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Effect of different regimens of early malnutrition on behavioural development and adult avoidance learning in Swiss white mice

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1. The effects of perinatal malnutrition on behavioural development and adult shuttle-box avoidance performance were studied in Swiss white mice.

2. Mice were malnourished (*a*) from the 7th day of gestation until birth, (*b*) from birth until weaning, or (*c*) during both gestation and the sucking period.

3. Pups born of protein-restricted mothers had reduced birth weights, retarded development and poor adult avoidance performance, even if reared from birth by well-nourished mothers.

4. Postnatal malnutrition, induced either by restricting maternal diet or by rearing in large litters, retarded development during the second half of lactation and lowered subsequent adult avoidance performance.

Many studies have attempted to identify the effects of early nutritional deprivation on experimental animals. Lát, Widdowson & McCance (1960) showed that physical maturation and the appearance of certain reflexes in the rat were delayed by nutritional deprivation provoked by rearing rats in large litters. Similar results may be observed if the mother's food intake is restricted (Smart & Dobbing, 1971 *a*) or if she is fed on a low-protein diet (Cowley & Griesel, 1966). Simonson, Sherwin, Anilane, Yu & Chow (1968) found that restriction of maternal diet during pregnancy delayed physical and behavioural development of the offspring, even when the young were adopted at birth by well-nourished mothers. However, Smart & Dobbing (1971 *b*) found that although restriction during gestation produced significant physical stunting at birth, effects on reflex ontogeny were negligible.

The long-lasting effects of early nutritional deprivation in rodents include physical stunting (Widdowson & McCance, 1963), changes in food-related behaviour (Seitz, 1954) and more intense reactions to aversive situations (Levitsky & Barnes, 1970). Mice undernourished by rearing in large litters show marked reductions in shuttle-box avoidance-learning ability (Leathwood, Bush, Berent & Mauron, 1974). This large litter design for inducing early malnutrition has several limitations: the pups are malnourished only during the sucking period and, probably more important, account must be taken of effects of litter size, per se, on adult behaviour.

The present experiments examine how different regimens of early malnutrition influence behavioural and physical development and adult avoidance performance of Swiss white mice. During the developmental study pups were handled every day from birth to 21 d of age. As early handling can influence adult behaviour (Levine &

Table 1. *Composition (g/kg) of the low-protein, casein diets given to mice*

Casein	50	100
Agar	20	20
Salt mixture*	30	30
Maize starch	550	500
Sucrose	250	250
Olive oil	100	100
DL-methionine	1	—
Vitamins (ml/animal per d)†		
Fat-soluble	0·1	0·1
Water-soluble	1·0	1·0

* Hawk & Oser (1931).
† Mauron & Mottu (1962).

Mullins, 1966), the avoidance performance of these previously malnourished, handled animals was compared with the performance of mice subjected to similar regimens of malnutrition but left undisturbed until weaning.

EXPERIMENTAL

Mice were drawn from a colony of randomly bred, Swiss white mice maintained in this laboratory. They were kept on a 12 h light–12 h dark cycle commencing at 06.00 hours. Nulliparous females aged 2·5–3 months were housed four/cage with one male/cage and the day of mating was determined by the presence of a vaginal plug. From the 7th day of gestation, one group of mice was given a diet with 50 g casein/kg until parturition and a diet containing 100 g casein/kg during lactation. The others received a pelleted stock diet (Nafag S.A., Gossau, Switzerland) with 200 g protein/kg throughout gestation and lactation. The pregnant mice were randomly assigned to these different treatments. In a preliminary experiment one control group was given a diet with 200 g casein/kg and another the pelleted stock diet with 200 g protein/kg. Growth, development and adult avoidance performance of the offspring were virtually identical.

The low-protein diets had compositions as shown in Table 1.

The food was prepared as a pudding (Mauron & Mottu, 1962) and supplied *ad lib.* to the mice so that they ate to appetite. Pups also had access to the diet and began to eat it at 15–17 d of age.

At birth all pups were sexed and cross-fostered into litters of five pups per mother giving the following experimental groups: (*a*) pups born to and reared by well-nourished mothers (G^+L^+); (*b*) pups born to well-nourished mothers but reared by malnourished mothers (G^+L^-); (*c*) pups born to malnourished mothers and reared by well-nourished mothers (G^-L^+); (*d*) pups born to and reared by malnourished mothers (G^-L^-); (*e*) (for comparison with the large-litter model for inducing early malnutrition) pups born to and reared by well-nourished mothers but with twenty pups in each litter (20's).

