Effects of Prenatal and Chronic Undernutrition on Aging and Survival in Rats[1]

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ABSTRACT The effect on aging and survival of mild (⅔ of ad lib) chronic prenatal and postnatal undernutrition over 10 generations, or prenatal undernutrition only has been studied in rats. Both regimes of undernutrition used decreased body weights of animals as compared to the controls. Both undernourished groups had lower cholesterol levels and tumor incidence than the controls. The effects on survival depended not only on the time period of undernutrition but also on the age of the animals examined: chronic undernutrition resulted in lower survival of young adult animals (4–8 months) but higher survival than the controls in old age. Prenatal undernutrition only had no effect on young adult animals, gave higher survival than the controls at ages 8–18 months but considerably lower survival than controls in the old age: the underdevelopment of vital organs whose cells proliferate only before birth might have been the cause of such decreased longevity. J. Nutr. *112:* 972–977, 1982.

INDEXING KEY WORDS prenatal undernutrition · chronic undernutrition · aging · aging and nutrition · aging and survival · longevity and undernutrition

Reports from several other laboratories have suggested that in the rat *postnatal* undernutrition in general leads to an increased life span (reviews in refs. 1, 2). However, not all the reports uniformly agree with this conclusion. Widdowson and Kennedy (3) found that underfeeding during the suckling period does not prolong life span but rather shortens it. A fixed diet high in protein and low in carbohydrate, when administered at a young age, was associated with a higher life expectancy compared with that obtained with diets containing relatively lower levels of protein (4). However, one must bear in mind that any potential benefit accruing from a diet during one stage of life could well be in part or entirely reversed by its life-shortening effect at a later age period (2). In general, animals that grow rapidly have a reduced life span (5), and an inverse relationship between body weight and longevity has been reported (1).

All these studies refer to postnatal undernutrition only. In our work we were interested in also studying the effects of aging on prenatal undernutrition alone or as a combination of prenatal and postnatal undernutrition for several generations; both of these undernutrition regimes as such were the subjects of several previous reports from our laboratory, in connection with problems other than aging (6–9).

MATERIALS AND METHODS

The rats were Sprague-Dawley derived, and were bred in our closed colony for 38 generations. The animals were housed two per cage. The vivaria, which housed only the animals used in this study, were temperature-

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(23.5°) and humidity-(62%) controlled. The vivaria light schedule was 12-hours light, 12-hours darkness. Virgin females 3 months old and weighing 200–260 g were mated; the presence of a vaginal plug was considered day 0 of pregnancy. The control group (969 animals), run concurrently with the experimental groups, was fed ad libitum (15.5 g/24 hours) a pelleted diet containing 20.5% protein. The diet, 2882 calories/1000 g, was Wayne Mousebreeder Block (Allied Mills, Chicago, IL). The experimental rats in one group (chronic undernutrition for 10 generations, 829 offspring animals) were fed ⅔ of ad libitum diet or 10 g/24 hours, which is a rather mild protein/energy malnutrition. The results for individual generations were not statistically different, and therefore they were pooled across generations. In the experimental group this feeding was started at the time of mating of F_0 generation, and continued throughout pregnancy and postweaning.[2] 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 0–60 days in F_1, and 0–15 days in all subsequent generations. The mothers of rats in another experimental group (prenatal undernutrition only, 104 offspring animals) were also fed ⅔ of the ad libitum food intake, but food restriction started when the animals were 60 days old, and continued until and throughout pregnancy. All food restriction regimes were on a 7 days/week schedule. The rats were weighed at birth and at indicated ages.

Blood serum cholesterol was analyzed with the automatic blood chemistry analyzer Accu-Stat (Clay-Adams, Parsippany, NJ). The data were analyzed statistically by means of the Student's paired *t* test. Both females bearing litters and those not bearing litters were included.

Pathological examination (necropsy procedure). Animals that were autolyzed were routinely excluded from any further necropsy procedure. The rats were examined for external lesions, and their general appearance and weight at the time of death were recorded.

After this examination, the rats were opened by midline incision. The following subcutaneous structures were examined for gross abnormalities: mammary tissue, superficial lymph nodes and subcutaneous fat deposits. After this examination, particular emphasis was on the gross and, in selected cases, the microscopic examination of internal organs, in particular the lung, liver and kidneys.

Tissue samples selected for microscopic examination were routinely fixed in 10% phosphate-buffered formalin, embedded, sectioned at 40 μm, and stained with hematoxylin-eosin or other appropriate stains.

