REPRODUCTION AND SURVIVAL OF MICE AT 23° AND 32°C

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Summary. Details of the fertility of two inbred strains of mice, the reciprocal F_1 hybrids between the strains and some F_2 mice under temperate and high environmental temperature conditions are presented. Infertility was rare in the control mice but common in those exposed to heat. Genotypic differences were greater in the heat and chiefly of male origin, being related to the duration of exposure to the heat. Prolonged heat exposure caused an impairment of fertility which persisted even when the temperature stress was alleviated. There was some prolongation of the oestrous cycle at high temperatures but it was not closely related to the greatly reduced mating frequency which was the prime determinant of the failure to bear litters.

The postimplantation and early postnatal mortality indicate a heterotic effect on survival. Prenatal mortality may have been greater in the mice exposed to heat than in the controls but the effect was too small to account for the environmental difference in the sizes of litters at birth. Mortality in the first 3 weeks of life was also greater in the heat where survival seemed to be a function of the inbred mother. The F_2 litters had low mortality rates compared with inbred and F_1 litters. Parental heterosis was apparent in both environments.

The postnatal survival of litters was influenced by the litter's size at birth and its parity and the survival of individual animals was affected by the interval between birth and feeding and the body weight at birth. Both genotype and the temperature of the environment influenced the extent to which these variables operated.

INTRODUCTION

It has been shown that exposure of mice to high temperatures, either intermittently (Ogle, 1934) or permanently (Ogle, 1934; Pennycuik, 1967), or to greatly reduced temperatures (Barnett & Manly, 1959) may decrease fertility. Only in the last experimental series, however, has the extent of the environmental stress on a variety of genotypes been examined, and then genotype was found to have a significant determining influence.

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From the literature, it would appear that the extent and the cause of the infertility are very closely related to the particular environmental conditions created. Thus a reduction of the environmental temperature by 11°, from 21°C, did not alter the fertility of mice, but a further reduction of 13°, to -3°C, could cause a decrease in the numbers of young born and failure to breed in a few pairs though these effects were strain-dependent (Barnett & Manly, 1959). Similarly a temperature of 32°, as compared with 21°C (Pennycuik, 1967) and 25°C (Knudsen, 1962), produced little change in performance when exposure to such conditions began before adulthood. However, adults exposed to a temperature of 32°C, and even immature males and females exposed to a temperature of 36°C, suffered serious impairment of fertility (Pennycuik, 1967).

A reduction in the sizes of litters at birth, in a high temperature environment as compared with temperate conditions, has been reported by Mills (1945), Biggers, Ashoub, McLaren & Michie (1958) and Pennycuik (1967) and a similar trend was observed by Knudsen (1962). Since preweaning mortality is more common among mice reared in a hot environment than in controls, the numbers of animals weaned at high temperatures is very greatly reduced (Mills, 1945; Biggers *et al.*, 1958; Pennycuik, 1967). The differential effect of a low environmental temperature on the sizes of litters of mice of a variety of strains has been examined (Barnett & Manly, 1959). The only similar comparative data for strains at high temperatures involved randomly bred mice and naked and hairless lines (Pennycuik, 1971).

The present paper gives details of the strain variation in fertility and in other aspects of reproductive performance, including the survival of offspring, of mice reared at high environmental temperatures with increased humidity also under conventional conditions. Some of the factors contributing to the observed effects have been analysed.

MATERIALS AND METHODS

Two inbred strains of mice, C57BL and BALB/c, the reciprocal F₁ hybrids between them and F₂ litters, derived from sib to sib matings of these F₁ hybrids, were maintained in two different environments. Only the temperatures of the environments differed, being 22.8±1.6°C dry bulb and 17.8±1.0°C wet bulb in the 'control' and 32.2±0.5°C dry bulb and 26.7±0.5°C wet bulb in the 'hot' condition. Water and food (Dixon's Diet 41B) were supplied freely and a supplement of lettuce and mixed grain was given once a week.

A functional assessment of fertility of all the mating types in both environments was made. Animals were classified as fertile or infertile according to whether or not the females produced any full-term offspring within 2 months of a mating being established. To determine the duration of the oestrous cycle and to ascertain whether the cycle was affected by the environmental temperature, vaginal smears were taken daily for 40 days from ten BALB/c females in the control conditions and for a similar period after their transference to the hot conditions. The lavage method was used to obtain the smears. Mating frequency was estimated from daily examination of selected females for the presence of vaginal plugs.

