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Dominant lethal mutations in rats fed on irradiated wheat

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1. Introduction

It is now generally accepted that the mutagenic potential of a substance is a serious health hazard. Processing foods by irradiation may introduce such risks, although foods preserved after irradiation can significantly increase the world's food resources by reducing spoilage. The consumption of such foods may be associated with greater risk in malnourished population in view of the fact that malnutrition can modify the toxicity of drugs and chemicals. Irradiated foods have been screened for their wholesomeness, using a variety of criteria but studies on their cytotoxic and mutagenic potentials have so far been relatively few.

It has recently been reported from this Institute (Vijayalaxmi and Sadasivan 1975, Vijayalaxmi 1975) that rats fed for 12 weeks on diets containing freshly-irradiated wheat had an increased incidence of polyploid cells in their bone-marrow than those fed on unirradiated or stored irradiated wheat diet. The results of an investigation dealing with dominant lethal mutations and germ-cell survival in male rats fed on irradiated wheat are presented here.

2. Materials and methods

Thirty-two weanling male rats of the Institute's colony (Wistar strain) were divided into two groups of 16 each. One group was fed on a diet providing 18 per cent protein (well-fed group); the other was given a 5 per cent protein diet (malnourished group). After a period of 8 weeks on these diets, four males in each group were kept for mating to determine the effect of low protein diet.

The remaining males in each group were divided into two sub-groups of six each. One sub-group was fed on a diet containing unirradiated wheat; the other received a diet containing irradiated wheat, both diets providing 9 per cent protein. At the end of 12 weeks of feeding the wheat diets, all males were used for mating experiments.

For four consecutive weeks, each male was caged with three virgin females for a period of 1 week. The females, from the stock colony, were 10–12 weeks old. During the period of mating, all animals received the stock colony diet which provided 20 per cent protein. Thus, throughout the 4 weeks of mating, all males were taken off of the test diet. The composition of the diets and the method of irradiation of wheat were similar to those used earlier (Vijayalaxmi and Sadasivan 1975). A cobalt-60 source was used for irradiation, and the radiation dose of 75 000 rad was checked by ferrous sulphate dosimetry.

The dominant lethal assay, as modified by Epstein and Rohrborn (1971), was followed. All females were killed 13 days after the mid-week of their

Weeks	Well-fed rats	Malnourished rats	Well-fed rats		Malnourished rats		Well-fed and malnourished rats combined	
	18 per cent protein	5 per cent protein	Unirradiated wheat	Irradiated wheat	Unirradiated wheat	Irradiated wheat	Unirradiated wheat	Irradiated wheat
1	1·1	—	3·3	4·5	3·6	4·3	3·5	4·4
2	2·3	—	3·6	5·6	4·3	5·8	3·9	5·7
3	2·4	—	4·5	9·6	4·9	9·1	4·7	9·4
4	3·6	—	5·2	12·0	7·1	9·0	6·2	10·5
Total	2·4	—	4·2	8·0	5·0	7·0	4·6	7·5
CD at 5 per cent for groups			2·69		1·98		1·64	
Analysis of variance								
Source			d.f.	(F. ratio)	d.f.	(F. ratio)	d.f.	(F. ratio)
Males			5	1·05	5	0·88	5	0·28
Groups			1	8·26‡	1	4·39†	1	12·67§
Weeks			3	2·71	3	3·62†	3	6·01§
Error			38		38		86	
Total			47		47		95	

† $p < 0{\cdot}05$. ‡ $p < 0{\cdot}01$. § $p < 0{\cdot}001$.

CD at 5 per cent indicate—confidence difference at 5 per cent level of significance for two groups. It is calculated with students *t* values at 5 per cent level of significance multiplied by pooled SE for 38 or 86 d.f. as given above.

