EFFECTS OF DAILY HANDLING AND OTHER FACTORS ON WEIGHT GAIN OF MICE FROM BIRTH TO SIX WEEKS OF AGE

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SUMMARY

The results of this experiment show that growth rate in mice is so sensitive to environmental factors that any change in the environment such as handling or diet could cause short-term changes in the rate of growth. It is also shown that if there is a period of restricted growth due to an environmental factor such as either handling or diet, this may be followed by a period of compensating rapid growth.

The development of young mice can be accelerated by various methods of stimulation as shown by Barnett & Burn (1967). On the other hand, Chantry (1964) found that when young mice were handled twice daily from birth to weaning, the average weaning weight per mouse was 0.5 g less than a corresponding group that was not handled or disturbed until weaning. He did, however, show that (contrary to general belief) frequent sympathetic handling of litters of young mice did not lead to cannibalism or affect the mothering ability of an inbred strain of CBA mice. Cowley & Widdowson (1965) studied the effect on weight gain in young rats of stroking for 30 seconds a day from 0-21 days. When they compared the average weights of the daily-handled rats with a similar group that had not been handled, there was no significant difference in the weight gain.

Monteiro & Falconer (1966) studied compensatory growth resulting from the action of maternal environmental factors, and showed that a high litter weight at four weeks was followed by low subsequent growth and vice-versa.

This present experiment was designed:

to study the weight gain of two strains of young mice from birth to maturity;

to compare the effects on early growth rate of two commercially-produced compounded diets;

to observe the effects of daily handling and weighing young mice from 0-42 days.

MATERIALS AND METHODS

Treatments

Two diets, two strains and two handling regimes were used, and the weight of the young male and female mice were recorded separately. Litters were assigned to the treatments at random as the litters became available. Each litter came from the third parity of the mother, and was adjusted to a total of seven young. The treatments used and week of birth of each of the litters are given in Table 1.

Table 1. Treatments and week of birth of the litters.

	Treatment			*Week of birth of the litter*			
Group	*Strain*	*Diet*	*Handling**	*1*	*2*	*3*	*4*
I	CBA	FFG	D	14†	18	19	20
II	CBA	FFG	W	15	18	19	20
III	CBA	CDD	D	17	19	20	20
IV	CBA	CDD	W	18	19	20	20
V	LACA	FFG	D	14	15	16	19
VI	LACA	FFG	W	15	16	18	20
VII	LACA	CDD	D	15	15	18	20
VIII	LACA	CDD	W	15	16	19	20

**D* handled daily. *W* handled once per week.
†Week 14 was the week commencing 2 April 1967.

Mouse strains employed

Strain LACA is a strain which, though previously inbred, had been random bred for a number of generations and had a high reproductive performance. Strain CBA/Ca is an inbred strain (Staats, 1968) sub-cultivated from the LAC Specific Pathogen Free Unit.

Diets

The nutrient content (Table 2) of diet FFG has been published elsewhere (Porter & Bleby, 1966). The formula of Carworth-Dixon diet (CDD) is also given in Table 2. Briefly, diet CDD has a higher protein and fat content than diet FFG. Both diets were supplied by E. Dixon & Sons, Crane Mead Mills, Ware, Herts.

Table 2. Formula and nutrient content of the diets.

*Formula of the Carworth-Dixon diet (CDD)**

	Per cent
Wheat, English soft milling, approximately 80% Cappelle type ...	33.75
Barley, English grown	20.00
Soya bean meal, extracted with hexane boiling range 62-63°C, residual fat content 1.5%; heated to destroy trypsinase	12.50
White fish meal 'Provimi 66' (British White-Fish Meals Ltd)	15.00
Yeast, English blended 40% protein	1.25
Fat supplement; a proprietary blend of 42% animal fat in a base of detoxicated decorticated groundnut meal	15.00
Inert binding agent	1.25
Salt, 99.85% NaCl	0.80
Vitamin premix*, a proprietary mixture of vitamins and trace elements in a suitable vehicle	0.45
	100.00

*Published in *Carworth Europe handbook* (no date): Alconbury: Carworth Europe. Nutrient content not published. This diet has now been modified and is available in two forms, CDDM and CDDR, the formulae of which can be obtained from Carworth Europe or the manufacturer.