Cages were examined daily for newborn litters and the sizes of litters born within each 24-hr period were recorded. By including dead animals and mutilated parts, a maximal estimate of litter size was obtained. At birth, each animal was weighed and marked so that it could be distinguished from its litter mates. The age of mortality of any animal was ascertained from daily observations of litters while they were between 1 and 21 days old.

RESULTS

Breeding performance

The incidence of infertile matings in each of the mating types in the control environment was generally low but did seem to vary according to the genotype of the parents (Table 1). In the two inbred strains, infertility had a lower incidence in BALB/c matings than in C57BL ones ($\chi^2 = 5.65$). Among intercross matings, no mating of a C57BL female to a BALB/c male was infertile, but infertility occurred in the intercross matings involving C57/BL males.

Table 1. The incidence of infertility in inbred, intercross and hybrid matings of mice

Matings	Control environment		Hot environment	
	No. of pairs	% infertile	No. of pairs	% infertile
Inbred				
C57/BL $\times$ C57/BL	68	10.3	41	80.5
BALB/c $\times$ BALB/c	102	2.0	46	32.6
Intercross				
C57BL $\varnothing$ $\times$ BALB/c σ	21	0	38	15.8
BALB/c $\varnothing$ $\times$ C57BL σ	35	8.6	33	84.8
Hybrid				
F ₁ sib $\times$ sib off-spring of C57BL $\varnothing$ $\times$ BALB/c σ	5	0	8	0

Data on the fertility of animals paired in the hot environment are included in Table 1. In all inbred and intercross matings, there was a very high incidence of infertility. This was particularly marked in those matings, both inbred and hybrid, which involved a C57BL male. In the hot environment, the difference in the fertility of the two inbred strains was increased and was highly significant ($\chi^2 = 18.20$); so too was the difference between the reciprocal cross matings ($\chi^2 = 31.04$). Although there was some indication that BALB/c males mated to BALB/c females were less fertile than those mated to C57/BL females, the difference was not statistically significant ($\chi^2 = 2.31$) nor was the difference in fertility of C57BL inbred and C57BL σ $\times$ BALB/c $\varnothing$ hybrid matings ($\chi^2 = 0.04$). There was therefore no evidence for a differential effect of maternal genotype on fertility under these conditions.

A contingency analysis indicated how great was the increase in the incidence of infertile matings in the hot compared with those in the control conditions: for C57BL, $\chi^2 = 51.27$; for BALB/c, $\chi^2 = 26.35$; for C57BL $\varnothing$ $\times$ BALB/c σ , $\chi^2 = 2.17$; and for BALB/c $\varnothing$ $\times$ C57BL σ , $\chi^2 = 36.82$. Only the value for C57BL $\varnothing$ $\times$

BALB/c♂ failed to be statistically significant. All matings of F₁ to F₁ hybrids were fertile in both environments.

Vaginal smears taken from a group of BALB/c females in the control environment and after they had been transferred to the heat indicated that in many animals transference was associated with a prolonged dioestrus. Following this, the oestrous cycle was again established but was longer ($P=0.001$) and of more variable duration than in the control environment. The mean duration of the cycle was 5.01 days, with a standard error of 0.22, in the control environment whereas in the heat it was 6.89 (S.E.=0.36). The increased variability was more apparent from comparisons of the variation of the duration of the cycle in the same animal over the period of examination. The estimated intra-individual variance in the control environment was 0.80 and in the heat was 1.91, indicating a statistically significant difference in variance at the 1.0% probability level. Further, there was no tendency for the cycle to shorten over the 40 days following transference to the heat.

Since earlier work indicated that testicular damage or degeneration result from heat exposure, the histology of the testes of males of the genotype most

Table 2. Numbers of vaginal plugs found and numbers of litters born to female mice with vaginal plugs in the hot environment

<i>Mating type</i>	<i>No. of pairs observed</i>	<i>Vaginal plugs</i>	<i>Litters born</i>
C57BL inbred	20	1	0
BALB/c inbred	13	13	5
F ₁ (BALB/c♀ × C57BL♂)	10	1	1

frequently involved in infertile matings, C57BL, was studied. No systematic structural variation was found. Among males which had been classified as infertile, all stages of spermatogenesis were represented and the overall picture was one of normality.

