The Effects of Chronic Undernutrition Over Generations on Rat Development[1]

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ABSTRACT Experimental rats were fed 2/3 (10 g/24 hours) of ad libitum diet throughout pregnancy and post-weaning, thus far for six generations; their brain and body development was compared with those of controls fed ad libitum (15.5 g/24 hours). As expected from previous reports, neonatal F_1 offspring exhibited highly significant decreases in body weight, cerebral wet weight, cerebral DNA and cerebral protein. However, neonatal decreases were not greater in F_2 through F_6 than in F_1 indicating that there was no cumulative effect of this undernutrition on offspring's parameters over generations. Maternal body weight at mating (90 days) and percentage of females that did not litter steadily decreased over generations. The observed high mortality in F_1 through F_6 and the resulting strong natural selection in favor of best mothers and weanlings could explain these findings. The phenomena contributing to high mortality are multiple and involve maternal factors during pregnancy and before weaning, as well as offspring factors. J. Nutr. *108:* 1719–1723, 1978.

INDEXING KEY WORDS chronic undernutrition · undernutrition, chronic · malnutrition, chronic · brain development · undernutrition over generations

The effects of *acute* maternal malnutrition on body and brain development of the offspring in one generation have been by now the subject of many studies. However, the effects of maternal malnutrition over several generations did not attract the attention which this problem deserves in view of its implications for the incidence of human malnutrition. Previous publications from our laboratory (1–3) as well as the work of Cowley and Griesel (4) have demonstrated that brain underdevelopment caused by prenatal malnutrition in females of one generation (F_1), can be transmitted to the next generation (F_2), even in absence of postnatal malnutrition of F_1. It was suggested (1–3) that this effect was due to various defects [kidneys (5), endocrines (6)] in the F_1 females, caused by prenatal malnutrition; these F_1 females then became defective mothers for F_2. With reference to *chronic* (continued) malnutrition over several generations, in a series of papers Stewart (7) has reported that the birth weight of offspring of rats fed a marginally low protein diet is not lower in subsequent generations than it is in the first generation. The values of brain parameters throughout generations were not studied.

The purpose of our work was to reassess the problem of chronic malnutrition through generations, using a somewhat different dietary regime, and studying in particular *brain* parameters in consecutive genera-

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TABLE 1

Assessment of newborn rats following chronic undernutrition[1]

Group	Offspring generation	Number[2]	Body weight	Cerebrum Weight	Cerebrum DNA	Cerebrum Protein
			g	*g*	*mg*	*mg*
Control		429	6.04±0.54	0.177±0.013	0.578±0.048	8.72±1.39
Exper.	F_1	22	5.05±0.5[a]	0.147±0.013[a]	0.470±0.048[a]	7.79±0.96[a]
	F_2	18	4.88±1.09[a]	0.148±0.019[a]	0.484±0.075[a]	7.71±1.40[b]
	F_3	37	4.85±0.66[a]	0.148±0.015[a]	0.547±0.024[a]	7.57±1.22[a]
	F_4	36	4.98±0.55[a]	0.139±0.011[a]	0.536±0.027[a]	7.88±0.79[a]
	F_5	31	5.54±0.68[a]	0.150±0.017[a]	0.513±0.046[a]	8.16±0.98[b]
	F^6	40	5.79±0.43[b]	0.160±0.013[a]	0.556±0.030[a]	8.73±0.72

[1] Mean±SD. [2] Number of rats examined. [a] Significant at $P < 0.001$ level with respect to control. [b] Significant at $P < 0.01$ level with respect to control.

tions. In this paper we report the studies on newborn rats; the study of older rats and of rehabilitation after chronic malnutrition will be reported at a later date.