RESULTS AND DISCUSSION

a) Pathological findings: macro-microscopic examination. Lesions in the examined livers were the commonly found age-associated alterations in hepatocytes: increased cell size within the foci, with small dark-staining nuclei. These alterations in cell size were found to the same extent in age-matched controls as in both undernourished groups of animals.

A high incidence (23%) of cyst formations in the liver has been found in the control animals. Forty-eight females were autopsied, all past 16 months of age: 11 females were found to have some cyst formation. It appeared that these cysts were benign.

In comparison, 16 females in the undernourished groups past 16 months of age were examined, and only one female had cyst formation (7%).

b) Incidence of spontaneous tumors. Tumors were only found in females that had one litter (none were found in virgins). The youngest control female with a tumor was 10 months old, whereas in the chronic malnourished group, one female developed a tumor at 4 months of age. The tumors examined were benign and were of epithelial origin. The incidence of tumors is listed in table 1. The average of tumor onset was 17.85 months for controls, 15.9 for chronic undernutrition, and 14.2 for prenatal undernutrition only; these differences between the undernourished groups and the controls were not statistically significant. No differences were found in tumor incidence among each

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

TABLE 1

Incidence of tumors

Group	n^1	Animals with tumors	Statistical significance
		%	
Control	755	7	
Chronic undernourished	325	2.1	$P < 0.001$
Prenatal	103	5.8	$P < 0.01$

[1] *n*, Total number of animals observed.

of 10 generations of chronic undernutrition. These studies should be considered preliminary, as more data are needed on this important subject.

c) Cholesterol. In view of the implied correlations between blood serum cholesterol levels and atherosclerosis, these levels were also investigated in our aging animals. The results are listed in table 2. No significant differences were found between these levels in males and females; the data for males and females were therefore pooled. Cholesterol levels rose with advancing age ($P < 0.001$). Both undernourished groups had lower levels of cholesterol than the controls; however, only in chronic undernutrition group was the difference to control statistically significant.

d) Weights of animals. The weights of animals in the three groups (control, prenatal undernutrition only and chronic undernutrition) at various ages, are represented in figure 1 (males) and figure 2 (females). The birth weights (both sexes together) were: control, 6.9 ± 0.5 g; chronically undernourished (all generations combined), 5.45 ± 0.81 g; prenatally undernourished only 6.00 ± 0.7 g. The weaning weights (30 days) were (females): control, 66.7 ± 12.0 g; chronically undernourished, 49.24 ± 14.28 g; prenatally undernourished only, 65.37 ± 13.0 g. The chronically undernourished males were significantly ($P < 0.001$ at all ages) lighter than the controls. It is of interest that while the controls continued to gain weight, the chronically undernourished males have actually started to lose weight after 22 months of age. The males that were mildly undernourished before birth only were also statistically significantly lighter until about 4 months of age; later on the weight deficiency became nonsignificant until about 18 months of age when they rapidly began to lose weight. Thus, a mild undernutrition before birth only, can have a profound effect on body weights late in life. The deficiencies in the females (figure 2) were not as pronounced as in the males ($P < 0.001$ until 28 months for "chronic" and not significant for "prenatal"); possibly, the females adapted better to the stress of undernutrition (9).

TABLE 2

Blood serum cholesterol levels

Group	n^1	Age	Cholesterol level	Significance[2]
		months	*mg/100 ml*	
Control	19	3	89 ± 25	
Control	15	20.1 ± 1.4	149 ± 28	$P_1 < 0.001$
Chronic undernourished	29	21.6 ± 1.1	122 ± 23	$P_2 < 0.01$
Prenatal undernourished	19	19.4 ± 1.3	127 ± 28	N.S.

[1] *n*, Total number of observations. [2] P_1, statistical significance compared to control 3 months old. P_2, statistical significance compared to control 20.1 months old. N.S., not significant.

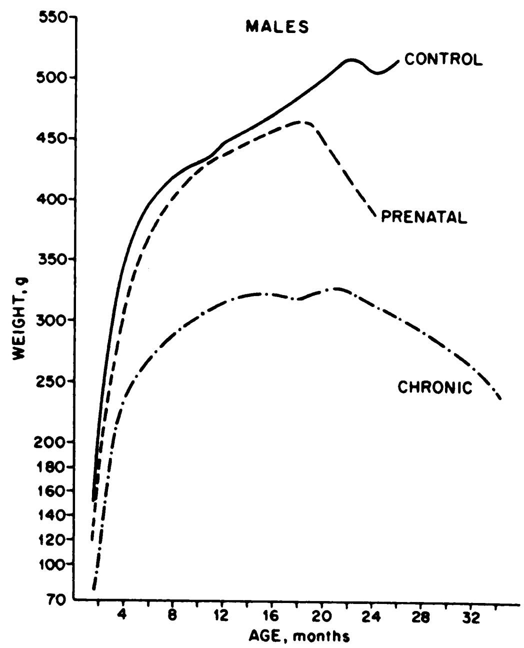

Fig. 1 Weight of males in control, chronically undernourished, and prenatally only undernourished groups. Abscissa, age in months; ordinate, weight in grams.