The frequencies of mating among inbred males were estimated from daily observations for vaginal plugs among females with which they were paired in the hot environment. The observations were made for a period of 6 weeks and are summarized in Table 2. Although the difference between the BALB/c matings and the two other mating types is minimized by the fact that, among the former, five of the vaginal plugs were associated with subsequent pregnancies while only one birth occurred in the thirty pairs involving C57BL males, the mating frequency of C57BL males was clearly much lower than that of BALB/c males. This difference was independent of the genotype of the female.

The effect of the duration of exposure to the heat on mating frequency and fertility was examined in the less fertile inbred strain. Under a regimen in which C57BL pairs were set up in the control environment and were transferred to the heat following copulation (as recognized by the presence of vaginal plugs), twenty-eight pairs produced eighteen litters within a 3-week period. Following transference to the heat, daily examination of the females was continued for 2 weeks when animals did not become pregnant, and for the 2 weeks after

parturition in those females which gave birth to litters. During the period of heat exposure, only six plugs were recorded for the twenty-eight pairs. None of the matings resulted in the birth of a litter. Following return to the control environment, however, only two of the pairs failed to produce a litter within 5 weeks.

In contrast to this, of seven male and seven female C57BL animals which had spent between 5 and 9 weeks in the heat following weaning, but which were paired in the control environment, only four produced litters, though at least one vaginal plug was observed in two of the remaining three pairs. Among a group of nine pairs, established initially in the heat and maintained there for 3 months without the production of a single litter, there was no evidence of mating even when the pairs were transferred to the control environment.

Survival of litters

The sizes of litters showed some variation with parity both at birth and at weaning. Since parity effects were distributed similarly in the two environments, however, data on litter sizes are presented in Table 3 as mean values

Table 3. The mean sizes of mouse litters at birth and at weaning in the control and hot environments

Mating type	Control environment		Hot environment	
	No. of litters	Mean $\pm$ S.E.	No. of litters	Mean $\pm$ S.E.
Birth				
C57BL	239	5.8 $\pm$ 0.21	18	4.7 $\pm$ 0.47
BALB/c	205	5.4 $\pm$ 0.16	133	3.5 $\pm$ 0.49
F ₁ (C57BL $\times$ BALB/c)	77	6.5 $\pm$ 0.32	141	4.4 $\pm$ 0.14
F ₁ (BALB/c $\times$ C57BL)	98	7.4 $\pm$ 0.23	14	4.5 $\pm$ 0.60
F ₂	20	9.8 $\pm$ 0.46	22	6.6 $\pm$ 0.30
Weaning				
C57BL	168	4.9 $\pm$ 0.15	3	2.3 $\pm$ 0.67
BALB/c	194	5.1 $\pm$ 0.16	27	2.5 $\pm$ 0.24
F ₁ (C57BL $\times$ BALB/c)	66	6.4 $\pm$ 0.27	32	3.2 $\pm$ 0.22
F ₁ (BALB/c $\times$ C57BL)	97	6.6 $\pm$ 0.24	8	3.5 $\pm$ 0.63
F ₂	20	9.6 $\pm$ 0.41	21	5.8 $\pm$ 0.28

with standard errors for each mating type. In the control environment, there was a small but significant difference between the mean sizes of inbred litters at birth, C57BL being the larger ($P=0.001$). Though there was a reciprocal hybrid difference in litter size, it was the litters of BALB/c mothers which were larger ($P=0.01$). The F₁ litters were generally larger than inbred litters ($P=0.01$) and the F₂ litters were largest of all ($P=0.001$). The ranked mean sizes of litters at birth were different in the hot from those in the control environment. The C57BL and the F₁ hybrids did not differ from each other, all being larger than BALB/c litters. Again, the F₂ litters were largest ($P=0.001$). For each genotype, exposure to the heat was associated with a significant reduction in the mean size of the litters and with a reduction in the between-strain variation in litter size.