Table 1. The percentage of intrauterine deaths during 4 weeks of mating. The male rats were fed experimental diets for 12 weeks before mating (square root, arcsin and log transformations were also used in the analysis to assess whether there is any change in the level of significance—it was found that in all cases, the level of significance remained the same).

being caged and presumptive mating. Their uteri were screened for live and dead embryos. The mutagenic index was calculated as

$$\frac{\text{Number of dead embryos}}{\text{Total implants}} \times 100.$$

Another set of four male rats in each group was given the same high and low protein as well as wheat diets for the same duration, and their testes were collected and fixed in Zenker's formal solution. Sections of 3 micron thickness were made and stained with periodic-acid–Schiffs with haematoxylin as nuclear stain. For each rat, one hundred round tubules with regular cell layers were selected and cell-counts, namely, spermatogonia of types A and B and resting primary spermatocytes of stage VII, were scored to estimate the number of surviving germ cells (Oakberg and Clark 1961).

2.1. *Design of the experiment and statistical analysis*

A randomized block design was adopted to distribute the males into different groups on the basis of body-weight. The mutagenic index was calculated for each male, and the values were expressed as percentages. Statistical analysis was carried out by the analysis of variance technique and F test. In addition, square root, arcsin and log transformations were used to assess whether there were any variations in the level of significance between groups.

3. Results

3.1. *Dominant lethal mutations*

The results are presented in table 1.

3.1.1. *Effect of malnutrition*

During all 4 weeks of mating, the mutagenic index of rats maintained on 18 per cent protein diet was consistently lower than in all other groups which received 9 per cent protein in the diet. None of the female rats which were caged with malnourished males (5 per cent protein) became pregnant, and their mutagenic index could, therefore, not be calculated. Probably these malnourished rats were not sexually mature.

The mutagenic index of well-fed rats when switched over to unirradiated wheat diet was not significantly different from that of malnourished rats fed on unirradiated wheat, the values being 4·2 and 5·0 per cent, respectively. Similarly, well-fed rats maintained on a diet of irradiated wheat showed a mutagenic index of 8·0 per cent, whereas the mutagenic index of malnourished rats fed on irradiated wheat was 7·0 per cent. These data suggest that malnutrition *per se* had little effect on the rate of intrauterine deaths.

3.1.2. *Effect of feeding irradiated wheat*

Well-fed rats, when switched over to a diet of irradiated wheat, showed a higher mutagenic index than those given unirradiated wheat, the values being 8·0 and 4·2 per cent, respectively. Similarly, malnourished rats given irradiated wheat showed more intrauterine deaths than malnourished rats given unirradiated wheat, the percentages being 7·0 and 5·0, respectively. Since the response to irradiated wheat is similar in both well-fed and malnourished groups, analysis

	Spermatogonia		Resting primary spermatocytes
	Type A	Type B	
Well-fed group (18 per cent protein)	305·0 ± 5·23	300·0 ± 2·78	364·5 ± 10·23
Malnourished group (5 per cent protein)	203·3 ± 3·57	166·3 ± 0·63	250·3 ± 6·09
Well-fed rats:			
unirradiated wheat	286·2 ± 6·81	261·2 ± 8·08	364·8 ± 5·45
irradiated wheat	285·5 ± 6·59	276·7 ± 5·34	376·2 ± 2·84
Malnourished rats:			
unirradiated wheat	285·5 ± 4·99	256·5 ± 6·31	357·2 ± 6·05
irradiated wheat	255·5 ± 6·61	237·3 ± 9·50	330·5 ± 4·65
F ratios:			
well-fed versus malnourished	88·07§	81·98§	118·01§
unirradiated versus irradiated	15·35§	6·30‡	42·48§
Statistical analysis:			
control well-fed versus control malnourished	§	§	§
well-fed unirradiated versus malnourished unirradiated	NS	NS	NS
well-fed irradiated versus malnourished irradiated	†	†	§
well-fed unirradiated versus well-fed irradiated	NS	NS	NS
malnourished unirradiated versus malnourished irradiated	†	NS	†

† $p < 0{\cdot}05$. ‡ $p < 0{\cdot}01$. § $p < 0{\cdot}001$.