Throughout this paper we have used a shorthand notation adapted from that of

Smart & Dobbing (1971 *a*, *b*). Thus G^+ denotes adequate nutrition during gestation; G^-, protein restriction during gestation; L^+, adequate nutrition throughout lactation and L^-, protein restriction during lactation.

In a pilot study we found that more deaths occurred in the G^+L^- and G^-L^- groups; therefore, in the complete development experiment a total of twenty-three litters was studied (four G^+L^+, five G^-L^+, six G^+L^-, six G^-L^- and two 20's). During the course of this study these animals were handled every day throughout lactation. In anticipation of deaths in the experimental groups, extra litters were set up and dead pups replaced as necessary. As each new pup was added to an experimental group it was marked by injection of indian ink under the skin of a hind paw so that it could be eliminated from subsequent statistical analyses. Those that were not so needed, six litters of G^+L^+, six G^+L^- and three 20's, were left undisturbed except when the bedding was changed each week. All litters were weaned onto stock diet at 3 weeks of age. Survival rates were recorded, males were separated from females and housed eight/cage in 0·25 × 0·20 × 0·14 m Macrolon cages until the end of the experiment. Shuttle-box avoidance learning began at 8 weeks old and continued until the 11th week. The following week five mice from each group were killed and regional brain weights and DNA content determined.

Physical and behavioural development

Growth. It was difficult to measure birth weight with live pups because most mothers suckled their pups soon after birth, and when each newborn litter was found, some pups had stomachs full of milk whilst others were empty. In order to obtain more accurate estimates of birth weight eight G^+ and nine G^- litters were killed at birth and their stomach contents removed before weighing. Also included in this repetition were eight litters whose mothers had been fed half the normal daily amount of stock diet from the 7th day of gestation.

Reflexes. The mice were examined daily for eye-opening, ear-unfolding, the appearance of fur and development of the auditory startle response. Behavioural development of pups was studied on alternate days until the 21st day. Every pup was tested once for a given response each day. We monitored the development of the following reflexes: grasping, righting, crawling, fore-limb placing response, free-fall righting, and auditory startle response. The testing procedure was exactly as described by Fox (1965), but the strength of the response was graded on a 0–4 scale: 0 no response, 1 weak response, 2 moderate response, 3 strong response and 4 adult-like response.

Avoidance learning. The apparatus was essentially as described by Bovet, Bovet-Nitti & Oliverio (1968). The shuttle-box consisted of a white rectangular Plexiglass box 0·4 × 0·1 m divided into two equal compartments and connected by a small opening (30 × 30 mm). The floor was a tilting platform of stainless-steel rods spaced 4 mm apart. In each trial the conditioning stimuli, light (from a 10 W bulb) and sound (approximately 5 kHz, 60 dB) preceded by 5 s the unconditioned stimulus (continuous 30 V a.c. shock through the grid floor scrambled through a silicon rectifier) and overlapped with it for 2 s. A resistance of 30 kΩ was mounted in line with the shock circuit of each cage to reduce fluctuation in current flow due to changes in

Table 2. *Effect of maternal diet on litter size and body-weights (minus stomach contents) of newborn mice*

Mothers' diet	No. of litters	Litter size Mean	Litter size SE	Total no. of pups	Birth weight Mean	Birth weight SE
Stock diet, *ad lib.*	8	12·5	0·98	100	1·54	0·013
Low-protein* (50 g casein/kg), *ad lib.*	9	6·0	0·71	54	0·84	0·013
Stock diet, 50 % of control intake	8	12·6	0·95	101	1·42	0·010

* For details see Table 1.

posture and contact of the animal with the grid floor and to limit current flow to 1·0 mA. The shock was switched off at initiation of the following trial. Mice avoided the shock by running into the adjacent compartment within 5 s of the onset of light and sound. Passage of the mouse from one side of the box to the other was recorded by a micro-switch tripped by movement of the tilting floor. All animals were given fourteen daily sessions of fifty trials each with an interval of 30 s between each trial.

Dissection and DNA determinations

At 80 d of age, five mice from each group were killed, their brains separated from the spinal cord at the foramen magnum and divided into forebrain, brain stem and cerebellum. The forebrain was separated from the brain stem just anterior to the corpora quadrigemina. Each region was weighed and stored frozen at −20° until required for DNA analysis. DNA was extracted and assayed as described by Margolis (1969).

RESULTS

Table 2 shows that the low-protein diet produced severe reductions in birth weight (30–40 %), whilst restricted intake of stock diet (50 % of control intake) produced only an 8 % reduction. With more severe restriction of stock diet it was not possible to obtain an accurate estimate of the reduction because the mothers ate their young shortly after birth.

