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Response of Cereal-Fed Guinea Pigs to Dietary Broccoli Supplementation and X-Irradiation[1]

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The presence in cabbage of a substance which reduces the radiosensitivity of guinea pigs fed a basal diet of oats and wheat bran was originally reported by Lourau and Lartigue ('50) and confirmed by Duplan ('53). Later, another member of the *Brassicaceae* family, broccoli, was shown to be an even more effective supplement (Spector and Calloway, '59). The present study was undertaken to determine in what way animals fed broccoli differed from those not receiving the supplement and which of the responses subsequent to radiation exposure were modified, as a means of elucidating the mechanism responsible.

PROCEDURE

Young, male, albino guinea pigs of the Aristocratic strain weighing 250 to 350 gm, were held for a two-week adjustment period upon receipt from a local supplier. During this period all animals received ad libitum a diet consisting of 50% of whole field oats and 50% of pure wheat bran and a solution of sodium ascorbate (75 mg per 100 ml) in lieu of drinking water. Four animals were killed on receipt and 4 at the end of the standarization period. Two dietary groups were then designated at random: one group (1) continued to receive the same diet and the second (2) was given a supplement of 50 gm of raw broccoli daily. This treatment was followed for two weeks before and after total-body exposure to 400 r x-radiation.[2] Radiation factors were: 180 kv, 15 ma, no filtration added, 21 to 22 r per minute, 105 cm target distance. Six animals, three from each diet, were irradiated simultaneously in a horizontal beam, half of the dose being applied to each side of the body.

Control animals were killed on the day of irradiation. All other controls were subjected to all treatments other than administration of the radiation dose. After irradiation, certain nonirradiated animals were fed an amount of food equivalent to the average food consumption of the irradiated animals of the same diet group each previous day; these were designated "restricted controls." All others were fed ad libitum. Randomly selected animals were killed one, three, 5, 7, 9 and 14 days after irradiation. Mortality experience was such that no irradiated, group 1 animals survived until the 14th day.

Food intake was recorded daily and body weight intermittently throughout the experiment. