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Effects of Genotype and Nutrition before Mating on the Reproductive Performance of Mice

Manika Wodzicka-Tomaszewska,[A] *S. K. Stephenson*[B] *and T. G. Truscott*[A]

[A]Department of Physiology, University of New England, Armidale, N.S.W. 2351.
[B]Department of Agricultural Biology, University of New England, Armidale, N.S.W. 2351.

Abstract

The effects of genotype and nutritional restriction before mating were studied in three lines of mice designated high (HL), control (CL) and low (LL). The HL and LL lines had been selected for differential body size and limb length relationships. This selection resulted in HL mice being larger and having relatively longer limb bones than the randomly bred control line, while the converse was true of LL mice.

All experimental mice in the LL group and approximately half the experimental mice from the HL and CL groups were fed *ad libitum* while the mice in the other halves of the HL and CL groups were placed on restricted intake for 18 days before mating. The intake was adjusted so that at the beginning of the mating period the HL mice on restricted intake (HLR) were of approximately the same weight as the CL mice on *ad libitum* intake (CLA), and the restricted control line (CLR) mice were of approximately the same weight as the LL mice on *ad libitum* intake (LLA).

Litter size was related to genotype, HL mice having the largest litters, CL litters being intermediate in size and the smallest litters being produced by LL mice. Restriction of intake prior to mating did not affect litter size. There were more LL mice which did not litter and losses between mating and littering were greater in LL mice than in HL and CL mice.

Restricting the feed intake increased the incidence of barrenness, mainly because of failure of many of the mice to copulate. Copulation and littering occurred later in groups on restricted intake; in fact, copulation did not take place until these mice regained the weight they lost during the period on restricted intake.

Introduction

Many workers (e.g. Mulinos *et al.* 1939; McClure 1963, 1966, 1967*a*, 1967*b*) have studied the effects of short-term (up to 48 h) starvation in rodents. Work on longer term undernutrition in mice was carried out by Lamond and Bindon (1969). These authors kept growing mice for periods of up to 7 weeks on planes of nutrition which maintained body weights without allowing growth. The mice on the higher nutritional plane that allowed growth had higher ovulation rates when treated with human chorionic gonadotrophin (HCG). However, when mice on restricted intake were subsequently fed *ad libitum* they produced more ova with HCG treatment than mice on the higher intakes.

In many experiments selection for larger body size or heavier liveweight in mice increased litter size whereas selection against these parameters decreased it (e.g. MacArthur 1949; Falconer 1953; Fowler and Edwards 1960; Rhanefeld *et al.* 1966; Elliott *et al.* 1968). In the work of Fowler and Edwards (1960), however, the percentage of sterile mated pairs was greater in both the high and the low body weight selection lines derived from one strain, but unaltered in similar selection lines derived from another strain. Sterility in the high body weight selection line was

due to the low libido of the males and not to female infertility, but sterility in the low body weight line appeared to result from the hypofunctioning of the anterior pituitary in some females. On the other hand, Bradford (1971) reported no increase in litter size when a high body weight line of mice was produced by selection for rapid post-weaning gain. It is evident, therefore, that the fertility response to differential selection for body size or liveweight in mice can vary depending on the strain being subjected to selection and possibly on the selection criteria, and that different physiological mechanisms may be involved in the responses.

The experiments described in this paper were designed to compare the effects of genotype and longer term nutritional restriction before mating on reproductive performance in three different lines of mice. The selection criteria used in the breeding of these three lines have been described previously (Dawson *et al.* 1972) and were not directly aimed towards producing differences in size or weight, but rather differences in body conformation and body composition. Nevertheless, mice from the high line (HL) were the heaviest, those from the low line (LL) the lightest, whereas the randomly bred controls (CL) were intermediate in weight. Although the reproductive performance of these lines had not been quantitatively characterized, LL mice were obviously the least fertile and only 12 animals were available for this study. Hence, there was no group from the low line on restricted intake.

Materials and Methods

Experimental Animals

The three lines of mice used in the experiment were those described by Dawson *et al.* (1972). Selection had been made using the index

$$I = 5{\cdot}07R + 0{\cdot}37T - \sqrt[3]{B},$$

where R = radius–ulna length, T = tibia–fibula length and B = body weight. The high line was selected for a high index number, the low line for a low index number and mating was random for the control line.