Development

All G^+L^+ and G^+L^- pups survived to adulthood but there were some deaths in the other groups. Most of these occurred within 24 h of cross-fostering, apparently because the pups were rejected by their foster-mother. Eleven G^-L^+ and six G^-L^- pups died in this manner, while two pups from the litters containing twenty pups died within 7 d of birth. Results for physical and behavioural development were obtained for all mice surviving at each day's observation.

The prenatally deprived pups, if nourished by adequately-fed mothers (G^-L^+), grew faster than the G^+L^- and 20's (Fig. 1) so that, at weaning, they weighed 10·3 ± 0·2 (SEM) g compared with 8·3 ± 0·06 g for the G^+L^- mice and 6·5 ± 0·025 g for the 20's. Although the G^-L^+ mice were heavier than G^+L^- and 20's at weaning, as adults they showed more marked weight reductions. This delayed consequence of prenatal

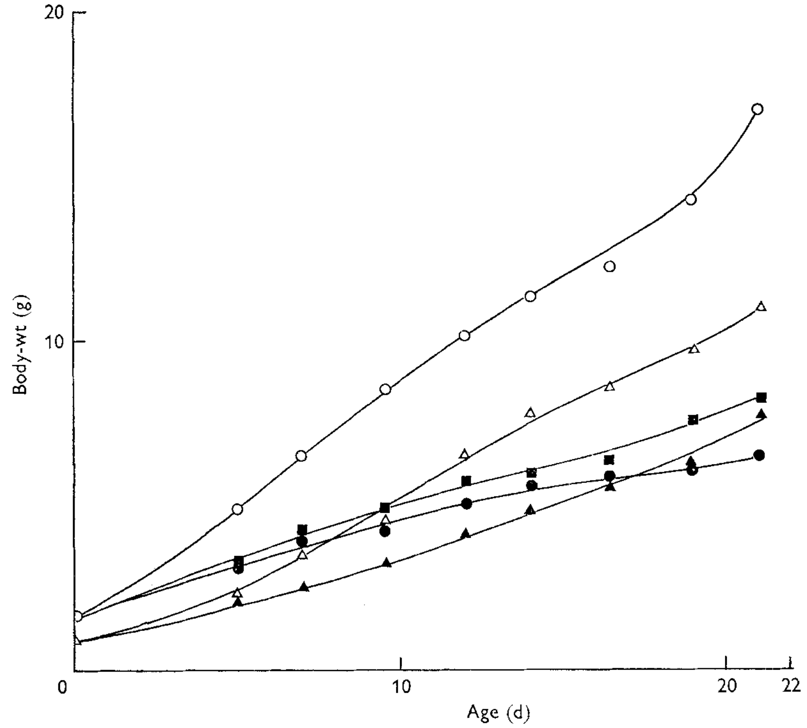

Fig. 1. The effects of different pre- and postnatal feeding regimens on the body-weights of mouse pups during sucking. —○—○—, G^+L^+ (pups born to and reared by well-nourished mothers); —■—■—, G^+L^- (pups born to well-nourished mothers but reared by malnourished mothers); —△—△—, G^-L^+ (pups born to malnourished mothers but reared by well-nourished mothers); —▲—▲—, G^-L^- (pups born to and reared by malnourished mothers); —●—●—, 20's (pups born to and reared by well-nourished mothers but with twenty pups in each litter).

nutritional deprivation has already been found in rats by Blackwell, Blackwell, Yu, Weng & Chow (1969).

The ages at which different physical features developed are shown in Fig. 2. Ear opening, the appearance of fur and opening of the eyes were all retarded in the prenatally deprived mice even when they were reared by well-nourished mothers. By 10 d of age the postnatally undernourished groups (G^+L^- and 20's) began to fall behind the controls. None of these physical features matured later than 15 d so it was not possible to evaluate the late (15–21 d) effects of postnatal deprivation.