To investigate some of the causes of genotypic and environmental variation in the sizes of litters at birth, the numbers of live and dead or necrotic embryos and moles, were ascertained for BALB/c and the reciprocal F_1 hybrids in the control environment and for F_1 (C57/BL♀) hybrids in the heat between the 8th and 12th days of gestation. Only that part of postimplantation mortality which occurred following extensive vascularization of the embryos could be detected. The data are presented in Table 4 with the mean sizes of litters at birth. Levels of mortality between vascularization and the period of uterine examination were low and did not differ according to mating type or the temperature of the environment. There were, however, marked variations in the numbers of live embryos recorded in females of the different mating types. The salient finding was that, although among F_1 (C57BL♀) litters in the control environment there was no evidence for any reduction in litter size from Days 8 to 12 of gestation and birth among the same mating type, there was a significant reduction in the heat. In this case, at least, the large

Table 4. The mean numbers of mouse embryos, per mother, between the 8th and 12th day of gestation and the mean sizes of litters at birth

	Control environment						Hot environment F_1 (C57BL ♀)	
	BALB/c		F_1 (BALB/c♀)		F_1 (C57BL♀)		No. of mothers	Mean no of young ± S.E.
	No. of mothers	Mean no. of young ± S.E.	No. of mothers	Mean no. of young ± S.E.	No. of mothers	Mean no. of young ± S.E.		
Live embryos	11	0.3 ± 0.75	8	9.3 ± 0.49	16	6.5 ± 0.32	6	6.0 ± 0.73
Dead embryos	11	0.6 ± 0.21	8	0.5 ± 0.38	16	0.4 ± 0.22	6	0.5 ± 0.34
Litter size at birth	2.05	5.4 ± 0.16	98	7.4 ± 0.23	77	6.5 ± 0.32	141	4.4 ± 0.14

environmental difference in litter size appeared to be due either to late intra-uterine mortality or to cannibalism shortly after birth. With the exception of F_1 (C57BL♀) hybrid litters in the control environment, the mean sizes of litters of all mating types in both environments were significantly smaller at 3 weeks of age, when the animals were weaned, than at birth. All the mean sizes of litters in the heat were smaller at weaning than when the same genotype was in the control situation ($P = 0.001$).

Between birth and weaning, there was mortality of complete litters and of animals within litters. The incidences of total and partial litter mortality and of total litter survival are shown in Text-fig. 1. There was marked variation in survival according to environment and to genotype. Survival, especially total litter survival in the control environment, was generally poor in C57BL as compared with BALB/c litters which were similar to F_1 and F_2 hybrids. Total litter mortality was rare in intercross matings in this environment though a maternal influence was apparent since more F_1 (C57BL♀) young than F_1 (BALB/c♀) young died before weaning, thus conforming to the inbred pattern. There was no mortality of complete litters among F_2 in the control environment.

So few C57BL and F_1 (BALB/c♀) young were born in the hot environment

that it was difficult to establish the variables which influenced their survival. Nevertheless certain trends emerged. With increasing outbreeding, the incidence of total litter mortality systematically decreased while total litter survival increased. Again a maternal effect on both partial and total survival of the reciprocal cross hybrids was found, with mortality being less in the litters of BALB/c mothers.

Comparison of the partial and total survival of litters showed a highly significant environmental influence when the parents were inbred, χ^2 values being 20.7 for C57BL, 202.2 for BALB/c, 30.7 for F_1 (C57BL♀) and 15.5 for F_1 (BALB/c♀) litters. By contrast, survival of the hybrid litters of F_1 parents was unaffected by the environment ($\chi^2 = 0.3$).

TEXT-FIG. 1. Percentage survival of mouse litters at (a) 23°C (control environment) and (b) 32°C (hot environment). Black columns represent total litter mortality; hatched columns, partial litter survival; open columns, total litter survival.

Of the total litter mortality, 97% occurred within a week of birth in all mating types in both the control and hot environments. Indeed, almost all these litters died within 48 hr of birth. Observation of many of the litters showed that mortality in the control environment was due approximately equally to mothers eating their litters and to the failure of the young to feed. In the heat, however, the mortality risk was due largely to a failure to feed.