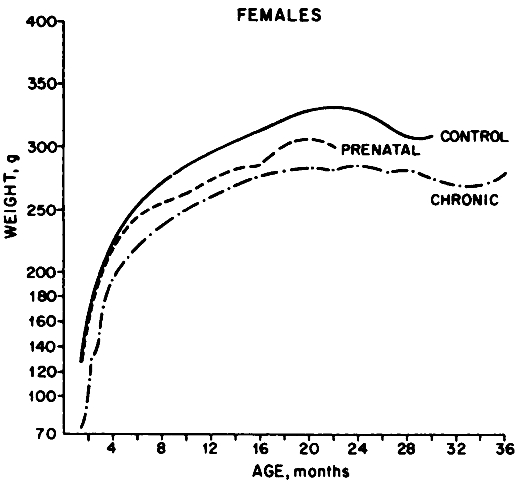

Fig. 2 Weight of females in groups as on figure 1.

e) *Longevity*. Mean ages at death are listed in table 3. It can be seen that the chronically undernourished animals (both sexes together; all generations pooled) have mean life span (mean age at death) significantly longer than the controls. The mean life span of prenatally undernourished animals was also longer than the controls, but the difference was not statistically significant (fewer animals).

The ages at various levels of survival are represented in table 4. In this and subsequent data, 100% is set at maturity (3 months). It can be seen that at high level of survival (75%), the chronically undernourished animals are at a definite disadvantage: they die sooner than the controls. This situation appears to be the continuation (tail end) of the period of high infant mortality in such chronically undernourished groups, as reported previously (9). In contrast, at lower survivals (50% and 25%), both chronically undernourished and prenatally undernourished animals are at a definite advantage: their survival times are longer. The age at zero survival (all animals dead) is shortest for prenatally undernourished animals (27 months), and longest for chronically undernourished (43 months). These results are also represented graphically in figure 3. It can be seen that at a young age, up to approximately 10 months, the chronically undernourished animals have highest mortality, but later on they show a definite advantage over the controls: all controls were dead at the age of 32 months, at which time 8 animals (0.97%) were still living in the chronic group. The average age of death for these 8 animals was 34.6 months; thus, one can estimate that chronic undernutrition increased the life span of approximately 1% of animals by 8% (2.6 months). The animals that were undernourished only before birth survive as well as controls up to approximately 8 months, survive longer than controls up to approximately 18 months, but then deteriorate rapidly and show survival shorter than controls after 20 months of age.

The survival of age groups 4–8 months, 9–12 months, etc., is represented in table 5. Young adult group (4–8 months) in the chronically undernourished group had the lowest survival; older adults (9–12 months) profited from both regimes of undernutrition, but above this age only the chronic undernutrition increased the survival, whereas the undernutrition only before birth considerably decreased the survival.

In evaluating all these results we must first state that any conclusions refer to the mild

TABLE 3

Mean age at death

Group	Age at death[1]	Difference to controls	Statistical significance
	months	%	
Control	13.73 ± 6.49		
Chronic undernourished	14.73 ± 7.78	+7.3	$P < 0.01$
Prenatal	14.44 ± 6.31	+5	N.S.[2]

[1] Means ± SD. [2] N.S., not significant.