Nutrient content of diet FFG†

Biotin (μg/g)	0.124
Nicotinic acid (μg/g)	31.7
Pantothenic acid (μg/g)	9.1
Riboflavin (μg/g)	8.9
Thiamine (μg/g)	4.3
Vitamin B_6 (pyridoxine) (μg/g)	3.58
Vitamin B_{12} (μg/g)	0.026
Folic acid (μg/g)	0.25
Vitamin A (μg/g)	0.97
Carotenes (μg/g)	0.36
α-Tocopherol (μg/g)	13.0
γ-Tocopherol (μg/g)	9.5
Methionine (g/16g nitrogen)	2.3
Histidine (g/16g nitrogen)	1.4
Comparative (untreated diet=100) nutritive value for *Streptococcus zymogenes*	100
Available lysine as % of crude protein	5.04
Crude protein (%)	19.5

†From Porter & Bleby, 1966. Formula not available.

Handling

The young animals were taken out of their nests and cages for one minute during which time they were weighed and handled. In order to disinfect the pan between weighings, and to ensure that the young animals were not chilled by being placed on cold metal, the weighing pan was repeatedly dipped in water maintained at a temperature of 30 °C and containing 2 per cent 'Cetavlon' (cetrimide: Imperial Chemical Industries Ltd, Alderley Park, Macclesfield, Cheshire). The animals were handled in complete litters, but the males and females of each litter were weighed separately.

Mice to be handled daily were removed from the nest and weighed at 11 00 hours every day from birth to the 42nd day of age. The same procedure was used for the mice that were handled weekly, except that they were handled and weighed on the day of birth and at intervals of seven days from then to the 42nd day of age.

In groups I, III, V and VII the young were out of their nests for 1 minute daily, a total of 42 minutes. Groups II, IV, VI and VIII were out of their nests for a total pericd of 7 minutes only (once at birth, and once every seven days for 42 days).

A total of only six baby mice, all of strain LACA, died during the course of the experiment. These deaths occurred during the first two weeks, and there was no indication that they were associated with a particular treatment.

Housing

One animal room measuring 5 × 3.5 × 3 m, heated partially by steam pipes and brought up to a temperature of 21 °C by thermostatically-controlled electric heaters, was used for the test. The relative humidity of the room varied from 65 to 70 per cent; this fluctuation was related to the functions taking place within the room, i.e. filling water bottles, or hosing down the floor. Natural daylight was supplemented by artificial lighting (controlled by a time switch) in order to provide a minimum of 12 hours light daily.

Cages

Translucent plastic cages (polypropylene, supplied by North Kent Plastic Cages Ltd, Home Gardens, Dartford, Kent) measuring 30 × 13 × 11 cm, fitted with wire-mesh tops incorporating food hoppers and drinking-bottle holders, were employed.

Husbandry

Bedding and nesting materials were supplied in the form of sawdust and shredded paper, both of which had been sterilized by high-vacuum autoclave at 134 °C (2.25 kg/cm^2) for 3 minutes. Sterile cages furnished with these materials were provided once weekly. In order to retain a reasonably uniform environment within the cage, the fully furnished sterile cages were taken into the animal room 48 hours before the animals were due to be transferred. The animals were then removed from the dirty cages, weighed, handled and placed in the clean cages which had during the previous 48 hours acquired the temperature of the animal room.

Observations of nest temperature and behaviour

The average temperature inside the nests of the young before disturbance was 33 °C. When the young were removed, the temperature of the nest dropped to 29.5 °C in 1 minute, and when the young were all returned to the nest the temperature rose slowly; on average, the time taken for the temperature to return to 33 °C was 2.5 minutes.

It was observed that when the young were returned to the nest the adult females invariably re-arranged them or carried them around the cage, but when they were placed on the floor of the cage, the females commenced replacing them in the nest. The animals that were handled daily were more docile then those that were handled once weekly. There was, however, a strain difference, the CBA inbred strain was more active than the LACA strain, even the daily-handled CBA mice were inclined to jump between the ages of 12-42 days.