At the time of this experiment there had been 17 generations of selection. The HL mice had a heavier liveweight than the CL mice and these in turn were heavier than the LL mice. HL mice had relatively long legs in relation to liveweight and a greater proportion of water, protein and ash in the body, while the LL mice had relatively short legs in relation to liveweight and a greater proportion of fat in the body. The CL mice were intermediate between the two selection lines in all these traits (Dawson *et al.* 1972).

The male and female mice used were individually identified. They were approximately 16 weeks of age and had not been mated previously. They were housed in the mouse colony at the University at a temperature of approximately 22°C, with uncontrolled humidity and a light to dark ratio of 14 : 10. The mice were fed a standard pelleted ration (Drug Houses of Australia Ltd.) with a minimum crude protein content of 20% and calcium, phosphorus and vitamin A, D and E supplements.

Experimental Design

The HL and CL lines of female mice were each divided randomly into two approximately equal groups. This resulted in the following five experimental groups:

HLA females—high line on *ad libitum* intake throughout the experiment (20 mice);
CLA females—control line on *ad libitum* intake throughout the experiment (24 mice);
LLA females—low line on *ad libitum* intake throughout the experiment (12 mice);
HLR females—high line on restricted intake for the first 18 days then on *ad libitum* intake (23 mice);
CLR females—control line on restricted intake for the first 18 days then on *ad libitum* intake (26 mice).

The liveweight of mice on restricted intake was controlled by weighing each mouse every second day and adjusting her ration accordingly, so that at the beginning of the mating period HLR mice weighed about the same as CLA mice, and CLR mice were approximately the same weight as LLA mice.

Management

During the 18 days of restricted intake, HLR and CLR mice were housed singly in small cages whilst the mice on *ad libitum* intake were kept in bigger cages (five to six per cage). During the mating period three female mice, all from the same group, were kept with one CL male for 5 days. After the males were removed each female mouse was placed in a single cage, where she remained until the completion of the experiment.

Measurements Made

Mice were weighed about an hour after feeding of the mice on restricted intake, to minimize differences in gut fill. Whilst mice on restricted intake were weighed every second day, all mice were weighed on days 3, 7, 9, 11, 18 (immediately prior to joining with males) and 23 (immediately after the males were removed).

During the mating period the mice were examined twice daily (at 9 a.m. and 7 p.m.) for the presence of copulation plugs and weighed when a plug was discovered. The number and weight of young born to each mouse and the number of barren mice were also recorded.

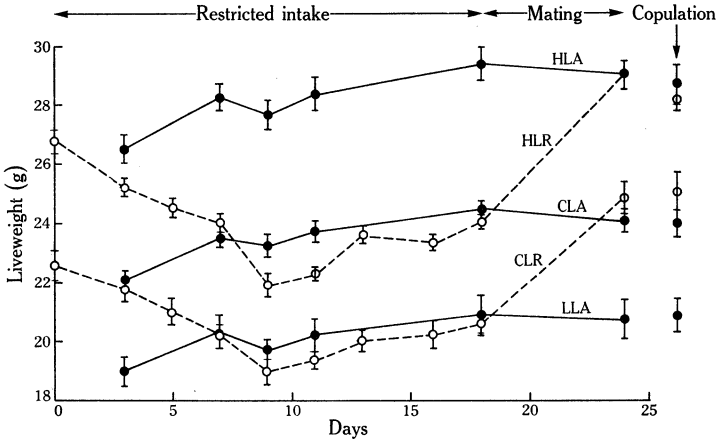

Fig. 1. Mean liveweights of the various experimental groups during nutritional treatment and mating and at copulation.

Results

Liveweights

Fig. 1 shows the mean liveweight of each group during the experiment. A rapid loss in weight occurred in the two groups on restricted intake (HLR and CLR) until the ninth day. From the seventh until the eighteenth day the mean weight of HLR approximated that of CLA mice, whilst that of CLR approximated that of LLA mice. HLR mice lost a mean of 5 g and CLR a mean of 3 g during this period. During the mating period the two restricted groups (HLR and CLR) gained weight rapidly, so that at the end of the mating period their weights were no longer different from the corresponding selection line on *ad libitum* intake throughout (HLA and CLA respectively).

