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Immune response in rats given irradiated wheat

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1. Rats given diets containing freshly-irradiated wheat showed significantly lower mean antibody titres to four different antigens, decreased numbers of antibody-forming cells in the spleen and rosette-forming lymphocytes as compared to rats given either unirradiated wheat or irradiated wheat stored for a period of 12 weeks.

2. The immune response in rats given 90 g protein/kg diet was essentially similar to that seen in animals given 180 g protein/kg diet.

Irradiation has been recommended as one of the methods for disinfestation and prolonged storage of food grains (WHO, 1970). A large number of investigations have so far been carried out which relate to the 'wholesomeness' of irradiated foods. These have included somatic, cytotoxic and mutagenic evaluation. Before the safety of consuming irradiated foods can be fully established, it is essential to determine that when such foods are included in habitual diets, they do not in any way seriously modify vital physiological functions.

Immunological competence is an important host defence mechanism and alterations in this mechanism may be expected to adversely affect resistance to infection. A variety of factors like malnutrition and chemicals are known to modify the immune status (Scrimshaw, Taylor & Gordon, 1968; Bluestein & Green, 1970; Nikolaev, Ponomareva, Geller, Rozgan & Garipova, 1972; Gopalan & Srikantia, 1973). Also, there is experimental evidence which suggests that several macromolecular dietary components exhibit distinct antigenicity in germ-free animals (Sell, 1964; Wostmann, Pleasants & Bealmear, 1971). It has been recently reported from this Institute that in children, rats and mice given freshly-irradiated wheat there were evidences of cytotoxicity and mutagenicity, and such changes were not seen when irradiated wheat was stored for 12 weeks before feeding (Bhaskaram & Sadasivan, 1975; Vijayalaxmi & Sadasivan, 1975; Vijayalaxmi & Visweswara Rao, 1975; Vijayalaxmi, 1976). The present study was designed to investigate the immune response in rats given irradiated wheat as the main ingredient of high(180 g/kg)- and marginal(90 g/kg)-protein diets. (In poor habitual Indian diets, approximately 80% of the energy is derived from cereals such as wheat or rice or both, and hence in the present study wheat was incorporated at 700 g/kg diet.)

MATERIALS AND METHODS

In the first study, forty weanling rats of the Wistar strain from the Institute's colony were divided into four groups of five males and five females each. They were maintained on one of the following four diets (protein content (g/kg) in parentheses): unirradiated wheat (180), unirradiated wheat (90), freshly-irradiated wheat (180) and freshly-irradiated wheat (90). The details of the irradiation procedures have been described previously (Vijayalaxmi & Sadasivan, 1975). The composition of the diets is given in Table 1.

All rats were immunized 12 weeks after they were started on the experimental diet. At intervals of 1 week, for a period of three successive weeks, two soluble antigens (tetanus and

Table 1. *Composition (g/kg) of the diets given to rats*

Dietary protein content (g/kg) ...	Unirradiated wheat		Freshly-irradiated wheat*		Stored irradiated wheat†	
	180	90	180	90	180	90
Wheat	70·0	70·0	70·0	70·0	70·0	70·0
Starch	9·0	20·0	9·0	20·0	9·0	20·0
Casein	11·0	—	11·0	—	11·0	—
Refined peanut oil	5·0	5·0	5·0	5·0	5·0	5·0
Salt mixture‡	4·0	4·0	4·0	4·0	4·0	4·0
Vitamin mixture§	1·0	1·0	1·0	1·0	1·0	1·0
Choline chloride	0·1	0·1	0·1	0·1	0·1	0·1

* Every 20 d, a fresh batch of irradiated wheat was used to prepare the diet.
† Irradiated wheat was stored at 10° for 12 weeks before preparing the diet.
‡ Association of Official Analytical Chemists (1965).
§ National Academy of Sciences/National Research Council (1963).

diphtheria), one bacterial antigen (typhoid) and one particulate antigen (sheep red blood cells) were injected into each rat. The following doses were used: 0·1 ml tetanus and diphtheria (6 Lf/ml each) (L_F dose of toxin is the appearance of rapid maximal flocculation in the presence of 1 unit of antitoxin), 0·2 ml typhoid (1000×10^6 *Salmonella typhii*/ml) and 0·2 ml sheep red blood cells, 25 ml/l saline (9 g sodium chloride/l) (washed three times with saline). The soluble and bacterial antigens (0·3 ml) were administered subcutaneously at four different sites, two on the back (0·1 ml/site) and two in both foot pads (0·05 ml/site) while the particulate antigen was injected intraperitoneally.

On the seventh day after final immunization, blood samples were obtained by heart puncture. Sera were separated and stored $-20°$ until assayed for antibody titres. Antibodies to tetanus, diphtheria and sheep red blood cells were measured by haemagglutination technique (Tasman, Vanramshorst & Smith, 1960) while the H and O antibodies to typhoid antigen were measured by the standard Widal procedure (Bryan & Bryan, 1961). In all instances, control tubes were set up using sera from unimmunized rats. The end-point was taken as the greatest serum dilution at which agglutination could be detected by the naked eye. The reciprocal of serum dilution at the end-point was recorded, converted to its logarithm before being subjected to analysis.