MATERIALS AND METHODS

The rats and their nutrition regimes were similar to those described in the previous work (1–3, 8, 9). The rats were Sprague-Dawley derived, and were bred in our closed colony for 38 generations. Virgin females 3 months old and weighing 200 to 260 g were mated; the presence of a vaginal plug was considered day 0 of pregnancy. The control group C was fed ad libitum (15.5 g/24 hours) a pelleted diet [2] containing 20.5% protein. The experimental rats were fed 2/3 or 10 g/24 hours of ad libitum diet, which is a rather mild protein/energy malnutrition. This feeding in experimental group was started at the time of mating of F_0 generation, and continued throughout pregnancy and post-weaning.[3] In preliminary experiments we found that malnutrition of this kind during nursing may result in 100% mortality, and therefore the mothers and offspring were fed ad libitum during the period 0 to 60 days in F_1, and 0 to 15 days in all subsequent generations. At birth or at various times thereafter the rats were weighed and decapitated; the brains were then immediately removed, weighed, frozen and subsequently used for analysis. The "brain", as dissected, was cerebrum without cerebellum and olfactory lobes.

DNA was determined by a modification of the diphenylamine colorimetric method (10, 11); protein was determined by a modification of the Lowry colorimetric method (12).

RESULTS AND DISCUSSION

The results for newborns F_1 to F_6 are represented in table 1. As can be seen here, and as expected from our previous reports, even this relatively mild malnutrition produced significant decreases in neonatal body weight, cerebral weight, cerebral DNA, and cerebral protein. This decrease is highly significant even in F_1 although undernutrition was started only as late as at mating. What is of interest is that there was no cumulative effect of this chronic undernutrition over six generations.

Table 2 represents postnatal body weight gains for the offspring. The experimental rats (F_1 to F_5) lag behind the controls. At 21 days, F_2 to F_5 show more severely decreased body weights than F_1. The mean body weights of the female rats at day 0 of pregnancy were: Controls 219 ± 22 g, F_0 226 ± 22 g, F_1 204 ± 24 g, F_2 198 ± 21 g,

[2] Pelleted diet (2882 calories/1,000 g) was Wayne Mousebreeder Block, supplied by Allied Mills, Chicago, Ill.

[3] The weaning in our colony is at 30 days.

TABLE 2

Postnatal body weights (g)

Group	Generation	Number	Age, days[1] 1	7	10	21
Control		130	6.9 ±0.5[2]	15.5±2.3	21.0±4.1	63.4± 8.7
Exper.	F_1	211	5.05±0.5 (−27)[a]	12.1±2.8 (−22)[a]	18.6±4.3 (−11)[a]	40.4±13.8 (−36)[a]
	F_2	104	5.9 ±1.0 (−14)[a]	12.4±3.1 (−20)[a]	17.6±4.0 (−16)[a]	33.2±13.1 (−48)[a]
	F_3	240	5.6 ±0.5 (−19)[a]	14.1±2.4 (−9)[a]	19.8±3.8 (−6)[b]	35.1± 7.1 (−45)[a]
	F_4	210	5.0 ±0.6 (−27)[a]	11.9±2.1 (−23)[a]	17.3±3.6 (−18)[a]	32.3± 7.2 (−49)[a]
	F_5	358	5.7 ±0.6 (−17)[a]	14.2±3.6 (−8)[a]	21.4±4.3 (+2)	33.0± 7.6 (−48)[a]

[1] In parentheses: difference to control, in percent of control. [2] Mean±SD. [a] Significant at $P < 0.001$ level with respect to control. [b] Significant at $P < 0.01$ level with respect to control.

F_3 198 ± 19 g, F_4 198 ± 22 g, F_5 225 ± 25 g. Thus, in F_1 to F_4 the rats had a decreased energy requirement for basal metabolism since their adult body weight did not reach normal control weight. That a natural selective process is favoring rats with a better food utilization is suggested by the results in the F_5 generation, in which the body weight has reached control values, in spite of five generations of chronic undernutrition.

Table 3 represents energy requirements for basal metabolism of the control and experimental mothers. Because of lower maternal body weight, the actual energy requirement was higher in F_0 than in F_1 and in subsequent generations (except in F_5); this, in further generations the mother becomes less handicapped.

