REPRODUCTION AND GROWTH OF MICE OF THREE STRAINS, AFTER TRANSFER TO −3° C.

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'Wild' house mice (*Mus musculus* L.) breed in cold stores kept at about −10° C (Laurie, 1946), and laboratory mice of the same species can be bred at an environmental temperature of −3° C., given nesting material (Barnett & Manly, 1954; Barnett, 1956). The response of mice to low temperatures varies with different inbred strains. The differences are reflected in mortality (especially of young), growth, numbers of young born and weaned, and in internal, especially endocrine, changes. All these, and other aspects of the maintenance of the lives of mice at low temperatures, are to be discussed in future communications.

This paper deals with the effect of low temperature on the reproduction of mice of three strains. The experimental mice were transferred as young adults from a 'normal' temperature of about 21° C. to 10° C., or to −3° C., and mated at the same time. The study of the reproductive performance and growth of these mice provides a quantitative expression of differences between the strains.

MICE AND METHODS

The mice were of the following strains: (i) A (from Imperial Cancer Research Fund, Mill Hill, London); (ii) C57BL (also from Mill Hill); (iii) GFF (from Glaxo Laboratories, Greenford, Middlesex). All three strains have a long history of rigorous brother-sister mating.

Three constant-temperature rooms were used. The conditions in the two main rooms, kept at −3 and 21° C. respectively, and cages, food and nest material, are described in a previous paper (Barnett, 1956). The third room, kept at 10° C., was, unlike the others, not designed for the research on mice, and had a fan which maintained a continuous air circulation. It therefore differed in air movement, and in noise, as well as in temperature, from the other rooms.

Breeding procedure was as follows. All the mice to be mated were reared at 21° C. At 3 weeks they were separated from their parents, and at 5 weeks the sexes were separated. At 7 weeks all experimental mice were transferred to the room kept at 10° C., while the control mice, also not yet mated, remained at 21° C. At 8 weeks the mice destined for −3° C. were transferred to that temperature, and all mice were mated. All pairs were litter-mates.

All the mice mated at −3° C. had litter-mate counterparts at 21° C., and all those mated at 10° C. had litter-mates at both the other temperatures.

Body weights of males, and reproduction, were recorded for 20 weeks, that is, up to the age of 28 weeks. All litters born in this period were included in the record, even if they were weaned only after the end of the period. Weaning weights, at 3 weeks, of litters of all pairs were recorded. A few of the litters were not weighed, and so the total numbers of young were slightly higher than the numbers of those whose weights are analysed.

RESULTS

Mice of all three strains survived the transfer to the cold room. Only in the C57 strain was there mortality attributable to the cold (Table 1); in this strain the mortality at $-3°$ C. is highly significant.

Table 1. *Mortality and reproduction up to age 28 weeks*

	A			C57BL			GFF		
	21° C.	10° C.	−3° C.	21° C.	10° C.	−3° C.	21° C.	10° C.	−3° C.
No. of pairs	12	4	12	14	8	14	13	3	13
Adult mortality ♂	0	0	0	0	0	4	0	0	0
Adult mortality ♀	0	0	0	—	1	1	0	0	1
No. of barren pairs	0	0	0	1	0	9	0	0	1
No. of pairs weaning no young	0	0	2	1	0	11	2	1	9
Mean litters per pair	4·6	3·5	3·6	3·2	2·8	1·0	3·3	2·3	1·5
Mean young weaned per pair	18·75	16·25	12·75	16·0	15·4	1·4	9·8	10·0	0·9
Mean young born per litter	5·0 ± 0·27	6·3 ± 0·64	5·2 ± 0·58	7·2 ± 0·58	7·2 ± 0·54	4·5 ± 0·48	3·9 ± 0·29	4·3 ± 0·93	3·4 ± 0·29
Mean young weaned per litter	4·1 ± 0·31	4·6 ± 0·74	3·5 ± 0·37	5·0 ± 0·37	5·6 ± 0·69	1·4 ± 0·53	3·0 ± 0·36	3·4 ± 1·23	0·6 ± 0·32