In the second study, seventy-two weanling rats of the same strain from the Institute's colony were divided into six groups of six males and six females each and maintained on the four diets described previously, in addition to stored irradiated wheat (180 g protein/kg) and stored irradiated wheat (90 g protein/kg). At the end of 12 weeks on the cooked diet (steamed for 5 min), each rat received 1 ml sheep red blood cells (100 ml/l, washed three times with saline) intraperitoneally and was killed on the fifth day.

Agar plates were prepared in triplicate and the technique of Jerne & Nordin (1963) was followed for enumeration of antibody-forming cells in the spleen. The method of Wybran, Chantler & Fudenberg (1973) was followed to determine the rosette-forming lymphocytes (%). Coded samples were used and 500 consecutive cells were examined for rosette formation, a rosette being defined as a lymphocyte surrounded by at least three sheep red blood cells.

The results were analysed using the two-factor analysis of variance, one factor being irradiation, the other factor was the protein content of the diet.

Table 2. *Antibody titres in rats given diets containing irradiated wheat†*

Group	Dietary protein content (g/kg)	Mean body-wt (g)		Mean antibody titres				
						Typhoid		
		Initial	Final	Tetanus	Diphtheria	H	O	Sheep red blood cells
Unirradiated wheat	180	50·2	229·0	1·87‡	1·87‡	2·44	2·44	1·84
	90	50·2	152·8	1·87	1·90	2·78	2·51	1·90
Freshly-irradiated wheat	180	50·2	218·8	1·57	1·39	2·02	1·95	1·42
	90	50·2	154·5	1·39	1·42	2·05	2·11	1·48
		Analysis of variance: Variance						
Source of variance	df							
Irradiation	1	0·1	180·625	1·480***	2·253***	1·776***	1·947***	1·776***
Protein level	1	0·1	49350·625***	0·064	0·019	0·009	0·121	0·036
Interaction	1	0·1	354·025	0·098	0·009	0·000	0·025	0·001
Error	36	22·36	1350·603	0·106	0·128	0·089	0·088	0·081

*** $P < 0·001$.

† For details, see Table 1.

‡ One rat did not develop antibodies to tetanus and diphtheria; hence the analysis of variance, df for 'error' will be 35.

Table 3. *Number of rats showing agglutination at different serum dilutions when they were given diets containing irradiated wheat**

(Ten rats/group)

Antigen	Dietary protein content (g/kg)	Unirradiated wheat: Serum dilution 1:5	1:10	1:20	1:40	1:80	Freshly-irradiated wheat: Serum dilution 1:5	1:10	1:20	1:40	1:80
Sheep red blood cells	180	10	10	10	9	9	10	9	8	4	3
	90	10	10	10	10	10	10	10	8	5	3
Tetanus	180†	9	9	9	9	8	10	10	8	6	5
	90	10	10	10	10	9	10	8	6	6	3
Diphtheria	180†	9	9	9	9	8	10	8	6	5	4
	90	10	10	10	10	10	10	8	7	6	3
		Serum dilution 1:20	1:40	1:80	1:160	1:320	Serum dilution 1:20	1:40	1:80	1:160	1:320
Typhoid:											
H	180	10	10	10	10	8	10	9	7	5	3
	90	10	10	10	10	9	10	10	7	5	3
O	180	10	10	10	10	8	10	10	7	5	3
	90	10	10	10	10	10	10	10	8	6	3

* For details, see Table 1.

† One rat did not produce antibodies to tetanus and diphtheria antigens.

RESULTS

The important observations made in these two studies were that at both levels of protein in the diet: (*a*) rats given freshly-irradiated wheat had significantly lowered mean antibody titres as compared to rats given unirradiated wheat (Table 2); (*b*) the number of rats showing reduced titres increased with increase in serum dilution (Table 3); (*c*) the number of antibody-forming cells as well as rosette-forming lymphocytes was significantly lower in rats given freshly-irradiated wheat as compared to rats given unirradiated and stored irradiated wheat. However, there were no significant differences between unirradiated- and stored irradiated-wheat groups (Table 4).

When the results were analysed to determine the effect of the level of protein in the diet, rats given 180 g protein/kg diet (unirradiated, freshly-irradiated and stored irradiated wheat) showed higher mean body-weight, lower thymus weight and increased number of antibody-forming cells as compared to rats given 90 g protein/kg diet. There were no differences in spleen weight, white blood cell count, lymphocytes (%), neutrophils, rosette-forming lymphocytes as well as antibody titres.