Statistical analysis

The original data consisting of the mean weight of each group of young mice at birth, and thereafter at weekly intervals until six weeks of age, were converted to weight gains during the first, second, etc. to the sixth week. The data were then analysed as a completely randomised $2^4 \times 6$ factorial experiment with four observations per cell (Cochran & Cox, 1957). The error term was, however, broken down into components appropriate to testing the effects associated with the age periods (litters among ages error) and that appropriate to testing the other effects (litters within ages), since it was considered possible that there was a correlation between growth rates in adjacent weeks.

All effects were considered to be fixed, and Bartlett's test was used to confirm that the variances were homogeneous.

RESULTS

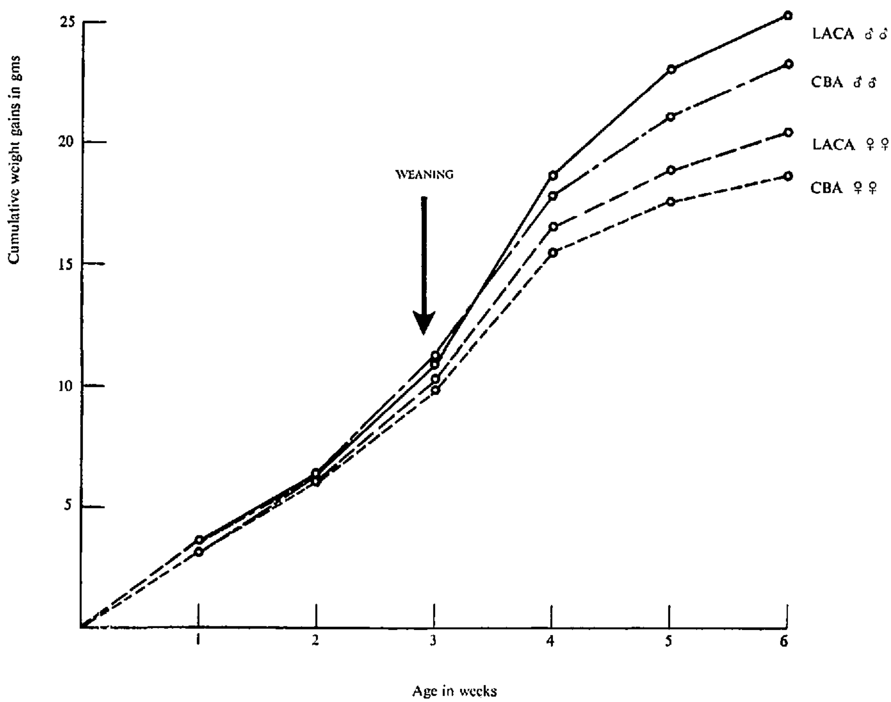
The mean weekly growth rate for the two strains, the two diets, the two sexes, the two handling regimes, and the six weekly periods are given in Table 3. There were highly significant strain, sex and age differences in mean growth rate, but there was no evidence that diet or handling affected the mean growth rate to six weeks of age. Growth curves for the male and female mice of the two strains are shown in Fig. 1.

Table 3. Effect of strain, diet, sex, handling and age on growth rate.

		Mean growth rate per week (g)
Strain***	CBA	3.50
	LACA	3.79
Diet	CDD	3.68
	FFG	3.62
Sex***	male	4.03
	female	3.27
Handling	daily	3.65
	weekly	3.64
Age***	1st week	3.43
	2nd week	2.81
	3rd week	4.29
	4th week	6.58
	5th week	3.04
	6th week	1.72

***Indicates heterogeneous at the 0.1 per cent probability level.

Fig. 1. Growth of male and female mice of two strains.



Analysis of variance, Table 4, indicates that four of the ten two-factor interactions were statistically significant.