At copulation there were no significant weight differences within each genetic line between the restricted mice and those on *ad libitum* feeding (HL: $F_{1,24}=0{\cdot}37$; CL: $F_{1,29}=2{\cdot}02$).

Distribution of Time of Copulation and Time of Littering

Fig. 2 shows the distribution of copulation over the 5-day mating period and the distribution of littering over a 6-day period. The two groups on restricted intake (HLR and CLR) copulated and littered significantly later than the three groups on *ad libitum* intake (copulation: $F_{1,61}=35\cdot97$, $P<0\cdot001$; littering: $F_{1,63}=39\cdot13$, $P<0\cdot001$).

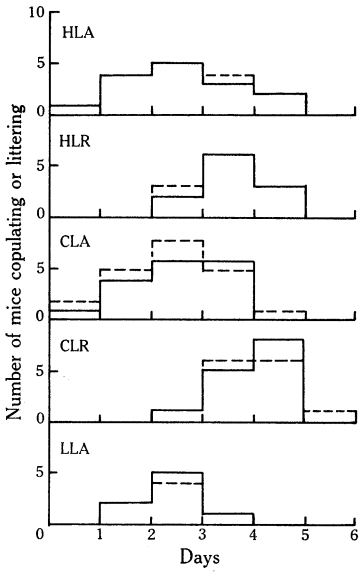

Fig. 2. Frequency distribution of numbers of mice copulating (——) and littering (– – –) over the 5-day mating period and the 6-day littering period.

Litter Size and Barrenness

The mean litter size and the total number of barren mice in each group was as follows:

Group	HLA	CLA	LLA	HLR	CLR
No. of mice per group	20	24	12	23	26
Mean No. of young per litter	10·0	8·4	6·4	9·8	8·8
Standard error	0·59	0·48	0·43	0·51	0·50
No. of barren mice per group	4	3	5	11	13

Litter size was greatest in the line with the heaviest genotype (HL), intermediate in the line of intermediate weight (CL) and smallest in the LL line. The differences between the lines were significant (HLA > CLA: $F_{1,64}=5\cdot54$, $P<0\cdot05$; CLA > LLA: $F_{1,64}=5\cdot19$, $P<0\cdot05$). Litter size was not affected by nutritional treatment prior to mating (HLA + CLA *v.* HLR + CLR: $F_{1,64}=0\cdot11$). However, it must be remembered that, on average, the mice on restricted intake did not copulate until they had regained the lost weight (see Fig. 1). The groups on restricted intake had a higher proportion of barren mice and a χ^2 test on the proportion of barren to fertile mice on restricted and *ad libitum* intake (HLR + CLR *v.* HLA + CLA) showed this difference to be significant at the 1% level [χ^2 (one degree of freedom) = 9·97]. There was no significant difference between HL and CL in the proportion of barren mice. Although the low line had a high proportion of barren mice (5 out of 12), the total number of mice in this group was small. However, Stephenson and Fredline (unpublished data) have found a significantly higher proportion of barren mice in the LL line during their selection experiments with mice from the same three lines.

The numbers of mice with copulation plugs which did and did not produce litters, and the numbers of mice without plugs which did not produce litters, were as follows:

Group	HLA	CLA	LLA	HLR	CLR
No. of mice	20	24	12	23	26
No. of mice with plugs which produced litters	14	17	6	9	13
No. of mice with plugs which did not produce litters	1	0	3	2	1
No. of mice without plugs which did not produce litters	3	3	2	9	12

It must be remembered that the detection of a copulation plug is conclusive evidence that copulation has occurred, but failure to detect a plug is by no means conclusive evidence that copulation has not taken place. In fact, in this experiment copulation plugs had been detected in only 85% of mice that littered. There was a significantly greater proportion of mice without plugs in the two groups on restricted intake (HLA+CLA+LLA *v.* HLR+CLR: $F_{1,61}=36{\cdot}0$, $P<0{\cdot}001$), suggesting that the main reason for the greater proportion of barren mice after restriction of intake was due to failure to mate.