The development of reflex behaviour followed a similar pattern. Reflexes that developed early in the control (G^+L^+) mice were delayed by prenatal nutritional deprivation whereas postnatal deprivation due to rearing in large litters or caused by sucking from malnourished mothers, only began to take effect after 8–10 d (Fig. 3)

As the development of most characteristics was rated for each animal on a 0–4 scale, it was possible to analyse the results using the one-tailed, Kolmogorov–Smirnov two-sample test (Siegel, 1956). Table 3 shows the ten possible comparisons between the five experimental groups, with the day of statistical testing indicated for each physical or behavioural feature. Both prenatally undernourished groups (G^-L^+ and

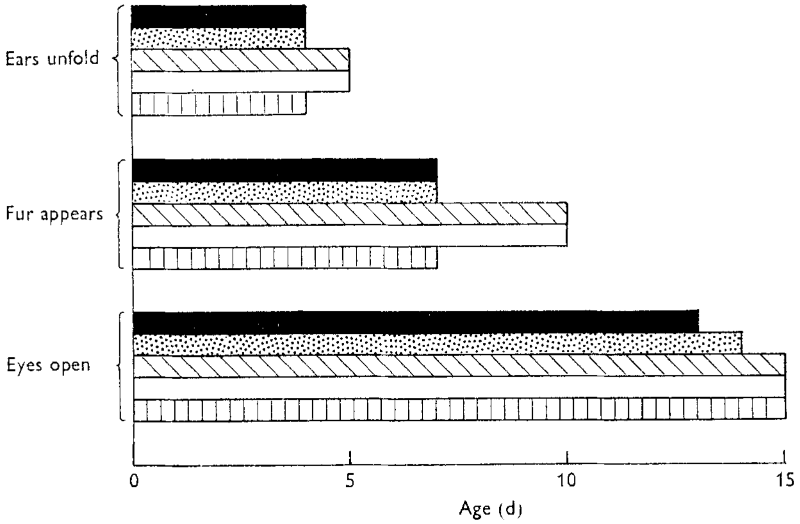

Fig. 2. Effect of different pre- and postnatal feeding regimens on the mean age of mouse pups at maturation of three physical features: ear unfolding, appearance of fur, eye opening. ■, G^+L^+ (pups born to and reared by well-nourished mothers); ▦, G^+L^- (pups born to well-nourished mothers but reared by malnourished mothers); ▧, G^-L^+ (pups born to malnourished mothers but reared by well-nourished mothers); □, G^-L^- (pups born to and reared by malnourished mothers); ▥, 20's (pups born to and reared by well-nourished mothers, but with twenty pups in each litter).

G^-L^-) were significantly retarded in every characteristic when compared with the control (G^+L^+) group and in almost all characteristics when compared with the G^+L^- mice. At 3 and 5 d the G^-L^+ mice were significantly behind the 20's but had overtaken them by 14 d. Postnatal undernutrition significantly retarded development in pups from large litters from the 9th day (column 4) while the G^+L^- groups were not significantly affected until 14 d old (column 1).

Avoidance learning

Conditioned avoidance performance in the shuttle-box was studied when the mice were 8–11 weeks old. Learning curves are shown in Fig. 4 and the results are summarized in Table 4.

All nutritionally deprived groups achieved significantly fewer successful avoidances than the controls (G^+L^+), although only the 20's were significantly worse than G^+L^- mice (t 2·31, $P < 0·05$).

Effects of early handling

The previous behavioural results were obtained using mice which had been handled in early life, during the development studies. To test for the possibility of an interaction between the effects of early handling and early nutritional deprivation, avoidance performance and body-weights of handled mice were compared with those of mice subjected to early nutritional deprivation but otherwise left undisturbed until weaning.