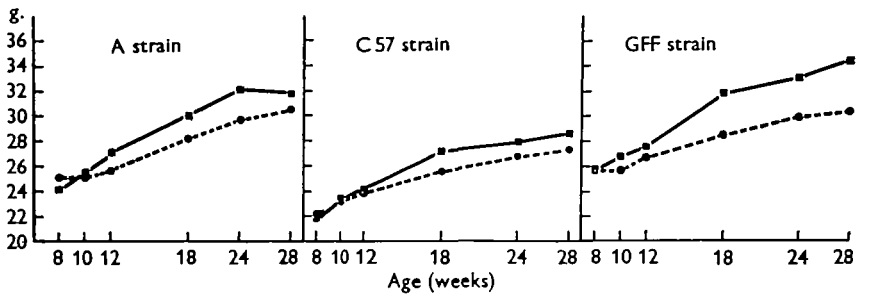

Fig. 1. Group means of weights of adult male mice. Continuous line: at 21°C. Broken line: at −3°C.

The group means of the weights of the males at six ages are shown in Fig. 1. At 21° C. the C57 mice are a smaller stock than the others at all ages; the GFF mice maintain a relatively high growth rate for longer than the A, and so are ultimately heavier in the warm. In the cold, total body growth is reduced in all three strains. The reduction is greatest in the GFF mice, and these, at the low temperature, no more than keep pace with the A mice. In both A and GFF mice

there was a marked check in growth during the first 2 weeks in the cold. Changes in relative growth will be reported separately.

Reproductive performance is summarized in Table 1. The figures for 21 and $-3°$ C. are the important ones, since the number of pairs at 10° C. is too small for full significance.

The number of litters born per pair was reduced at $-3°$ C. for all strains, but especially for C57 and GFF. There was no indication, from the weight records, of resorption of litters *in utero* in any of the mice at 21° C. At $-3°$ C. weight fluctuations suggest that this occurred in one A female, one C57 female and three GFF females. Complete failure to reproduce at $-3°$ C. occurred in none of the A pairs; but 64% of the C57 matings and 38% of the GFF matings were barren. The ability of the C57 mice to breed continuously in the cold (Barnett & Manly, 1954) therefore depends on the performance of a minority of successful pairs.

There is also, in the C57 and GFF strains, a heavy mortality in the cold during the first 3 weeks of life of the young, shown in the difference between the number of young born, and the number of young weaned, per litter. Data on conditions in the nest, relevant to this loss, are given in a previous paper (Barnett, 1956). The effect of cold on the number of young born *per litter* was slight; indeed, in the A mice no effect was evident.

Table 2. *Weights of young at 3 weeks*

	21° C.	10° C.	$-3°$ C.
A ♂ No.	111	34	70
Mean wt., g.	10·07 ± 0·20	9·41 ± 0·31	8·79 ± 0·19
♀ No.	110	31	87
Mean wt., g.	9·91 ± 0·20	9·10 ± 0·27	8·74 ± 0·20
C57BL ♂ No.	101	37	6
Mean wt., g.	7·88 ± 0·14	8·14 ± 0·27	6·08 ± 0·65
♀ No.	82	33	6
Mean wt., g.	7·60 ± 0·18	7·91 ± 0·29	6·83 ± 0·38
GFF ♂ No.	67	17	4
Mean wt., g.	9·96 ± 0·24	8·32 ± 0·33	9·50 ± 1·24
♀ No.	56	8	8
Mean wt., g.	9·79 ± 0·36	9·75 ± 0·50	10·31 ± 0·49

The weights of the young were significantly reduced at $-3°$ C. in the A and C57 strains, but not in the GFF (Table 2). We have evidence, to be published separately, that (as would be expected) resistance to cold at weaning is positively correlated with relatively high body weight. The weanling mice from the cold room, of which the weights are given in the right-hand column of Table 2, probably represent a group from which the lighter members have been eliminated during the 3 weeks in the nest.

The most important criterion of fertility is the number of young weaned per pair. In this respect there is little difference between the A and C57 strains at 20° C., but a marked difference at $-3°$ C. The A mice were notably successful at

the low temperature, showing a reduction of only 32% in the production of viable young; while the C57 mice showed a reduction of 91%. The performance of the GFF mice in the cold was even worse than that of the C57 mice, but comparison with their performance in the warm shows that the reduction in fertility was the same as that of the C57 mice, namely, 91%.

