CONCEPTION AND PARTURITION OF MICE AT TWO TEMPERATURES

S. A. BARNETT AND M. J. LITTLE

Department of Zoology, Glasgow University, Scotland

(Received 10th July 1967)

Summary. Mice of strains A/Tb, A2G/Tb, C57BL/Tb, the F_1 generation from the last two, and a genetically mixed stock have been bred in permanently mated pairs. One colony of each type was in a room kept at 21° C, another at –3° C. The dates of birth of their litters, and the number of young born and weaned (at 3 weeks), were recorded. Information on 2708 intervals between parturitions has been analysed.

The distribution of intervals was always bimodal. The first mode, which represented conceptions at post-partum oestrus, was at 20 or 21 days, at both temperatures, with the following exceptions: at 21° C, the C57BL mice had a mode at 24/25 days after lost litters, and at 26 days after surviving litters; at –3° C, the A2G mice had a mode of 31 days, and the C57BL of 28 days, both after surviving litters. Evidently, the C57BL mice were particularly subject to lactation delay. After post-partum conception, delayed birth, due to lactation, was proportional to the number of young weaned in the previous litter, except in strain A at 21° C. The influence of lactation was greater at –3° C than at 21° C in all three inbred strains and also in the mixed stock, when the effect was measured by the percentage of intervals of less than 25 days. But in terms of modal intervals between parturitions only strains A and A2G displayed an effect of temperature. There was no evidence that the cold environment lengthened gestation in the absence of sucklings.

Second modes, representing post-lactation conceptions, were at 45 to 50 days. The proportion of post-lactation conceptions (about 25% in inbred strains at 21° C) was higher at –3° C, but only when the previous litter survived. At 21° C percentages of post-lactation conceptions were higher among the F_1 and mixed stocks than among inbreds. Dispersions about both modes were greater at –3° C than at 21° C.

It is suggested that the supply of foodstuffs for anabolism was a crucial influence in determining the interval between parturitions at –3° C.

INTRODUCTION

Mice, *Mus musculus*, breeding in rooms kept at –3° C, have been compared, in previous work, with controls at 21° C. The age at which the vagina opens and the first oestrus are later in the cold; the oestrous cycles may be longer; and the age of first parturition is later (Barnett & Coleman, 1959, 1960). For one strain

(A2G) at least, the age at which reproduction ceases is later in the cold environment (Barnett, 1962). The number of litters produced in a given time is lower in the cold (Barnett, 1961, 1962). The accumulated information from about 12 years of this work now allows an analysis of the effect of cold on the distribution of intervals between parturitions, and hence some inferences on the times of effective conceptions, and the influence of lactation on pregnancy, in two environments.

MICE AND METHODS

The mice were of three inbred strains, A2G/Tb, A/Tb and C57BL/Tb, and a mixed stock obtained by crossing these three and one other, GFF. (In the account which follows, the substrain symbol, Tb, is omitted.) Some observations were also made on F_1 mice derived from matings between strains A2G and C57BL. These are called ' F_1 ' in the tables. Table 1 summarizes the material used. All the mice belonged to colonies breeding permanently in rooms kept at one of two temperatures, namely, 21° C (controls) and -3° C. The inbred

TABLE 1
SUMMARY OF MATERIAL

Class of mice	Temperature (°C)	No. of pairs	Generations	No. of intervals after litters		Age at first parturition (days)	
				Surviving	Lost	Mean	Range
A/Tb	21	102	17 to 31	326	26	72.4	55-120
	-3	96	1 to 12	127	29	88.3	58-148
A2G/Tb	21	188	1 to 23, 30 to 37	628	137	69.5	53-107
	-3	66	6 to 13	108	10	97.4	55-158
C57BL/Tb	21	199	3 to 34	609	47	74.8	53-151
	-3	124	2 to 21	214	39	89.7	55-154
F_1 *	21	10	-	37	0	59.7	56-68
	-3	18	-	47	0	74.3	62-122
Mixed	21	76	6, 9 to 13, 20	167	18	72.2	51-87
	-3	84	3 to 6, 12, 14, 20, 22	120	19	83.3	63-138

