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Chromosomal abnormalities in starved and marginally malnourished rats and in utero upon rehabilitation

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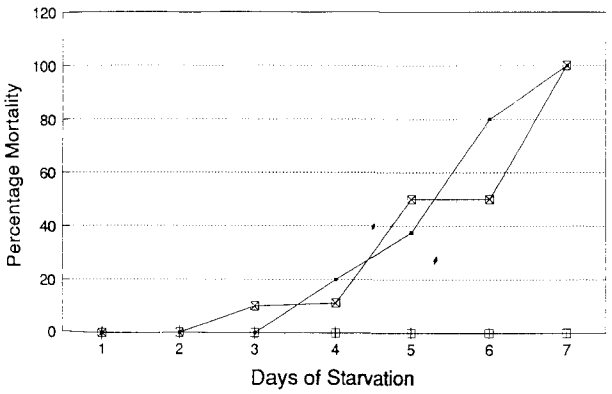
Abstract. The effects of starvation and marginal malnutrition (MN) on the lymphocytes of rats were evaluated by chromosomal analysis before and after rehabilitation. The effect of parental starvation or malnutrition on chromosomal aberrations in the foetus was also studied. Wistar rats, 30–35 days old, were starved for 5 days or fed a minimally restricted or a severely restricted diet for three weeks. At the end of the period of starvation or malnutrition, lymphocytes were isolated and chromosomal analysis was performed.
Starved and severely restricted rats showed significantly higher mean chromosomal aberrations than the controls. These aberrations returned to a normal level when the experimental groups were rehabilitated for a month, indicating that the damage was transient. A chromosomal aberration study done on foetal cells from rehabilitated rats which had previously been starved or fed a severely restricted diet showed significantly increased values, indicating that some damage was permanent. A low number of implantations was also recorded in these experimental groups. These observations clearly indicate that young animals exposed to conditions like starvation or chronic malnutrition are prone to permanent damage of the genetic system.
Key words. Starvation; marginal malnutrition; chromosomal aberrations; foetal chromosomal aberrations; rats.

Protein energy malnutrition (PEM) is one of the nutritional deficiency disorders most extensively studied. Studies on chromosomal aberrations (CA) in PEM children suggested both positive and negative effects[1–7]. Since PEM is commonly associated with other factors like vitamin deficiency, anemia or infection, the role of these factors in causing aberrations was also suspected[8], until experiments conducted in controlled environmental conditions on rats by Sadasivan and Raghuram[9] proved that PEM per se had caused the damage.
Microscopic examination of chromosomes for structural aberration is one of the established procedures for investigating the adverse effect of environmental agents on mammalian cells. Chromosome aberrations are due to lesions in DNA which lead to discontinuities of the DNA double helix. Depending on the time of induction within the cell cycle, the aberrations observed at the succeeding metaphase are of either the chromosome or the chromatid type[10]. Though a majority of the aberrations result in cell death, non-lethal events may also occur in the form of balanced exchange of material between two chromosomes, as reciprocal translocation. The available literature on chromosomal aberrations in malnutrition has been confined mainly to the possible occurrence of increased genotoxicity in severe PEM, but starvation and marginal malnutrition (MN) have not been examined. Therefore this study was designed to investigate the effect of starvation and MN on chromosome integrity, and also whether the offspring of pregnant rats previously starved and malnourished exhibit an altered frequency of CA in utero.

Materials and methods

Animals and diet. Healthy Wistar rats were obtained from the Institute's animal house. The rats were caged individually in polypropylene cages with paddy husk as bedding and a 12 h light, 12 h dark cycle. The temperature was 22–24 °C and the relative humidity 50–70%. All the rats were fed with purified pellet diet consisting of 22% protein (from wheat and casein) and the diet was complete with respect to vitamins and minerals. At 5 weeks of age, the animals were used for the experiment.
Starvation experiment. Animals were randomly divided into three groups with 22 males and 22 females in the control group and 30 males and 30 females in each of the test groups I and II. The weights of all the animals were recorded on day zero and every day thereafter until they were killed. Animals of groups I and II were subjected to starvation for 5 days. The 5-day period was chosen because a pilot study showed 50% mortality by the end of the 5th day (fig.). Controls were fed ad libitum. Clinical signs of starvation were recorded and deaths noted. After 5 days, 50% of the surviving animals of group I were sacrificed along with 8 males and 8 females from the control group, and the occurrence of CA investigated. The remaining animals of groups I and II were rehabilitated with the complete diet. After a