Deaths of isolated animals in litters which had some survivors to weaning age were largely confined to the 1st week after birth, though about 12% of this mortality occurred later, a few animals dying even in the 3rd week. The age distribution of partial litter mortality was also unaffected by the environmental temperature.

The association between feeding shortly after birth and the survival of animals in the heat was examined closely. Table 5 shows the association between whether an animal had milk in its stomach when it was first observed and whether the animal survived. The mortality risk of animals which had not fed was much greater than that of those which had fed. Nevertheless, an offspring and a maternal genotypic effect are apparent. Where the maternal genotype was constant, a greater proportion of hybrid than inbred offspring had fed. There was also a marked difference in the proportions of the reciprocal F_1 hybrids which had fed. Hence, with respect to this feeding variable, F_1 hybrid litters with BALB/c mothers were at least risk while the inbred litters of C57BL mothers were at greatest risk.

The number of animals within a litter also appeared to influence their survival. In both environments, total litter mortality was high in very small litters. This may be partly an artifact, small litters being ascribed to mothers

Table 5. The association between milk in the stomach when newborn mice were first observed, and their subsequent survival in the hot environment

<i>Genotype</i>	<i>No. with milk</i>	<i>No. without milk</i>	<i>No. of deaths among:</i>	
			<i>animals with milk</i>	<i>animals without milk</i>
C57BL	8	27	1	24
BALB/c	70	71	39	65
F_1 (C57BL♀)	86	41	43	25
F_1 (BALB/c♀)	91	15	14	9

which had already eaten some of their offspring before litter size could be established and, having eaten some animals, they would be more likely to eat the remainder. As litter size increased, the incidence of total litter mortality appeared to decrease. In the hot environment, however, there was a limit to this trend for when there were more than four or five animals, total litter mortality again increased. The frequency with which animals died in litters which had some survivors was not influenced by the numbers of animals within the litter in either environment.

The direct influence of litter size on survival was rather small but bodyweight at birth (which was inversely related to litter size in all mating types in both environments) was related to survival. Litters of the four mating types in the heat were tested, but, in the control environment, only C57BL was tested since this was the only strain in that environment with a sufficiently high incidence of total litter mortality. There was no difference in the mean birth weights of litters that survived and those that died before 1 week of age. By contrast, when the birth weights of individuals that died were compared with the weights of surviving litter mates, by a statistical weighting procedure that compensated for numerical differences, the surviving animals were found to be much the heavier (see Table 6).

Table 6. The mean weighted amount by which the birth weights of surviving mice exceeded those of litter mates which died before 3 weeks of age

<i>Genotype</i>	<i>d.f.</i>	<i>Weighted mean difference</i>	<i>S.E. of the weighted mean</i>	<i>Significance of the difference</i>
Control environment				
C57BL	160	+0.147	0.018	0.1%
BALB/c	64	+0.234	0.027	0.1%
F ₁ (C57BL♀)	121	+0.085	0.030	1.0%
F ₁ (BALB/c♀)	122	+0.063	0.022	1.0%
Hot environment				
C57BL	18	+0.025	0.057	N.S.
BALB/c	37	+0.164	0.033	0.1%
F ₁ (C57BL♀)	60	+0.119	0.025	0.1%
F ₁ (BALB/c♀)	20	+0.126	0.060	N.S.

N.S. = not significant.

DISCUSSION

This work has confirmed that genetic and environmental temperature factors and the interactions between them profoundly affect fertility (Steive, 1923; Ogle, 1934; Pennycuik, 1967). Though, in general, the adverse effect of high temperature on reproductive performance is in agreement with the results of other workers, the degree of impairment is greater than that found at approximately the same dry-bulb temperatures (Pennycuik, 1967). Indeed, the severity of the temperature effect at 32.2°C in the present study approaches that described by Pennycuik at 36.1°C. This difference might well be explained by the relative humidity in the two environments, the greater humidity in the experiments reported here imposing a more severe heat stress than the environment with the higher dry-bulb temperature but without greatly increased humidity. In addition, Pennycuik used a random-bred stock of mice and, as the results of the present study indicate, the extent of the adverse effect of temperature is profoundly influenced by genotype.