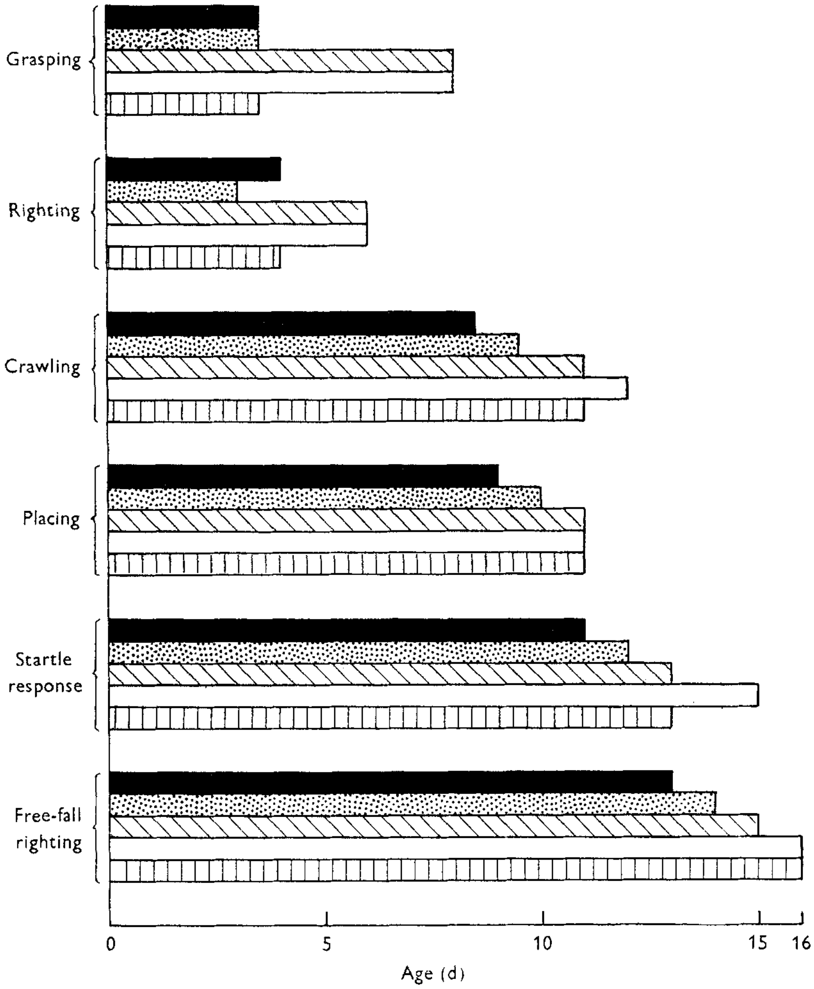

Fig. 3. The effect of different pre- and postnatal feeding regimens on the age of mouse pups when they achieved a mean group score of 2 (on a 0–4 development scale) for six behavioural reflexes: grasping, righting, crawling, placing, startle response and free-fall righting. ■, G^+L^+ (pups born to and reared by well-nourished mothers); ░, G^+L^- (pups born to well-nourished mothers but reared by malnourished mothers); ▧, G^-L^+ (pups born to malnourished mothers but reared by well-nourished mothers); □, G^-L^- (pups born to and reared by malnourished mothers); ▥, 20's (pups born to and reared by well-nourished mothers, but with twenty pups in each litter).

Early handling was associated with significantly higher body-weights in two of three comparisons (Table 5). A two-way analysis of variance with handled mice *v.* non-handled mice as one factor, and with early nutritional status as the other, showed that body-weight was significantly affected by early nutritional status ($P < 0·01$) but there was no interaction, suggesting that early handling did not differentially influence control and malnourished groups.

The previously handled mice performed the avoidance task slightly, but not significantly, better than their non-handled counterparts (Table 6). Analysis of

Table 3. *The significance of differences in maturation of physical features and reflex behaviour between groups* of mice subjected to different pre- and postnatal feeding regimens*

	Significance of difference† (*P*) for:										Day of testing
	$G^+L^+ > G^+L^-$	$G^+L^+ > G^-L^+$	$G^+L^+ > G^-L^-$	$G^+L^+ >$ 20's	$G^+L^- > G^-L^+$	$G^+L^- > G^-L^-$	$G^+L^- >$ 20's	$G^-L^+ > G^-L^-$	20's $> G^-L^+$	20's $> G^-L^-$	
Ears unfolding	—	0·01	0·01	—	0·01	0·01	—	—	0·05	0·01	3
Fur appearance	—	0·01	0·01	—	0·01	0·01	—	—	0·01	0·01	5
Eye opening	—	0·01	0·01	0·01	0·01	0·01	0·05	—	—	—	14
Grasping	—	0·01	0·01	—	0·01	0·01	—	—	0·01	0·01	3
Righting	—	0·05	0·05	—	0·01	0·01	—	—	—	—	5
Auditory startle	—	0·01	0·01	0·01	0·05	0·01	0·01	—	—	—	12
Crawling	—	0·01	0·01	0·01	0·01	0·05	0·05	—	—	—	9
Vibrissae placing	—	0·01	0·01	0·01	0·01	0·01	0·01	—	—	—	12
Free-fall righting	0·01	0·05	0·01	0·01	—	0·01	0·01	0·01	0·01‡	—	14

G^+,well-nourished during gestation; G^-, malnourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's, well-nourished, twenty pups/litter,

* For details, see p. 374.

† Difference expressed in terms of the retardation of the 2nd group relative to the 1st.

‡ This difference was opposite to that indicated i.e. the 1st group was retarded relative to the 2nd. During the 1st few days of life, development of the G^-L^+ mice was retarded in comparison with the 20's, but they grew rapidly and overtook the 20's by 14 d of age.

variance showed that effects due to early nutritional status were significant ($P < 0·01$) but there were no significant handling effects or interaction.