Effect of starvation on mortality in Wistar rats. Approximately 50% mortality is seen in starved rats (both sexes) by the end of 5th day. □, Control males; ⊞, control females; —■—, starved males; ⊠, starved females.

month, the animals of group I along with 7 males and 7 females from the control group were sacrificed for CA study. The animals of group II and the remaining controls (7 males and 7 females) were rehabilitated for three more months, after which they were paired within their groups and left to mate. Females were checked daily for the presence of a copulation plug and the day on which a plug was found was designated as day zero of gestation. Pregnant rats were killed by cervical dislocation between the 11th and 13th day of gestation. The uterine horns were opened and the number of surviving and dead embryos counted. A few live foetuses from each animal were processed for CA study.

Marginal malnutrition experiment. Animals were randomly divided into five groups with 6 males and 6 females in the control group and in groups I and II, and 14 males and 14 females each in groups III and IV. The control group was given a purified diet as described earlier, ad libitum. Groups I and III were given 80% (restricted diet) and groups II and IV were fed 60% (restricted diet) of the amount consumed by control rats. Weights of all rats were checked on day zero and then weekly, and the diet was revised accordingly. All groups were provided with drinking water. The animals were maintained on restricted diets for three weeks. Clinical signs of malnutrition were observed and deaths recorded. 6 males and 6 females from the control group along with all the live animals of groups I and II were sacrificed after three weeks and cytogenetic studies were done. Group I rats did not show significant cytogenetic changes, so the group III rats were discarded.

The group IV rats were rehabilitated for a month with the normal diet. One male in group IV died during the study. 6 males and 6 females from group IV and the same number from the control group were sacrificed to study chromosome aberrations. The remaining control animals and those of group IV continued to receive the control diet ad libitum for another two months, after which they were paired within their group in the ratio of 1 male:1 female. Two females in group IV died during rehabilitation and one male in this group was found to be sterile. The experiment was completed as described for the starvation experiment.

Foetal chromosome preparations. The foetus was horizontally divided into two and visceral tissues were collected in a petri dish. The tissue was transferred under aseptic conditions into sterile RPMI-1640 medium, and 1 g of tissue was then cut out and minced into smaller pieces in 0.2% collagenase, incubated for 2 h at 37 °C, and then washed with medium free of collagenase. The cells were processed for CA study.

Chromosome preparations. 1.5–2 h before sacrifice, animals were injected i.p. with colchicine (0.07 mg/100 g b. wt) to accumulate metaphases. Bone marrow cells from the femur of each animal were aspired into RPMI. The cell suspension was washed in 0.075 M KCl solution and fixed in methanol:acetic acid (3:1). Chromosomes were prepared by placing a drop of cell suspension on a cold slide and drying on a hot plate. Slides were then stained with Giemsa, mounted with De Pex and analysed.

Results

The frequency of occurrence of chromosomal aberrations in starved rats is presented in table 1. A total of 582 metaphases from starved males and 640 metaphases from starved females exhibited a mean aberration rate of 6.53% and 6.25% respectively, compared with 1.94% and 1.36% in control males and females. The difference between experimental and control groups was statistically significant. After a month of rehabilitation, 637 cells from 7 males and 656 cells from an equal number of females showed a mean aberration rate of 1.93% and 1.43% respectively against 1.41% and 1.37% shown by control males and females. These differences were not statistically significant.

Rehabilitated rats were paired and foetuses were analysed for CAs between the 11th and 13th day of gestation. The results (table 2) showed 53 embryos from 6 dams in the starved rehabilitated group with a mean percent aberration rate of 6.52 ± 0.95 (mean $\pm$ SD). In contrast, 7 control dams gave 65 embryos with a mean percent aberration rate of 1.14 ± 1.12. These differences were statistically significant.