* C57BL × A2G and reciprocal cross.

and F_1 mice were in permanent brother-sister pairs from the age of 5 weeks. The mixed stock was outcrossed at the age of 5 to 8 weeks. The birth of litters was recorded daily, at about 10.00 hours; hence the time of birth was accurate to 24 hours. The number of young in each litter was recorded at birth, and on weaning at 21 days. Pairs were usually killed at the age of 28 weeks, or later if they had an unweaned litter at that age. A few were kept to the end of their breeding period; observations on these have been analysed separately. Some analyses are based on smaller numbers than the totals given in Table 1; these numbers are given in the relevant tables.

Diet 41 (Bruce & Parkes, 1949) and water were provided in excess; at -3° C the water was either in open jars or in the form of blocks of ice which the mice licked. Further information on methods is given elsewhere (Barnett, 1956; Barnett & Manly, 1959).

TABLE 2
MEAN INTERVALS BETWEEN PARTURITIONS

	Temperature (°C)	Previous litter	A2G/Tb		A/Tb		C57BL/Tb		Mixed		F ₁	
			No.	Mean	No.	Mean	No.	Mean	No.	Mean	No.	Mean
Post partum	21 — 3	Weaned	456	23.5	249	23.9	444	26.6	107	22.8	16	22.6
		Lost	114	21.9	25	22.8	38	25.5	18	21.4	—	—
		Weaned Lost	41 8	27.4** 25.8	68 24	24.8 22.9	84 33	29.7** 23.8	48 15	25.4* 22.7	9	28.4
Post lactation	21 — 3	Weaned	161	45.7	73	45.2	155	45.8	55	43.5	20	43.2
		Weaned	51	49.9*	48	49.2**	107	49.4**	63	44.9*	32	48.1*

Statistical significances were calculated by the Kolmogorov-Smirnov one-tailed, two-sample test.

* Significantly different from 21° C ($P < 0.001$).

** Significantly different from 21° C ($P < 0.02$).

RESULTS

Age at first parturition

Table 1 gives figures on ages at first parturition. The distributions were very skewed, as the ranges show. Most pairs had their first litter within 40 days of mating, but in all groups, except the F_1 and mixed stocks at 21° C, some females went beyond 100 days before giving birth. Table 1 shows also: (i) there were differences among the inbred strains; (ii) the mixed stock was quicker to conceive than any inbred strain; and (iii) low environmental temperature led to longer delay before parturition in each class.

Bimodality of parturition intervals

Text-figure 1 gives the distributions of parturition intervals, after weaned litters only, for all classes. Each is bimodal. The mean intervals associated with each mode are given in Table 2. The first mode for all classes except three was at 21 days (Table 4). The A2G mice at -3° C had a mode at 31 days; the C57BL mice had a mode at 26 days at 21° C, and 28 days at -3° C. The distribution about these atypical modes was nearly symmetrical, unlike those of the other classes of mice. The second modes were at 45 to 50 days; the distributions about them were fairly symmetrical, but the second modes were less clearly defined than the first.

Each first mode represents conceptions at post-partum oestrus; the second, at post-lactation oestrus. The latter very rarely occurs until 12 or 13 days *post partum* (Bruce & East, 1956). If the uncomplicated gestation of *M. musculus* is taken as about 20 days, all parturition intervals of 33 days or less represent post-partum conceptions; of these, intervals of 21 to 33 days represent varying delays of implantation due to lactation (Lataste, 1887; Kirkham, 1916; Mirskaia & Crewe, 1931; Enzmann, Saphir & Pincus, 1932; Brambell, 1937; Bruce & East, 1956). The very few intervals of 34 to 37 days are difficult to evaluate: they may represent unusually delayed post-partum conceptions (Enzmann *et al.*, 1932), or unusually early post-lactation conceptions. In the analysis that follows we have taken all intervals below 37 days as representing post-partum conceptions; this allows for a maximum consisting of 20 days gestation, plus 16 days delayed implantation, plus recording error of 1 day.