A larger proportion of the LL mice failed to produce a litter after copulation, suggesting greater losses between copulation and littering. These data on the increased barrenness of LL mice are supported by results of Stephenson and Fredline (unpublished data) obtained with mice from generations 12, 13 and 14 of the selection experiment that produced the lines of mice used in the present study. Their data showed that a larger proportion of mice from the LL line failed to produce a litter after pairing, but they did not determine whether this barrenness was caused by a failure to copulate or by greater losses between copulation and littering. As in the present experiment, the average litter size was smallest in the LL mice.

The mean weight of pups born to CL mice was significantly lower than that of pups born to HL and LL mice. There were no effects of nutritional treatment on the weight of the pups at birth.

Discussion

In this experiment, genetic differences in liveweight were present in the three selection lines which were kept on *ad libitum* intakes. Liveweight differences due to nutrition were obtained by restricting intakes of approximately half the mice in two lines. Both genotype and nutrition considerably affected reproductive performance, but appeared to act in different ways, and so it is likely that different mechanisms were involved.

There were significantly more barren mice in the groups on restricted intake. These mice did not copulate until, as a group, they had regained their original liveweight. Perhaps if the males had been kept with these mice for longer than 5 days more HLR and CLR mice would have copulated. Those that did copulate became pregnant and litter size was unaffected by undernutrition for the 18 days prior to the introduction of the male. Thus, longer term undernutrition, like short-term (24–48 h) starvation (e.g. McClure 1966), produced a reduction in the incidence of mating. This could be due to silent ovulation (without behavioural oestrus) or to suppression of ovulation.

With undernutrition there may be several possible causes of barrenness. There

may be a lowered production or release of the hypothalamic releasing factors, or a lowered production or release of gonadotrophins. It is probable that the pituitary and the hypothalamus are involved in longer-term undernutrition, since Howland (1972*a*) has found decreased basal levels of plasma LH after 20 days of restricted intake in the rat. Howland (1972*b*) has also produced evidence to suggest that, with restricted feeding, the hypothalamus and the pituitary are unable to respond to a reduction in ovarian steroids. Piacsek and Meites (1967) found that 30 days of undernutrition in rats reduced LH levels in the pituitary to one-third and the amounts of luteinizing hormone releasing factor in the hypothalamus to one-quarter of those of *ad libitum* controls. On the other hand, FSH content of the pituitary was unaltered.

Lamond and Bindon (1969) and Cooper *et al.* (1970) found that, when rodents on restricted intakes were subsequently fed *ad libitum* for short periods (12–24 h), there was an increase in the number of ovulations, suggesting a sudden release of FSH rather than an increased rate of synthesis. The study of Lamond and Bindon (1969) suggests a lowered sensitivity of the ovaries to HCG and so perhaps the involvement of the ovaries as well.

It has been mentioned before that selection in these lines was not directly for or against liveweight, litter size or conception rate. Nevertheless, the resulting lines were of different weight with the heaviest line (HL) having the largest litters and the lightest (LL) having both the smallest litters and a greater number of barren mice. Such associations between body weight and fertility have been noted by many experimenters (e.g. Falconer 1953; Fowler and Edwards 1960; Elliott *et al.* 1968).

In the present work, the differences in litter size seen between the genetic lines were probably due in part to differences in ovulation rate since, in the study reported by Fowler and Edwards (1960), variations in the number of ova shed showed a high correlation with body weight and these variations were a major component of the differences in litter size in their selection lines. Losses between ovulation and littering may also have contributed to the low litter size of LL mice. Falconer (1960) found that, in mice selected for low litter size, the decrease was entirely a result of increased embryonic mortality. The observed increase in the number of mice that copulated but did not produce litters in our experiment also suggests increased embryonic mortality.

The physiological mechanisms causing the differences in our genetic lines of mice are not known, but other work (McLaren 1962; Murr *et al.* 1973) suggests that the main effect of selection for fertility is to alter ovarian sensitivity to FSH and hence to increase ovulation rate. On the other hand, selection for small litter size appears to increase embryonic loss without having much, if any, effect on the number of ova shed (Falconer 1960; Falconer *et al.* 1961; Bradford 1971). The correlated responses in fertility occurring through genetic changes in body size appear, however, to operate through changing the size of the pituitary (Edwards 1962). This change, by altering the production of growth hormone and gonadotrophins, might affect both growth rate and ovulation rate and probably produces the high correlation between body weight and ovulation rate.

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