Data on CA frequency in MN rats are shown in table 3. An aberration rate of 1.71% and 1.81% was found in 6 males and 6 females of the control group. In group I rats (on 80% of control diet) the rates were 1.32% and 1.69%. These differences were not significant statistically. However, the group II rats (on 60% of control feed) showed aberration rates of 5.18% and 5.00% in males and females respectively, which differed statistically by nearly 3-fold from the respective control values (table 3).

Table 1. Chromosomal aberrations (CA) in starved rats

Group	Sex	Number	Total cells analysed	Chromatid aberration Gap	Break	Chromosome aberration Gap	Break	Dicentric	Fragment	Polyploidy	Rings	No. of aberrations (without gap)	% aberrations
Control	M	8	826	5	3	3	3	1	5	4	0	16	1.94
	F	8	806	4	5	5	0	0	5	1	0	11	1.36
Experimental group I													
	M	7	582	7	9	4	6	4	13	6	0	38	6.53[a]
	F	7	640	5	9	5	3	5	16	7	0	40	6.25[a]
Upon rehabilitation													
Control	M	7	724	3	6	2	0	0	6	2	0	14	1.93
	F	7	700	4	7	1	1	0	1	1	0	10	1.43
Experimental group II													
	M	7	637	4	3	1	2	0	3	1	0	9	1.41*
	F	7	656	7	5	2	0	0	3	1	0	9	1.37*

[a]Statistically significant from controls at $p < 0.001$ by Student's t-test and Mann Whiteney 'U' test.
*Not statistically significant from controls at $p > 0.05$ by Student's t-test and Mann Whiteney 'U' test.

Table 2. In utero chromosomal aberrations (CA) in rehabilitated rats previously starved

Group	Number of pregnant dams	No. of dead embryos/ Total no. of embryos (11–13th day)	% of dead embryos recorded	% of chromosomal aberrations X ± SD
Control	7	1/65	1.54	1.14 ± 1.12
Experimental group	6	5/53	9.43*	6.52 ± 0.95[a]

*Chi-square analysis presented significant difference, $p < 0.025$.
[a]Significantly different from controls by Chi-square, $p < 0.05$.

Table 3. Chromosomal aberrations (CA) in marginally malnourished rats

Group	Sex	Number	Total cells analysed	Chromatid aberration Gap	Break	Chromosome aberration Gap	Break	Dicentric	Fragment	Polyploidy	Rings	No. of aberrations (without gap)	% aberrations
Control	M	6	642	4	3	1	0	0	4	4	0	11	1.71
	F	6	609	6	3	1	0	0	5	3	0	11	1.81
Experimental group I (on 80% of control feed)													
	M	6	604	2	1	1	1	0	4	2	0	8	1.32*
	F	6	591	3	2	1	0	1	3	4	0	10	1.69*
Experimental group II (on 60% of control feed)													
	M	6	617	11	7	4	5	3	11	6	0	32	5.18[a]
	F	6	600	9	8	5	4	3	10	5	0	30	5.00[a]
Upon rehabilitation													
Control	M	6	636	4	2	1	1	0	3	1	0	7	1.10
	F	6	600	5	1	0	0	0	3	1	0	5	0.83
Experimental group IV (on 60% of feed and on normal diet later)													
	M	6	607	3	3	1	0	0	2	1	0	6	0.99*
	F	6	591	6	2	2	0	0	3	1	0	6	1.02*

[a]Statistically significant from controls at $p < 0.001$ by Student's t-test and Mann Whiteney 'U' test.
*Not statistically significant from controls at $p > 0.05$ by Student's t-test and Mann Whiteney 'U' test.

After rehabilitation, group IV rats showed aberration values which did not differ from the control values.