There is no physiological reduction in fertility in the strains investigated which can be solely ascribed to females despite their somewhat extended oestrous cycles in the heat. But the magnitude of the effect of the high temperature environment on the oestrous cycle was much less than that reported by Pennycuik (1968), who associated the low frequency of oestrus with the reduction in the frequency of mating behaviour and regarded the failure of pairs to produce litters as chiefly of female origin. This conflicts with the data reported here that females of the strain whose males were almost all infertile were quite capable of conceiving hybrid litters.

Although high environmental temperatures can result in severe testicular damage (Steive, 1923; Moore, 1924; Harrison & Harris, 1957), this was not the cause of the observed high levels of infertility, for the reproductive system of males of the less fertile strain was morphologically completely normal. It is

probable that infertility arose mainly from behavioural changes, especially as manifest in the reduced frequency of mating. This view has also been expressed by Steive (1923), Sundstroem (1922) and Pennycuik (1967).

The environmental effect on litter size was marked and probably of maternal origin. The small sizes of litters in the heat appear, as Biggers *et al.* (1958) suggested, to be due mainly to late abortion or to parental eating of young almost immediately after parturition. The study of the mid-term fetuses indicated no differences in the two environments in the numbers of live fetuses or in the number which had been aborted between implantation and examination. By implication, therefore, the heat does not affect the number of eggs ovulated, the number fertilized or the number implanted. Hence, it would seem that the effect of the environment on litter size operates by acting on the physiological state of the mother. Though prenatal mortality was greater in the hot than in the control environment, the difference was not so great as to account for the failure of many pairs to give birth to any offspring in the heat. It is not apparent whether males or females were more implicated in the trend towards a reduction in the numbers of matings resulting in pregnancy. This trend agrees with the findings of Pennycuik (1971).

The marked genetic influence on the size of litters (Falconer, 1960; McCarthy, 1965, 1967; Festing, 1968) has been confirmed. Litter size is, in general, related to the degree of heterozygosity of both the parents and the offspring. There was no exception in either environment to the widely reported finding that inbred litters are characteristically smaller than the hybrid ones of genotypically similar mothers. Inbred animals are, as a function of their homozygosity, less able to obviate adverse fluctuations in their environment and are consequently less viable than hybrid animals. Whilst this phenomenon has been reported chiefly in postnatal development and adulthood, it may also be expected to occur prenatally and may account for the smaller size of inbred litters at birth (Falconer & Roberts, 1960; McCarthy, 1965) though Bateman (1966) failed to find an effect of female hybridism on the frequency of fetal deaths. In the case of the BALB/c strain, which is characterized by particularly small litters at birth, it would appear that the factors influencing survival operate before, or immediately after, implantation for it has been shown in this investigation that there is very little difference between the numbers of BALB/c and F_1 (BALB/c $\times$) embryos which have aborted by mid-term. The observed strain-characteristic differences in the sizes of litters at birth, and subsequent survival to weaning age, is in agreement with the report of Festing (1968) on a number of SPF strains.

In addition to strain differences in the sizes of litters within each environment, the extent of the change in performance that is associated with exposure to the hot, as opposed to the control, environment shows characteristic differences according to strain. In both absolute and proportional terms, the adverse effect of the heat was much greater in F_1 hybrid than in inbred litters. Although the genotype of the offspring may influence the demand made on the mother, the data seem to indicate that it is the physiological state of the mother which severely limits the number of animals in a litter in the heat. It was, therefore, the comparatively large control F_1 hybrid litters, as compared with inbred ones,

which showed the greater reduction in size in the heat, both at birth and at weaning.

This interpretation is supported by the observations on the reproductive performance of matings involving heterozygous (F_1 hybrid) animals. Hybrid parents were both more fertile and had larger litters than homozygous ones in the control conditions but it was in the heat that these genotypic effects were particularly evident.

The data on postnatal survival of litters followed a similar pattern to those on litter size. In both environments, F_2 offspring survived better than F_1 offspring. The survival of inbred litters was the poorest. Again, the magnitude of the difference between the litters of F_1 and inbred parents was much greater in the hot than in the control conditions.

It can be concluded that among the strains investigated, in both environments, heterozygosity of the parents and of the offspring have marked effects upon reproductive performance. In the control situation, it would seem that the genotype of the offspring may be the more important factor but in the heat there is clear evidence that the heterozygosity of the mother is of paramount importance.

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