Regional brain weights and DNA content

Prenatal nutritional deprivation had more effect on forebrain weight than did deprivation during sucking (Table 7). Similar results have been reported by Smart, Dobbing, Adlard, Lynch & Sands (1973). Malnutrition during sucking had more effect on cerebellum weight than on forebrain weight. Both postnatally malnourished groups (20's and G^+L^-) had similar patterns of brain- and body-weight reductions although the 20's were more severely affected. Regional reductions in brain DNA content were similar to those for wet weight (Table 8). Again, the forebrain DNA (cell number) was most affected by prenatal deprivation while the cerebellum DNA content was equally diminished in G^-L^+ and G^+L^- groups (although the G^-L^+ mice were well-nourished and were growing rapidly throughout the cerebellum growth spurt). Winick (1970) and Smart *et al.* (1973) found in rats that under-nutrition throughout gestation and lactation produced a greater reduction in cell number than in either of these periods alone. In mice this 'synergistic' effect was not found with our technique of nutritional deprivation.

DISCUSSION

The different pre- and postnatal feeding regimens and the consequent malnutrition had markedly different effects on birth weight, development and adult behaviour. It

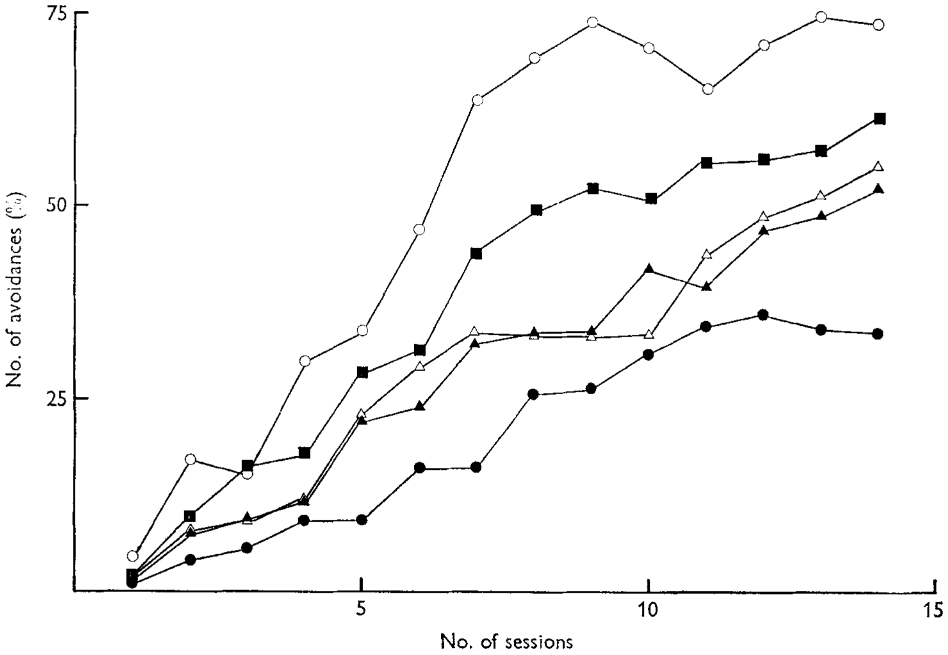

Fig. 4. The effect of different pre- and postnatal feeding regimens on the avoidance-conditioning performance of adult mice (8–11 weeks old). —○—○—, G^+L^+ (born to and reared by well-nourished mothers); —■—■—, G^+L^- (born to well-nourished mothers and reared by malnourished mothers); —△—△—, G^-L^+ (born to malnourished mothers and reared by well-nourished); —▲—▲—, G^-L^- (born to and reared by malnourished mothers); —●—●—, (born to and reared by well-nourished mothers, but with twenty pups in each litter).

Table 4. *Effects of different pre- and postnatal feeding regimens on avoidance performance of mice at* 8–11 *weeks of age*

Group*	No. of mice	No. of successful avoidances (%) Mean	SE	*t*†	Significance of difference between groups: *P*
G^+L^+	16	50·8	3·3	—	—
G^+L^-	22	38·4	4·5	2·19	$< 0·05$
G^-L^+	12	29·7	5·8	3·14	$< 0·01$
G^-L^-	21	29·1	4·2	4·02	$< 0·01$
20's	19	22·7	4·7	4·88	$< 0·01$

G^+, well-nourished during gestation; G^-, malnourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's, well-nourished, twenty pups/litter.

* For details, see p. 374.

