animals of the same genotype that had been reared in the heat ($P < 0{\cdot}001$). A similar situation prevailed among F_1 (C57BL♀) offspring at weaning though not at birth, for then animals in small litters were heavier in the heat but animals in large litters were heavier in the control conditions.

The regression analysis also indicated that among animals in the hot environment the difference in the mean body weights of inbred and F_1 hybrid litters that have had a similar maternal influence was greater than among those in the control. This difference was greatest in small litters but was evident in larger ones too and was more marked at weaning than at birth.

Partial regression analyses indicated that in both environments mean weight at birth is the chief determinant of the mean weight of a litter at weaning. Only in the inbred and hybrid litters of C57BL mothers in the control conditions was there a secondary, inverse effect of the size of a litter at birth on its weight at weaning.

The effect of body weight at weaning on the mature (12-week-old) animals was ascertained. In the litters of all mating types in both environments weights at this age were positively and significantly related. Thus litters with the heaviest animals at weaning also had the heaviest adults.

Among females there was also a distinct environmental influence on the relationship between body weight at weaning and the weight increment during the next 9 weeks. The variables were inversely related in the control environment ($P < 0{\cdot}001$ for each regression) but not significantly associated in the heat, indicating that early weight differences tended to decrease in mature females under control conditions but that differences in the body weights of immature animals persisted in the heat.

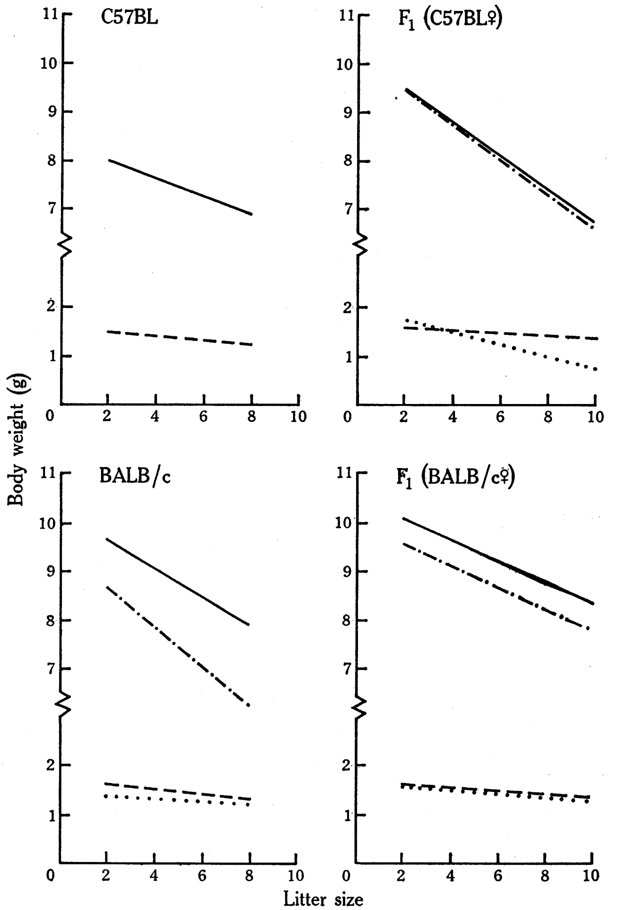

Fig. 2. Regressions of mean birth weights and of mean weaning weights on sizes of litters at birth. - - - - - Birth weight, control environment. · · · · · · Birth weight, hot environment. ——— Weaning weight, control environment. -·-·-·- Weaning weight, hot environment.

Among male BALB/c and F_1 (C57BL♀) offspring in both environments the post-weaning increment in body weight was independent of weight at weaning, so there was no change in the relative weights of litters during this period. In contrast, in F_1 (BALB/c♀) male offspring these variables were positively related to each other. Thus those litters which had relatively heavy animals at weaning became progressively heavier than other litters between 3 and 12 weeks of age.

To ascertain the effect upon body weight of direct competition between inbred and hybrid animals, composite litters of inbred and hybrid offspring were formed. The weights of animals in these mixed BALB/c and F_1 (BALB/c♀) litters at birth (before they were subdivided) and at 1, 2 and 3 weeks of age (after subdivision) are presented as the means of litter means, for each genotype and disregarding sex, in

Table 2. The table also includes the mean weights of BALB/c and F_1 (BALB/c♀) litters which were not fostered. Since there were no statistically significant differences between the birth weights of any of the groups, the fostered BALB/c offspring can be regarded as typical of that strain and similarly the hybrids as typical F_1 (BALB/c♀) offspring. It appears that fostering reduced the rate of growth, for by 2 weeks in the case of the inbred, and a week later in the case of the hybrid, fostered animals were significantly less heavy than non-fostered ones of the same genotype. Hybrids in mixed litters grew more rapidly than inbreds. This was tested more rigorously by estimating a mean within-litter difference which was weighted according to the size of the litter. Using this estimate we found that the difference in the body weights of inbreds and hybrids at birth was still small and not significant, but at each of the three following weekly intervals hybrids were heavier than the inbreds with which they had been reared ($P < 0{\cdot}001$).