Effects of temperature

The cold environment left the distributions bimodal, but increased the dispersion around each (Text-fig. 1). The first modes of the mixed stock and of strain A remained at 21 days in the cold; that of A2G was at 31 days, and of C57BL at 28 instead of 26 days.

In the cold the second mode of all strains except A was one or more days further to the right than in the warm. This was perhaps due to a longer lactation at -3° C and a later occurrence of post-lactation oestrus.

Table 3 gives the percentages of successful implantations associated with the first mode. Since the mice were discarded 23 weeks after mating, the pairs which had shorter intervals between parturitions, and therefore more intervals in the time during which they were bred, are excessively represented in the distributions. Hence all first modes are probably more marked than they would be if this effect were allowed for.

Nevertheless, Table 3 shows the following: (i) At 21° C most conceptions were post-partum; only the F₁ mice were exceptions. (ii) At both temperatures the proportion of successful post-partum conceptions was higher when the

TEXT-FIG. 1. The distributions of interval lengths between parturitions, after weaned litters only, at 21° C (left-hand graphs) and -3° C (right-hand graphs) for four strains of mice.

previous litter was totally lost. (iii) The proportion of successful post-partum conceptions after weaned litters was much lower at -3° C than at 21° C. Nevertheless, the first modes after lost litters (Table 4) were usually close to

those after weaned litters: they were at 21 days for A2G and the mixed stock at both temperatures; and at 20 days for A at both temperatures. Strain C57BL was atypical and is considered further below.

Variation in lactation delay

Our records do not allow us to distinguish between neonatal deaths and those that occurred later. However, most deaths of nestlings occur before 10 days (unpublished). Biggers, Finn & McLaren (1961) eliminated the effect of lactation by killing all litters of random-bred TO mice at birth. During the first half of reproductive life of their mice, 83.5% of parturition intervals were of less than 25 days; a mode at 20 days represented post-partum conceptions.

TABLE 3
PERCENTAGES OF EFFECTIVE POST-PARTUM CONCEPTIONS

	Strain of mice	21° C		-3° C	
		No. of intervals	% post partum	No. of intervals	% post partum
After weaned litters	A2G/Tb	628	73.5	108	37.0
	A/Tb	327	76.0	131	52.1
	C57BL/Tb	609	75.9	213	39.2
	Mixed	166	64.2	119	39.4
	F ₁	36	44.4	41	22.0
After lost litters	A2G/Tb	138	83.0	10	80.0
	A/Tb	26	92.3	29	93.2
	C57BL/Tb	47	83.2	39	84.4
	Mixed	18	100.0	19	78.8
	F ₁	—	—	—	—

Accordingly, in Table 4, we give the percentages of intervals of less than 25 days after (a) lost litters, and (b) surviving litters. Our mixed stock at 21° C resembled that of Biggers and his colleagues. As expected, loss of the previous litter tended to reduce lactation delay; this effect was more marked in the cold than in the warm environment in all classes of mice. This may be because most total losses at -3° C, but not at 21° C, occurred within 3 days of birth. This supposition is supported by the figures for C57BL mice: this strain had a mode of 21 days only in the distribution of intervals after total losses at -3° C.

Evidently, the C57BL mice had more frequent and longer delayed implantation than the other strains. Since some intervals were of 21 days or less, post-partum oestrus can evidently occur at the usual time in this strain, and uncomplicated gestation can be of the usual length. At 21° C C57BL gestations of 21 days or less constituted 5.2% of all intervals after weaned litters, and 12.4% of those after lost litters. The corresponding figures for -3° C were 6.0 and 35.9%. The first modes after weaned litters, of 26 days and 28 days, respectively, in the two temperatures, show that the modal lactation delay was 5 days in the warm and 7 days in the cold. The C57BL mice at 21° C, therefore, resembled the Swiss mice described by Mantalenakis & Ketchel (1966): their mean gestation after mating was about 24 days. The distribution about the second

mode of the C57BL mice in the cold suggests that many conceptions took place 30 to 36 days after the birth of the previous litter.