TABLE 4

Survival times

Percent survival	Survival time: Control	Undernourished: Chronic	Undernourished: Prenatal
	months		
75	8	7	8
50	13.2	14.2	15.0
25	17.3	19.7	19.0
0	32.0	43.0	27.0

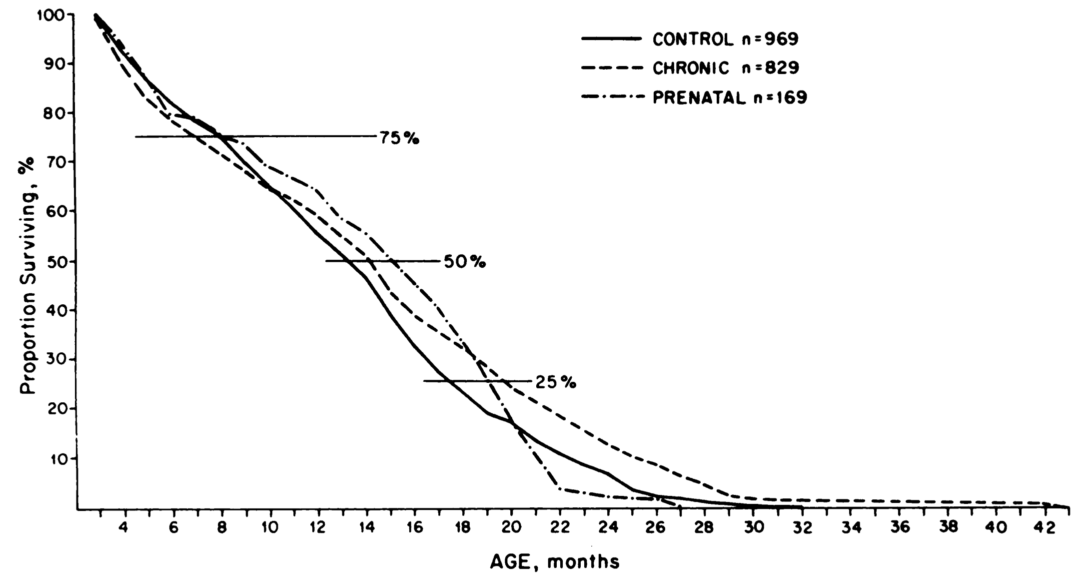

Fig. 3 Survival of controls and two undernourished groups at various ages. Abscissa, age in months; ordinate, proportion surviving, percent. 100% for all groups set at 3 months of age.

undernutrition regime used in this work; it is conceivable that other kinds and other periods of undernutrition will give different results. We must also point out that most of the results in tables 4 and 5 and figure 3 would look different if the survival 100% were set at birth instead of at 3 months of age (adulthood, or sexual maturity) as was done in this work. Which of these approaches is the "correct" one depends on our goals: in this work it was decided to study the aging of adult animals rather than the aging that begins at birth. The age at zero survival (tables 4 and 5, and fig. 3) would be, of course, the same in both approaches.

TABLE 5

Percentage of living animals in various age groups

Age group	Percent living: Control (n = 969)[1]	Undernourished: Chronic (829)	Undernourished: Prenatal (104)
months		%	
4–8	75.13	71.89	75.00
9–12	55.32	59.71	65.38
13–18	23.23	31.97	33.65
19–24	6.51	12.43	2.88
25–30	0.32	1.57	0
31–42	0	0	

[1] In parentheses, numbers of animals.

The first conclusion from the results is, that even excluding infant mortality (9), any given nutritional regime does not result in an unequivocal improvement or deterioration of survival: rather, it may result in an improvement at one age and a deterioration at another age (2). For instance, chronic undernutrition decreases the survival in young adults but increases the survival and the life span in older and senescent animals. In this context one might conclude that weight deficiency (figures 1 and 2) is harmful for survival in young adults but beneficial for survival in old and senescent animals.

Undernutrition only before birth produces different results. As mentioned (table 5 and figure 1) such undernutrition had practically no effect on survival of animals younger than 8 months; older animals appear at first to survive better than the controls, but after 18 months of age their survival becomes much worse than the controls. The weight deficiency of such prenatally undernourished males (figure 1) appears to be negatively correlated with the length of survival in younger animals but positively in senescent animals.

Thus, even such a short period (prenatal only)[3] of undernutrition had a profound effect at senescence. Such results are difficult to explain. One possibility is listed. In many organs (brain, pituitary, thymus) the cells proliferate mainly before birth. We have shown in the past that prenatal undernutrition results in a permanent deficiency in neuron number in cerebral cortex, and others have reported similar neonatal deficiencies in pituitary and in thymus (for a review, see ref. 10). Possibly, full development of these organs is essential for full life span; if so, then these early deficiencies may show at the end of life, even though the reduced body weights have had some advantages for younger adults.

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[3] Chronically undernourished animals had also a period of prenatal undernutrition; however, they are very different from the "prenatally undernourished" group in that: *1*) they underwent a rigorous selection [elimination of the "weakest" by high infant mortality (9)], and *2*) they gradually adapted to an undernutrition regime (9).