† *t* Values were calculated by comparing each group with the G^+L^+ mice.

has been suggested (Smart & Dobbing, 1971*b*) that reflex ontogeny is unaffected in prenatally undernourished rats fostered onto well-nourished dams, although Simonson *et al.* (1968), using a similar model, found significant delays in the appearance of most developmental indices. Our experiments show that in mice, postnatal development is severely retarded by feeding the mother on a low-protein diet during the last 2 weeks

Table 5. *Effects of early handling and different pre- and postnatal feeding regimens on body-weight of mice*

Group*	No. of mice	Body-weight at 56 d (g)		t	Significance of difference between values for handled and non-handled groups: P
		Mean	SE		
G^+L^+					
Handled	17	40·3	0·78	1·25	NS
Non-handled	23	39·1	0·51		
G^+L^-					
Handled	22	34·8	0·49	2·11	$< 0·05$
Non-handled	23	33·4	0·49		
20's					
Handled	19	33·1	0·49	3·387	$< 0·01$
Non-handled	23	30·7	0·45		

NS, not significant; G^+, well-nourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's, well-nourished, twenty pups/litter.

* For details, see p. 374.

Table 6. *Effects of early handling and different pre- and postnatal feeding regimens on shock-avoidance performance of mice at* 8–11 *weeks of age*

Group*	No. of mice	No. of successful avoidances (%)		t
		Mean	SE	
G^+L^+				
Handled	16	50·8	3·3	1·31
Non-handled	23	43·7	4·1	
G^+L^-				
Handled	22	38·4	4·5	1·05
Non-handled	22	32·5	3·4	
20's				
Handled	19	22·7	4·7	0·36
Non-handled	23	20·6	3·4	

G^+, well-nourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's well-nourished, twenty pups/litter.

* For details, see p. 374.

of gestation. If prenatally malnourished pups (G^-) were less vigorous than controls, it is possible that they would not suck efficiently, and therefore would not benefit immediately from cross-fostering onto well-fed mothers; but in all surviving litters, the pups' stomachs were bloated with milk from the 1st day of adoption. None of the pups used in the developmental study were premature but the possibility cannot be ruled out that during the first few days of life the prenatally deprived mice could not metabolize their mother's milk efficiently and so continued to be malnourished postnatally. Even so, a comparison of growth curves of G^-L^+ and G^-L^- mice shows

Table 7. *Effects of different pre- and postnatal feeding regimens on body-weights and wet weights of forebrain and cerebellum of mice at 80 d of age*

(Mean values with their standard errors, where given, for five mice/group)

Group†	G^+L^+	G^+L^-	G^-L^+	G^-L^-	20's
Body-wt					
(mg)	43·7±0·75	37·1±1·3	33·1±0·80	31·2±1·6	34·2±0·52
Relative to G^+L^+ value (%)	—	−15	−24	−29	−22
Value different (*t*) from that for group:					
G^+L^+	—	4·43**	9·80**	7·05**	10·52**
G^+L^-	—	—	2·62*	2·81*	2·02*
G^-L^+	—	—	—	1·05	1·14
G^-L^-	—	—	—	—	1·77
Forebrain wt					
(mg)	349±3·1	338±7·0	305±8·4	294±2·8	328±6·7
Relative to G^+L^+ value (%)	—	−3	−13	−16	−6
Value different (*t*) from that of group:					
G^+L^+	—	1·43	4·87**	13·26**	2·84*
G^+L^-	—	—	2·99**	5·74**	1·02
G^-L^+	—	—	—	1·21	2·09*
G^-L^-	—	—	—	—	3·11**
Cerebellum wt					
(mg)	71·6±2·6	65·2±1·1	61·0±1·6	57·0±1·0	61·4±1·5
Relative to G^+L^+ value (%)	—	−9	−15	−20	−14
Value different (*t*) from that for group:					
G^+L^+	—	2·23*	3·41**	5·17**	3·36**
G^+L^-	—	—	2·12*	5·48**	2·05*
G^-L^+	—	—	—	2·08*	0·18
G^-L^-	—	—	—	—	2·46*

G^+, well-nourished during gestation; G^-, malnourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's, well-nourished, twenty pups/litter.

* $P < 0·05$, ** $P < 0·01$; with 8 df $P = 0·05$, when $t = 1·86$; $P = 0·01$, when $t = 2·89$, by one-tailed tests.

† For details, see p. 374.

that those sucking from a well-fed mother grew faster and were significantly heavier within a few days of birth (Fig. 1).