The peculiar pattern of the C57BL mice was not displayed by the F_1 hybrids of that strain with A2G. At 21° C their distribution of intervals resembled that of the A2G mice, with a mode of 21 days.

TABLE 4
EFFECT OF LACTATION AFTER POST-PARTUM CONCEPTION

	Temperature (°C)	After lost litters		After surviving litters	
		Modal interval (days)	Intervals of <25 days (%)	Modal interval (days)	Intervals of <25 days (%)
A2G/Tb	21	21	71.4	21	50.0
	-3	21	62.5	31	34.1
A/Tb	21	20	60.7	21	53.2
	-3	20	51.5	21	29.1
C57BL/Tb	21	24 to 25	29.7	26	25.2
	-3	21	56.5	28	6.1
Mixed	21	21	83.0	21	54.7
	-3	21	67.8	21	24.2

Numbers of intervals in each class as in Table 1.

The long lactation delay of the C57BL mice might be attributed partly to the fact that this strain has larger litters than the other two inbred strains (Barnett & Manly, 1959; Barnett, 1961, 1964). Yet the mixed stock, which had a first mode at 21 days, had larger litters still. Accordingly, we examined the relationship between lactation delay and the number of young weaned, by

TABLE 5
REGRESSIONS OF PARTURITION INTERVALS ON SIZE OF PREVIOUS LITTER AT 3
WEEKS: POST-PARTUM CONCEPTIONS

Class of mice	Temperature (°C)	No. of intervals	No. in litter		Mean interval (days)	Regression coefficient	P<
			Mean	Range			
A2G/Tb	21	242*	5.1	1 to 11	23.2	+0.56	0.001
	-3	40	4.0	1 to 7	26.7	+1.47	0.001
A/Tb	21	75*	5.2	2 to 11	24.7	-0.04	-
	-3	65	4.5	1 to 10	24.6	+0.61	0.01
C57BL/Tb	21	75*	6.9	2 to 10	27.8	+0.86	0.001
	-3	75*	4.3	1 to 11	29.4	+0.67	0.01
Mixed	21	96	7.7	2 to 14	22.9	+0.63	0.001
	-3	38	5.7	1 to 10	25.8	+1.02	0.01

* Samples of the material available.

calculating the regressions of parturition interval on size of the previous litter at 3 weeks (Table 5). After post-partum conception, strain A showed no effect at 21° C, but all the remaining regressions were highly significant. The regression coefficients in Table 5 give the increases in intervals between parturitions, in days, per weanling. In this respect, if in no other, the C57BL mice resembled

other strains. The only significant regression after post-lactation conceptions was given by the mixed stock at -3°C ($P < 0.001$). It is surprising that there was even one significant regression when conceptions were at post-lactation oestrus. Perhaps the larger litters of this strain matured more slowly and sucked longer, and so postponed post-lactation oestrus. Alternatively, there may have been lactation delay due to restoration of the milk flow after conception.

Influence of age and parity

Most of our observations are unsuitable as evidence on the effects of age or parity, since they refer only to about the first half of reproductive life. Later parturition intervals were on average shorter than earlier ones, but this is probably because only the females which had short intervals produced fifth, sixth or (rarely) seventh litters at 21°C , or fourth and fifth at -3°C . This is supported by observations on twenty A2G pairs which were bred at 21°C to the end of reproductive life. Their mean interval lengths in days follow:

<i>First</i>	<i>Second</i>	<i>Third</i>	<i>Fourth</i>	<i>Fifth</i>	<i>Sixth</i>	<i>Seventh</i>
30.1	34.4	27.7	26.4	33.8	29.2	29.8

There is no evidence here of a decline in interval length with parity (cf. Biggers, Finn & McLaren, 1962).