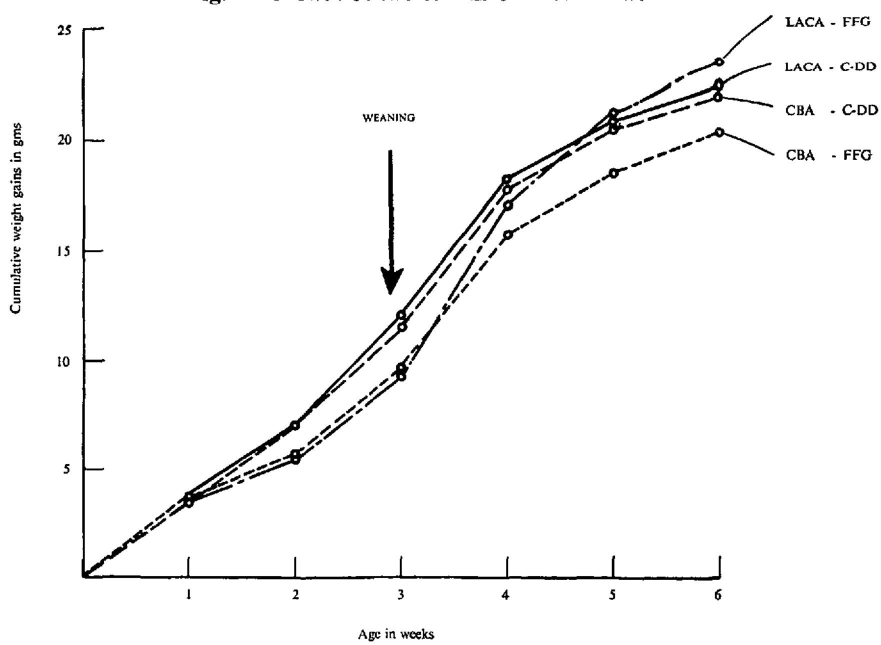
Table 4. Analysis of variance of weekly growth rates.

Source	*Degrees of freedom*		*Mean squares*	
Strain (S)	1		8.31***	
Diet (D)	1		0.17	
Handling (H)	1		0.00	
Sex (X)	1		55.43***	
Age (A)		5		177.27***
S × D	1		4.32**	
S × H	1		0.04	
S × X	1		0.03	
S × A		5		3.34
D × H	1		0.33	
D × X	1		0.07	
D × A		5		11.99***
H × X	1		0.39	
H × A		5		6.22**
X × A		5		6.12**
S × D × H	1		0.23	
S × D × X	1		0.07	
S × D × A		5		5.07*
S × H × X	1		0.14	
S × H × A ...		5		2.29
S × X × A		5		1.47
D × H × X	1		0.37	
D × H × A		5		4.37*
D × X × A		5		3.50
H × X × A		5		0.28
S × D × H × X ...	1		0.16	
S × D × H × A ...		5		1.87
S × D × X × A ...		5		1.05
S × H × X × A ...		5		1.83
D × H × X × A ...		5		1.71
S × D × H × X × A ...		5		0.00
Litters among ages error		240		1.58*** (versus 'within')
Litters within ages error	48		0.48	
	383			

*Significant at 5 per cent level. **Significant at 1 per cent level. ***Significant at 0.1 per cent level.

The highly significant strain × diet interaction was caused by a faster average growth rate of strain LACA on diet FFG (3.88 g/week) than on diet CDD (3.72 g/week), whereas strain CBA grew faster on diet CDD (3.67 g/week), than on diet FFG (3.37 g/week). There was some evidence that this difference in the response of the two strains was limited to the post-weaning growth rate. This is illustrated in Fig. 2 where it can be seen that both strains grew more slowly on diet FFG in the pre-weaning period than on diet CDD, but whereas strain LACA subsequently grew considerably faster, CBA continued to grow slowly on FFG.

Fig. 2. Growth of two strains of mice on two diets.



The highly significant diet × age interaction was caused by a higher average growth rate of the mice on diet CDD in the first three weeks (3.88 g/week) than in the last three weeks (3.43 g/week), whereas the mice on diet FFG grew more on average in the last three weeks (4.08 g/week) than in the first three weeks (3.17 g/week).