DISCUSSION

'Wholesomeness' of irradiated foods has been examined using a variety of criteria but studies undertaken to specifically investigate their effects on the immune system are few. Ehrenberg, Lofroth & Ehrenberg (1965) and Lofroth, Hanngren, Ehrenberg & Ehrenberg (1966) have reported lymphopenia in rats given diets irradiated at total doses of 3–9 Mrads, irradiation levels which are several times higher than those recommended by the Joint FAO/IAEA/WHO Expert Committee (1970). Shillinger (1975), however, has reported

Table 4. *Immune status of rats given diets containing irradiated wheat*†

(Mean values for twelve rats/group)

Group	Dietary protein content (g/kg)	Mean body-wt (g)		Spleen wt (g/kg body-wt)	Thymus wt (g/kg body-wt)	White blood cells (10^3/mm^3)	Lymphocytes (%)	Neutrophils (%)	Antibody-forming cells (per 10^6 spleen cells)	Rosette-forming lymphocytes (%)
		Initial	Final							
Unirradiated wheat	180	52·2	202·8	2·2	1·7	7·91	73·5	23·6	246	49·7
	90	51·9	160·0	2·0	2·1	7·84	72·9	24·5	233	48·0
Freshly-irradiated wheat	180	51·9	196·8	2·0	1·5	7·64	73·5	24·2	152	30·4
	90	52·1	161·7	1·9	1·8	7·69	72·5	25·1	139	30·5
Stored irradiated wheat	180	52·0	198·0	2·0	1·7	7·78	74·2	23·2	240	49·9
	90	52·1	166·3	2·0	1·9	7·61	73·7	23·8	228	47·8
Analysis of variance: Variance										
Source of variance	df									
Irradiation	2	0·0142	53·8477	0·0009	0·0027	0·3159	5·5416	7·5973	66407·521***	2710·321***
Protein level	1	0·0006	23980·5009***	0·0009	0·0128***	0·0800	8·6805	12·5002	2900·694***	30·031
Interaction	2	0·2969	193·2912	0·0002	0·0004	0·0732	0·4306	0·1249	2·340	7·895
Error	66	51·3838	591·9141	0·0010	0·0010	0·2686	29·5795	30·0581	64·918	32·379

*** $P < 0·001$.

† For details, see Table 1.

adverse effects on the reproduction function and on the survival rate of young rats of the fifth generation when the dams were given a diet containing irradiated flour, oats (100 krad), potatoes (20 krad) and meat (600 krad). Furthermore their experimental rats showed a threefold increase in the incidence of pneumonia compared to control animals. They have suggested that this may have been due to reduced resistance in the test rats. Results presented in this paper indicated that at both levels of dietary protein, rats given freshly-irradiated wheat had decreased numbers of antibody-forming cells and rosette forming lymphocytes as well as lowered mean antibody titres as compared to rats given unirradiated- and stored irradiated-wheat diets; observations which may be considered as generally supporting the reports of these earlier workers. It must be emphasized that the irradiation dose used in this study was 75 krad, a dose which has been recommended by the Joint FAO/IAEA/WHO Expert Committee (1970).

The observation that antibody titres and rosette-forming lymphocytes in rats maintained on 90 g protein/kg diet were similar to those seen in animals given 180 g protein/kg diet needs explanation, since numerous studies have shown that both humoral and cell-mediated immune responses are reduced in experimental protein-energy malnutrition (Mathur, Ramalingaswami & Deo, 1971; Gebhardt & Newberne, 1974; Chandra, 1975). In all these studies the level of protein in the diet was very low, less than 30 g/kg, whereas in the present investigation the low-protein diet provided 90 g protein/kg. It would appear that at 90 g protein/kg, the immune response is not impaired. At the time of killing, rats given the 90 g protein/kg diet had a mean body-weight deficit of 30% as compared to the animals given 180 g protein/kg diet. The finding that they had normal numbers of rosette-forming lymphocytes and could produce satisfactory antibody titres is somewhat similar to the normal humoral and cell-mediated immune responses seen in children whose body-weights were between 70–80% of the standard (Reddy, Jagadeesan, Raghuramulu, Bhaskaram & Srikantia, 1976). Results obtained in this study showed that the effect of dietary protein on antibody production and on the antibody-forming cells were somewhat different. This could have been because the former was measured after three repeated injections while the latter were enumerated after primary immunization. The decreased numbers of antibody-forming cells may have been the result of prolonged cell generation time after primary immunization (Mathur *et al.* 1971).

The mechanism(s) by which the observed effects were brought about are not known. Also the functional significance of the lowered antibody titres, decreased numbers of antibody-forming cells and rosette-forming lymphocytes in rats given freshly-irradiated wheat in relation to increased susceptibility to infection needs further evaluation since it may be argued that although they were low, the antibody titres that were achieved and the number of antibody-forming cells and rosette-forming lymphocytes that were present may have been adequate. It is necessary to carry out in vivo studies to answer this point. Whatever may be the functional significance, results presented in the present paper show that the consumption of irradiated wheat is associated with changes in the immune status of the animal.

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