DISCUSSION

Our observations give some evidence on the influence on gestation of (a) genotype, (b) concurrent lactation, and (c) low environmental temperature. The effects of genotype and a cold environment, on reproduction in general, have been reviewed elsewhere (Barnett, 1965).

Our records were not designed to give an estimate of the length of gestation, but, when conception is at post-partum oestrus, the interval between parturitions gives the gestation period over-estimated by about 18 hr (Runner & Ladman, 1950; Biggers, Curnow, Finn & McLaren, 1963). One question is whether our observations allow any conclusions on the length of gestation uncomplicated by concurrent lactation. Since the females were permanently mated, most pregnancies were prolonged by the presence of sucklings. When the previous litter was totally lost, the parturition interval tended to be less than after a surviving litter, but there was still usually some lactation delay (Table 4). The effect of losing a litter, in reducing lactation delay, was more marked at -3°C than at 21°C . This was perhaps because at -3°C most total losses were during the first 3 days after birth, while at 21°C they were often later. Probably, the best estimates of the length of uncomplicated gestation are those given by the modes at -3°C after lost litters. Since these were intervals between parturitions, they must over-estimate gestation by about 1 day. On this basis, even at -3°C , uncomplicated gestation took 19 or 20 days, and so there is no evidence that gestation was prolonged by the cold in the absence of lactation.

If 19 or 20 days represents the uncomplicated gestation of all strains at both temperatures (cf. Grüneberg, 1952), the effect of lactation is clearly shown in Text-fig. 1. At 21°C it was most marked among the C57BL mice. At -3°C it

caused a flattening of the distribution curve, and a shift to the right, in all strains. Consequently, the C57BL pattern at -3°C differed less from those of the other strains.

Our observations suggest that lactation delay in *M. musculus* is usually proportional to the number of sucklings present. All the regressions, except one, were highly significant (Table 5), even though they were on number in litter at 3 weeks and could not allow for deaths of some litter members between birth and weaning. The one anomaly was the absence of a regression in strain A at 21°C . Such genetically determined aberrations may account for contradictory results in the literature.

Not all conceptions were post-partum, and the percentages of effective post-partum conceptions again showed the influences of genotype, lactation and temperature (Table 3). At 21°C , in the three inbred strains, post-partum conceptions were successful on about 75% of occasions on which the previous litter was weaned. The mixed stock had a lower percentage, and the F_1 lower still; these mice are more fertile than the inbreds (Barnett & Coleman, 1960; Barnett, 1961); hence high fertility, measured by output of young, was in these instances correlated with rather high incidence of post-lactation conceptions. When a litter was lost at 21°C , the probability of a successful post-partum conception was increased. At -3°C the proportion of successful post-partum conceptions was much lower than at 21°C , but only when the previous litter was weaned. The low figures at the top of the right hand column of Table 3 reflect the combined effects of cold and the presence of a viable litter. The figures indicate that these two factors, acting together, not only delayed implantation, but often either prevented it, or caused loss of early implanted embryos (cf. McCarthy, 1965). A less likely hypothesis is that there was often no post-partum oestrus when a viable litter was present.

It would be conventional to try to explain our observations on both lactation delay and the failure of post-partum conceptions at -3°C by endocrine changes. But Barnett & Little (1965) have brought evidence that the reproductive performance of A2G female mice at -3°C is limited by the amount of food they can eat; and Barnett & Coleman (1959) have suggested that the reproductive cycle of female mice at -3°C resembles that of mice on a low plane of nutrition. It is, therefore, at least plausible that the crucial factor is the supply of metabolites from food.

ACKNOWLEDGMENTS

We are grateful to the Nuffield Foundation, the Wellcome Foundation, the Royal Society and the Medical Research Council for generous grants in aid of this research.