Number of rats in each group: 4. NS: Not significant.
(The values indicate the total numbers per 100 round tubules.) (Mean ± S.E.)

Table 2. Germ cell survival in rat testes.

of variance was carried on the combined data for well-fed and malnourished rats. The differences due to feeding irradiated wheat were found to be highly significant ($p < 0{\cdot}001$).

3.2. *Germ-cell survival*

Data on germ-cell survival are presented in table 2.

3.2.1. *Effect of malnutrition*

Malnourished rats had significantly fewer germ cells than did well-fed rats. All rats, irrespective of whether they were well-fed or malnourished, when switched over to a diet of unirradiated wheat (which provided 9 per cent protein), had almost the same number of germ cells. But malnourished rats, when switched over to irradiated wheat, which also provided 9 per cent protein, showed significantly fewer germ cells than the well-fed animals given the same irradiated wheat diet. This suggests that, though the protein content of the unirradiated and irradiated wheat diets are identical, giving irradiated wheat to malnourished rats was associated with some reduction in the number of germ cells.

3.2.2. *Effect of feeding on irradiated wheat*

Giving wheat diets to well-fed rats, whether unirradiated or irradiated, did not result in significant differences in the number of germ cells. On the other hand, malnourished rats, when switched over to irradiated wheat, had significantly fewer germ cells than those given unirradiated wheat diet.

4. Discussion

Dominant lethal mutations have been widely used as convenient indicators of major genetic damage which result in pre-implantation losses of non-viable zygotes, early foetal deaths, sterility and semi-sterility of the parents exposed to test substances (Bateman and Epstein 1971). Reports on the induction of dominant lethal mutations by irradiated foods in mammals are few and controversial. Ericksen and Emborg (1972) found no evidence for dominant lethals when they fed rats with radiation-sterilized food. But Moutschen-Dahmen, Moutschen and Ehrenberg (1970) reported an increased rate of pre-implantation deaths in mice fed on irradiated food. The present investigation also shows an increase in dominant lethality after feeding on irradiated wheat.

The genetic basis for dominant lethality is mainly the induction of structural and numerical chromosomal anomalies (Rohrborn 1970). Legator and Malling (1969) suggested that the usefulness of the dominant lethal tests can be enhanced by carrying out cytogenetic studies. Vajayalaxmi and Sadasivan (1975) recently reported neumerical aberrations in the bone-marrow chromosomes of rats fed on freshly-irradiated wheat. Bugyacki, Deschreider, Moutschen, Moutschen-Dahmen, Thijs and Lafontaine (1968) reported the results of detailed cytogenetic examination of spermatogonial preparations obtained from mouse given irradiated wheat. They showed several types of chromosomal aberration, and statistical analysis indicated that the proportion of damaged cells in mice given irradiated flour was much greater than in control animals. The increased intrauterine

deaths reported in this study might also have been caused by chromosomal abnormalities in the male germ cells.

The results of this investigation also show that the number of primordial germ cells, as measured by the germ-cell survival, were affected by giving irradiated wheat to malnourished animals. Although a variation was observed in the cell numbers between treated and control groups, Reddi, Reddy, Krishna, Syamala and Premalatha (1972) reported that the reduction in the different gonadal cells was not significant in the mice fed on irradiated wheat. However, the level of protein in the diet before the animals were given irradiated wheat was not indicated. Also, the wheat was stored for 3 months after irradiation. The results of this study indicate that feeding malnourished rats on irradiated wheat results in reduced number of germ cells in the testes. It may be relevant to point out that in this study we began to feed the animals on wheat within 20 days after irradiation (fresh batches of irradiated wheat were used in the preparation of diet every 20 days). The possible effects of feeding irradiated wheat stored for longer period needs further investigation.

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