A few of the MN rats (group IV) which had been rehabilitated and allowed to mate with males of the same group showed a significantly higher number of dead embryos (12.20% as against 3.77% in controls) (table 4). In addition, the mean percentage ±SD of the CA rate in their foetal cells was 7.00 ± 0.58 against 1.33 ± 0.75 seen in foetal cells from controls.

Table 4. In utero chromosomal aberrations (CA) in rehabilitated rats previously marginally malnourished (on 60% of control feed)

Group	Number of pregnant dams	No. of dead embryos/ total No. of embryos (11–13th day)	% of dead embryos recorded	% of chromosomal aberrations X ± SD
Control	6	2/53	3.77	1.33 ± 0.75
Experimental group	6	5/41	12.20[a]	7.00 ± 0.58[a]

[a] Significantly different from controls by Chi-square, $p < 0.05$.

The starved and MN (group II) rats in general showed both chromatid and chromosomal types of aberrations. A major percentage was constituted by gaps and fragments followed by breaks, polyploid cells and dicentrics. No rings or exchange figures were recorded in any group. On rehabilitation the aberration rate was not statistically significant compared to the controls.

Discussion

Analysis of bone marrow lymphocytes from starved and MN rats, both male and female, showed a frequency of CAs significantly higher than that in controls (tables 1, 3). After rehabilitation of both these groups for a month with normal diet, the rate of aberrations returned to a level within the control values, which demonstrated that the causative factor of CAs was nutritional deprivation and deficiency. Since the study was conducted under controlled experimental conditions, the possibility that infection and other associated factors like deficiency of folic acid and vitamin B_{12} played a role was ruled out. Sadasivan and Raghuram[9] also showed that rats malnourished under controlled experimental conditions had elevated CAs. The frequency of aberrations was found by Vijayalaxmi[11] to be inversely related to the protein content in the diet. The significant reduction in the percentage of aberrant cells in rehabilitated rats indicated that it was nutritional deficiency per se which caused the genetic damage.

Several explanations have been advanced for the high rate of CAs in PEM. Free radical formation on exposure to UV radiation could be a causative factor[12] for high CAs in PEM children, but this was not supported by observations of Betancourt et al.[6]. Free radical formation could not have been the causative factor for higher incidence of CA in our experiments because the rats were not exposed to UV at any time. Moreover, in the body free radicals can be detoxified by antioxidant enzymes[13,14].

Severe malnutrition depresses the immune system, resulting in defective T cell function. Whether increased frequency of CA has anything to do with decreased T cell function is not yet known[15]. Various mutagenic factors can easily produce chromosomal damage within a biochemically altered 'milieu', which could be caused by nutritional deficiency.

The effect of starvation and severe MN in the parent rats was reflected in a high level of CAs in foetal cells. There were also fewer implantation and a greater percentage of resorptions in the experimental groups compared to the controls. Further, pregnancy was delayed and 14% of the males were found to be sterile.

It has been found in other studies that undernutrition delays the maturation of the reproductive system and may impair its function after puberty. A low level of protein in the diet also delays puberty in both sexes[16]. Under extreme conditions of protein deficiency, the oestrus cycle in females ceases or becomes irregular. Fetal resorptions are common and the newborn are often weak or are born dead. The reproductive capacity of the male is impared, but little is known about the exact nature of these effects[17].

Experiments by Murthy[18] showed that the cells of foetuses of malnourished pregnant female rats exhibited an increased CA frequency, with an increase in the foetal mortality. Severe dietary protein restriction is known to affect foetal survival in pregnant rats and woman[19]. The role of PEM in causing CAs is not clear, but it was shown that PEM during pregnancy in rats caused a decrease in cellular DNA content and mitosis in foetal tissue[20]. Defective mitosis has been implicated in the etiology of structural aberrations seen in malnourished children and animals[8,11].

The most important difference between earlier studies and the present report is that we worked with rats which were subjected to starvation and severe malnutrition at a young age and then rehabilitated. In former studies, PEM was first induced during pregnancy. From our studies it can be concluded that when young animals are subjected to severe conditions like starvation and chronic MN, the effects can be reflected in the next generation even after the animals have been completely rehabilitated.

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