REFERENCES

- BARNETT, S. A. (1956) Endothermy and ectothermy in mice at -3°C . *J. exp. Biol.* **33**, 124.
BARNETT, S. A. (1961) Some effects of breeding mice for many generations in a cold environment. *Proc. R. Soc. B*, **155**, 115.
BARNETT, S. A. (1962) Total breeding capacity of mice at two temperatures. *J. Reprod. Fert.* **4**, 327.
BARNETT, S. A. (1964) Heterozygosis and the survival of young mice in two temperatures. *Q. Jl exp. Physiol.* **49**, 290.

- BARNETT, S. A. (1965) Adaptation of mice to cold. *Biol. Rev.* **40**, 5.
- BARNETT, S. A. & COLEMAN, E. M. (1959) The effect of low environmental temperature on the reproductive cycle of female mice. *J. Endocr.* **19**, 232.
- BARNETT, S. A. & COLEMAN, E. M. (1960) 'Heterosis' in F_1 mice in a cold environment. *Genet. Res.* **1**, 25.
- BARNETT, S. A. & LITTLE, M. J. (1965) Maternal performance in mice at -3°C : food consumption and fertility. *Proc. R. Soc. B*, **162**, 492.
- BARNETT, S. A. & MANLY, B. M. (1959) Effects of low environmental temperature on the breeding performance of mice. *Proc. R. Soc. B*, **151**, 87.
- BIGGERS, J. D., CURNOW, R. N., FINN, C. A. & McLAREN, A. (1963) Regulation of the gestation period in mice. *J. Reprod. Fert.* **6**, 125.
- BIGGERS, J. D., FINN, C. A. & McLAREN, A. (1961) Regulation of total reproductive output in the female mouse. *Proc. IVth int. Congr. Anim. Reprod., The Hague*.
- BIGGERS, J. D., FINN, C. A. & McLAREN, A. (1962) Long-term reproductive performance of female mice. II. Variation of litter size with parity. *J. Reprod. Fert.* **3**, 313.
- BRAMBELL, F. W. R. (1937) The influence of lactation on the implantation of the mammalian embryo. *Am. J. Obstet. Gynec.* **33**, 942.
- BRUCE, H. M. & EAST, J. (1956) Number and viability of young from pregnancies concurrent with lactation in the mouse. *J. Endocr.* **14**, 19.
- BRUCE, H. M. & PARKES, A. S. (1949) Feeding and breeding of laboratory animals. 9. A complete cubed diet for mice and rats. *J. Hyg., Camb.* **47**, 202.
- ENZMANN, E. V., SAPHIR, N. R. & PINCUS, G. (1932) Delayed pregnancy in mice. *Anat. Rec.* **54**, 325.
- GRÜNEBERG, H. (1952) *The genetics of the mouse*. Nijhoff, The Hague.
- KIRKHAM, W. B. (1916) The prolonged gestation period in suckling mice. *Anat. Rec.* **11**, 31.
- LATASTE, F. (1887) *Recherches de zoéthique sur les mammifères de l'ordre des rongeurs*. Durand, Bordeaux.
- MANTALENAKIS, S. J. & KETCHEL, M. M. (1966) Frequency and extent of delayed implantation in lactating rats and mice. *J. Reprod. Fert.* **12**, 391.
- MCCARTHY, J. C. (1965) Effects of concurrent lactation on litter size and prenatal mortality in an inbred strain of mice. *J. Reprod. Fert.* **9**, 29.
- MIRSKAIA, L. & CREW, F. A. E. (1931) The pregnancy rate in the lactating mouse and the effect of suckling on the duration of pregnancy. *Proc. R. Soc. Edinb.* **51**, 1.
- RUNNER, M. N. & LADMAN, A. J. (1950) Time of ovulation and its diurnal regulation in the mouse. *Anat. Rec.* **108**, 343.