Table 2. Weights of fostered and non-fostered litters in the control environment

Number of litters of fostered mice before subdivision and allocation to foster litters are given. N, non-fostered; F, fostered

	BALB/c litters		F_1 (BALB/c♀) litters			BALB/c litters		F_1 (BALB/c♀) litters	
Age	N	F	N	F	Age	N	F	N	F
Birth					2 weeks				
No. of litters	104	14	49	14	No. of litters	63	27	40	27
Mean body wt	1·40	1·37	1·45	1·40	Mean body wt	7·01	5·17	6·65	6·16
Standard error	0·017	0·040	0·020	0·032	Standard error	0·167	0·222	0·217	0·212
1 week					3 weeks				
No. of litters	91	25	43	25	No. of litters	63	27	36	27
Mean body wt	4·25	3·65	4·22	4·36	Mean body wt	8·71	6·71	8·83	8·18
Standard error	0·250	0·193	0·115	0·177	Standard error	0·174	0·248	0·246	0·175

Discussion

A high environmental temperature, in conjunction with high relative humidity, altered the growth pattern of the mouse. Although in the conditions created the magnitude of the environmental effect was influenced by genotype, the same pattern of alteration was evident in all strains. Apart from the transitory phase of rapid growth around weaning age in the heat, growth in the temperate conditions was the greater (see also Biggers *et al.* 1958; Harrison *et al.* 1959; Knudsen 1962; Pennycuik 1971).

Two factors contributing to the observed differences in the growth in weight of mice in the experimental environments are differences in the ability of immature animals to maintain body temperature in hot and in temperate conditions, and the availability of food, especially as provided by the mother. It has been demonstrated by Barnett and Manly (1959) and by Pennycuik (1966) that body weight at weaning is usually reduced under conditions of temperature stress. That weaning weight in the hot environment in this study was either similar to that of controls, or was actually increased in some genotypes, suggests that the total stress exerted by the hot environment, up to this age, was less than that due to exposure to the control conditions. The lower variability in the body weights of 2–5-week-old mice in the hot compared with the control conditions also indicates that the former is the less stressful environment (Harrison 1963). The period of relatively rapid growth in the heat probably coincides with the time when problems of heat conservation occur in mice reared under temperate conditions, a problem recognized in the rearing of rats (Filippova 1956; Hahn *et al.* 1956; Vacek *et al.* 1961).

From the weights of reciprocal hybrids at birth it is apparent that there is no differential maternal effect at this age. However, maternal factors exerted a strong influence on growth immediately after birth, irrespective of the environmental temperature, reciprocal cross differences probably being due to strain-specific differences in lactation and other aspects of maternal care.

The comparative growth of inbreds and hybrids confirms that under standard environmental conditions hybrid growth is the more luxuriant. The genotypic effect was more marked when allowance had been made for differences in the sizes of litters, but was greatest when hybrids and inbreds competed directly with each other and had the same numerical mother to offspring ratio within mixed foster litters. Hybrid luxuriance was detected in the pre-weaning period when animals were still dependent on maternal performance, as well as in the post-weaning period in the control environment, but not under the high temperature conditions, where inbred maternal genotype appeared to be the prime determinant of pre-weaning growth and a limiting factor in the expression of the genotype of the offspring.

The extent of the maternal limitation was also apparent from the total weights of litters, mothers in the heat supporting a much smaller mass of offspring than those in the control environment. This reduction in the mass of animals supported was equally apparent in inbred mothers of both strains. It is possible that the greater weights of individual animals of C57BL, but not BALB/c, mothers in the heat may be a function of the much greater reduction in the size of litters of mothers of the former strain than in those of the latter. If this is so then the largest environmental effect on body weight is mediated through those factors that are associated with the reduction in the sizes of litters in the heat.

It was especially in later growth, after approximately 5 weeks of age, that the greatest disruption, both in terms of increased variation in weight and in the smaller magnitude of weight increments, occurred in the heat. Heat exposure also influenced the amount of compensatory growth that occurred between weaning and maturity. There were differences in the extent to which the mature weights of the inbred lines were altered by the environment, though, as reported by Mather (1953), McLaren and Michie (1954, 1956) and Biggers and Claringbold (1954), the growth of both lines is more environmentally labile than that of hybrid animals.

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