Postnatal deprivation by sucking from a mother fed on the low-protein diet led to marked physical stunting even at 5 d old but had no discernible effects on reflex ontogeny until the 14th day. Undernutrition by rearing in large litters was much more severe. Stunting was more pronounced and delays in the appearance of developmental markers began at 7–9 d of age. We could find no behavioural characteristics maturing between 14 and 21 d, the period when effects of postnatal malnutrition were most marked.

As each behavioural characteristic developed over several days, the analysis in Table 3 was carried out when the Kolmogorov–Smirnov test was most sensitive to differences between the control and experimental groups. That is, when most of the G^+L^+ mice scored in the middle range of the 0–4 development scale (see Experimental section), and the scores for all groups were most widely spread. This method

Table 8. *Effects of different pre- and postnatal feeding regimens on DNA content in forebrain and cerebellum of mice at* 80 *d of age*

(Mean values with their standard errors, where given, for five mice/group)

Group†	G^+L^+	G^+L^-	G^-L^+	G^-L^-	20's
Forebrain DNA					
(mg)	377 ± 3·1	358 ± 7·9	332 ± 5·7	319 ± 3·5	359 ± 8·8
Relative to G^+L^+ value (%)	—	−5	−12	−15	−5
Value different (*t*) from that for group:					
G^+L^+	—	2·10*	6·96**	12·44**	1·89*
G^+L^-	—	—	2·93**	4·88**	0·02
G^-L^+	—	—	—	1·94*	2·62*
G^-L^-	—	—	—	—	4·27**
Cerebellum DNA					
(mg)	381 ± 4·6	324 ± 6·5	320 ± 6·5	292 ± 10·0	324 ± 12·4
Relative to G^+L^+ value (%)	—	−15	−16	−23	−15
Value different (*t*) from that for group:					
G^+L^+	—	7·18**	7·60**	8·13**	4·23**
G^+L^-	—	—	0·44	2·77*	0·02
G^-L^+	—	—	—	2·44*	0·23
G^-L^-	—	—	—	—	2·01*

G^+, well-nourished during gestation; G^-, malnourished during gestation; L^+, well-nourished during lactation; L^-, malnourished during lactation; 20's, well-nourished twenty pups/litter.

Difference between mean values was statistically significant: * $P < 0·05$, ** $P < 0·01$; by one-tailed tests.

† For details, see p. 374.

of analysis could obscure differences between the retarded groups, particularly between G^-L^+ and G^-L^-, and accordingly all possible comparisons were made over the whole period of development for each physical and behavioural characteristic. These analyses did not reveal other significant differences between any of the groups.

The physical consequences of perinatal undernutrition may perhaps most profitably be analysed on the basis of the vulnerable period hypothesis. In its simplest form this hypothesis states: 'If a developmental process be restricted by any agency at the time of its fastest rate, not only will this delay the process, but will restrict its ultimate extent, even when the restricting influence is removed and the fullest possible rehabilitation obtained' (Dobbing, 1968).

Our results confirm the predictions of this hypothesis in almost all respects. Peak growth rate of the mouse brain occurs between the 5th and 15th days after birth and undernutrition during this period produced marked reductions in body-weight, brain weight and brain cell number (see Tables 7 and 8). Undernutrition after weaning had no effect on the final body-weight or brain weight. The growth patterns and eventual physical deficiencies of the prenatally deprived mice (G^-L^+), however, did not fit the hypothesis as well. During the period of the brain growth spurt they grew faster than both postnatally undernourished groups, in fact, their growth rate was almost the same as that of the control group (Fig. 1). However, their ultimate body-weight, brain weight and brain cell number were all below those of the mice subjected to postnatal undernutrition alone (Tables 7 and 8). The most likely explanation for this finding is that vulnerability is not limited simply to the peaks of each developmental process but is a continuous state from organogenesis until at least the peak of the growth spurt.

Simonson, Stephan, Hanson & Chow (1971) and Smart *et al.* (1973) showed that prenatal growth restriction does influence subsequent adult behaviour in the rat. The present investigation shows that the mouse is also affected. Again, prenatal restriction (G^-L^+) had more effect than postnatal restriction (G^+L^-) but this time the effects were not additive (i.e. the G^-L^- and G^-L^+ mice reached similar levels of avoidance performance). The 20's produced by a long way the poorest performance, tending to confirm suggestions (Rajalakshmi & Ramakrishnan, 1972; Leathwood *et al.* 1974) that the behavioural effects of rearing in large litters arise from a combination of nutritional and social factors.

In this study, the early handling involved in the developmental study had no significant influence on avoidance behaviour. However, the consistent, but slight, improvement it did produce justifies further investigation of the relationship between early nutritional deprivation and early handling.

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