The change in some other parameters throughout generations is shown in table 4. The percentage of females that did not litter steadily decreased from F_0 to F_4. On the other hand, litter size has decreased after maternal generation F_0, in agreement with reports on wild animal populations during prolonged periods of starvation; this results in some improvement in birth weight. The mortality before weaning as compared with the control was very high. Similar results were obtained by Stewart et al. (13). The high mortality and the resulting strong natural selection in favor of best mothers and best weanlings could par-

TABLE 3

Energy requirements for basal metabolism of control and experimental mothers

Group	Food intake	Generation	Newborns per litter	Energy[1] required for mother and her fetuses	Total excess[2]
	kcal			*kcal*	*kcal*
Control	966	(47)[3]	9.2±2.3	532	434
Exper.	592 in 22 days	F_0 (44)[3]	10.0±2.6	504	88
		F_1 (68)	8.3±1.6	479	113
		F_2 (32)	8.4±1.9	482	110
		F_3 (43)	8.1±2.7	473	119
		F_4 (46)	7.9±2.3	475	117
		F_5 (49)	8.3±2.1	519	73

[1] Energy requirement for basal metabolism calculated from Kleiber's formula $70 \times (W)^{\frac{3}{4}}$, in kcal/day, where W is body weight in Kg. [2] Excess of food intake over requirement, in kcal. [3] Numbers of litters.

TABLE 4

Outcome of pregnancy and mortality over generations following chronic undernutrition

Group	Generation		Mothers that did not litter	Newborns per female	Stillborn	Mortality between days	
	Mother[1]	Offspring[1]				0–30	30–90
			%		%	%	
Control	(60)	(429)	23	9.2	2.6	18	22
Exper.	F_0 (77)		43	10.0			
		F_1 (440)			1.8	42	26
	F_1 (88)		23	8.3			
		F_2 (563)			2.1	66	9
	F_2 (37)		14	8.4			
		F_3 (270)			2.5	34	39
	F_3 (51)		16	8.1			
		F_4 (346)			4.3	50	0
	F_4 (48)		4	7.9			
		F_5 (358)			4.5	51	28
	F_5 (52)		6	8.3			
		F^6 (398)			10.2	64	

[1] Generation and total number of rats.

tially explain some of the findings such as near normal mortality rates during age 30 to 90 days and the elimination of mothers that do not maintain pregnancy.

All these results indicate that in the offspring of undernourished mothers there is a sharp decline in the first generation, but no progressive deterioration in future generations. High mortality and the resulting

TABLE 5

Survivors in experimental groups through generations

Generation	Age	Survivors[1]
F_1	Newborns	80
	30 days	58
	90 days	55
F_2	Newborns	49
	30 days	20
	90 days	24
F_3	Newborns	24
	30 days	19
	90 days	15
F_4	Newborns	14
	30 days	9
	90 days	11
F_5	Newborns	11
	30 days	7
	90 days	6

[1] In percentage of control animals surviving to the same stage.

strong natural selection was offered as a partial explanation of this phenomenon. The other trends are: decrease in body weight of the mother, with concomitant decrease in basal metabolism requirement and saving on food requirement; and possible adaptive phenomena such as better food utilization, including better intestinal absorption and lower destruction of essential amino acids, both known to occur during starvation.

The overall phenomenon is actually quite complex, because of the multitude of factors involved. They include:

Maternal factors

During pregnancy. Some females do not litter, presumably embryos or fetuses were resorbed; in litters carried to term there is an increased incidence of still births.

Before weaning. Unfit females die, and so do their litters; surviving females partially cannibalize their litter; surviving weak neonates do not get enough milk and die of starvation.

Offspring factors. Between days 30 and 90 young rats that are weaker or have less efficient metabolism die; undernourished rats have higher susceptibility to diseases and therefore higher mortality rates.

Our calculations are based on the actual data, such as proportions of rats that did not litter, litter size, percentage of stillborns, neonatal mortality rates, etc.: the hypothetical losses at specific developmental stages if there had been 100 control and 100 experimental F_0 females mated are shown in table 5.

The data indicate that the highest losses occurred: First, in F_0, because of females that did not litter (20% loss); then before weaning of F_1 generation (22% loss), and also before weaning of F_2 generation (29% loss). In F_5 at 90 days, the numbers of survivors in the experimental group was only 6% of that in the controls.

It appears, then, that a multitude of factors contribute to strong natural selection that is responsible for lack of progressive deterioration; nevertheless, the undernourished population can be readily distinguished from the normal, if only on the basis of its smaller size and higher mortality.

It is of interest that the effect of chronic administration of tritiated water through generations gives essentially similar picture (no cumulative effect).[4]

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