Similarly, the significant age × handling interaction was caused by a faster average growth rate in the daily-handled mice in the first three weeks than in the last three weeks, with the converse in the weekly-handled mice:

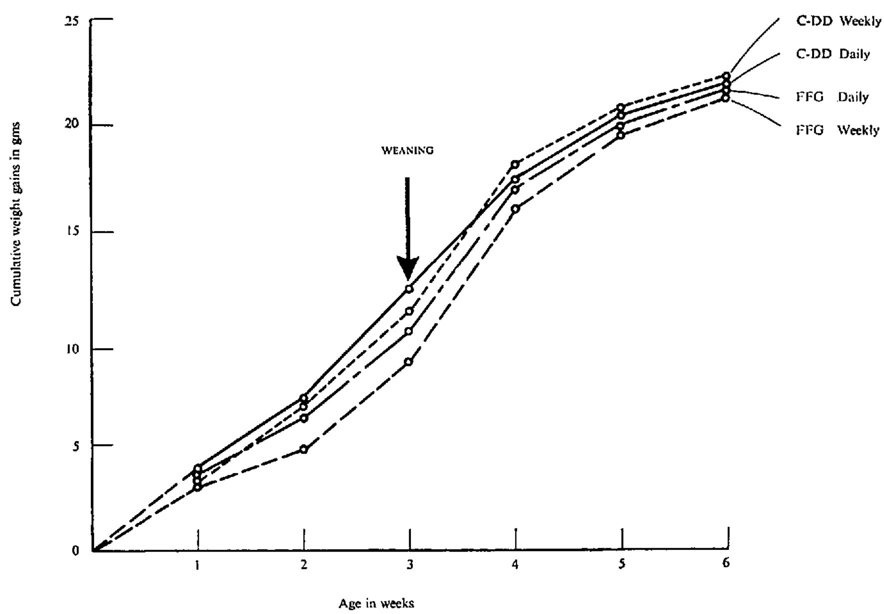
	Mean weekly growth rate (g)	
	first 3 *weeks*	*last* 3 *weeks*
Handled weekly	3.32	3.95
Handled daily	3.73	3.57

The highly significant sex × age interaction is a reflection of the relatively much faster growth rate of the males than the females in the last three weeks compared with the first three weeks (see Fig. 1).

Two of the three-way interactions were statistically significant. The strain × diet × age interaction has already been dealt with, and is illustrated in Fig. 2.

The significant diet × handling × age interaction is shown in Fig. 3, where it can be seen that the effects of daily versus weekly handling (given above) were apparently confined to the mice on diet CDD.

Fig. 3. Growth of mice on two diets and two handling regimes.



DISCUSSION

The results of this experiment show that growth rate to six weeks of age in mice can be influenced by several factors, and these factors do not always act independently. Moreover, a factor which stimulates growth at an early stage may even retard growth (relative to another treatment) at a later stage.

A clearer understanding of the relationships between the various factors described in this experiment may be obtained when it is remembered that growth rate in the pre-weaning period is probably largely dependent on maternal factors, whereas the growth in the post-weaning period is probably mainly dependent on the growth potentiality of the young mice themselves. One added complication is that in some cases there may be a negative correlation between growth in the pre- and post-weaning period, with a removal of the restriction after weaning, causing compensatory growth (Monteiro & Falconer, 1966).

In this experiment there were four sets of mice which differed in their intrinsic growth rates. Thus, there were two strains differing in growth rate, and the males of each strain grew faster than the females (Fig. 1). Superimposed on this basic set of mice were the two diet and two handling regimes. In the pre-weaning period diet CDD caused a higher rate of growth than diet FFG, presumably because it was a better diet for lactation. The beneficial effect of handling in the pre-weaning period is in contrast to the results of Chantry (1964). One hypothesis that could account for the increased growth rate with daily handling is that the mother mice spent more time caring for and suckling their young if these had recently been handled, thus stimulating growth. In the post-weaning period, a diet and handling regime which acts through maternal effects may not be satisfactory for maintaining growth directly, hence the reversal of relative growth rate with the two diets and handling regimes. The extent to which compensatory growth will occur will depend on the intrinsic capability of the mice to respond to a better set of conditions, hence the significant strain × diet × age interaction, and a diet × sex × age interaction which approaches significance.

In conclusion, the results of this experiment emphasize the complexity of a character such as growth rate, which may respond in opposite ways to a set of treatments depending on the time at which the treatment is applied. Thus, if an experiment is to be conducted to find the effect of a certain treatment on growth rate, this treatment should be replicated over a range of strains, diets and other environmental factors if the results are to have any general significance.

ACKNOWLEDGEMENT

We are indebted to Mr L. A. Mundy for valuable technical assistance.

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