The results of breeding at 10° C. indicate that, in the conditions of the experiment, living at this temperature does not constitute a severe handicap for any of the three strains. This conclusion is further supported by the results of continued breeding, which will be published separately.

DISCUSSION

There is little published information on the effects of low environmental temperature on *reproduction* in mammals. Lee (1926) found that the oestrous cycle in albino rats was lengthened by exposure to outdoor winter temperatures. Parkes & Brambell (1928) found that transfer of laboratory mice from an environment at about 18° C. to one at about 0° C. led to a disturbance of the oestrous cycle, but this was only temporary: the length of dioestrus rose from a mean of about 4 days to one of about 11 days, but after the first week the normal cycle was restored. The mice were also mated at 0° C., and were found to be 'normally fertile'; they were however removed from the low temperature once pregnancy had been established. These observations are in conformity with our observations on A mice: in particular, we have found that the oestrous cycle in these mice, once they are established in the cold room, is normal (unpublished).

Laurie (1946) examined a large number of mice trapped in cold stores, and compared them with mice trapped in other habitats. Her estimated number of litters per female per year for mice in ricks (the most favourable habitat) was 10·2; the corresponding figure for our A mice at 21° C. is 11·5, assuming that breeding would continue at the same rate for 52 weeks as for 20. Laurie's figure for cold store mice was 6·7, ours for A mice at −3° C. is 9·0. The cold store mice, unlike our inbred mice at −3° C., were heavier than those in other habitats. However, the cold store mice studied by Laurie, besides being genetically heterogeneous, were living on a purely meat diet, at an environmental temperature well below that of our cold room. The comparison can therefore not be pursued very far.

A more potentially fruitful comparison is that between the three inbred strains used in the present experiments. The differences they show, in response to cold, while not surprising, suggest that a comparative approach to the study of reproduction in a cold environment is possible. Young (1953) has described an experiment in which genotype-environment interaction was studied in three inbred mouse strains; he points out that, given the use of several strains and environments, nature-nurture interactions of interest can be detected. In general, a decline in reproductive capacity, measured by the number of live 3-week young produced per pair, could be due to a great variety of effects. Sawin & Curran (1952) have studied reproduction in a number of rabbit strains at ordinary temperatures. They describe differences in maternal behaviour, in milk production and in growth rate,

as well as in production of young. In our mice the cold undoubtedly influenced the separate physiological processes involved in reproduction to varying degrees in the different strains. Not only were the A mice less affected at all points than the other strains, but, for example, the GFF mice showed a smaller reduction than the C57 mice in the young born per litter in the cold; on the other hand, the loss of young in the nest was higher for GFF than for C57 mice.

The present study deals only with the reproductive performance of mice born and reared in a warm environment. The full record of A and C57 stocks maintained for several generations in the cold will be published separately. It can be said here, however, that such stocks do not recover their lost fertility in later generations in the cold: in other words, the reproduction of pairs introduced into the cold as young adults is quite similar to that of pairs reared at $-3°$ C. The later generations of C57 mice are of course descended from those pairs (as we have seen, a minority) which reproduce well in the cold. There may be involved, therefore, selection of mice genetically different from the rest, in cold resistance or reproductive capacity. This is possible, even though the mice are rigorously inbred, since it is unlikely that complete genetical uniformity exists in these strains. The question of selection for cold resistance will receive attention in later work.

SUMMARY

Mice of strains A, C57BL and GFF were mated at environmental temperatures of 21 and $-3°$ C., with a few pairs also at 10° C. All pairs had nesting material.

Growth was reduced at $-3°$ C. in all strains. Weights of A and C57, but not GFF, young on weaning at 3 weeks were reduced at $-3°$ C.

The number of litters per pair, and of young weaned per pair, was reduced at $-3°$ C. for all strains, but especially C57 and GFF. The fertility of the GFF strain at 21° C. is lower than that of the other strains: consequently, the GFF mice did very badly in the cold.

Reproductive performance at 10° C. was similar to that at 21° C. for all strains.

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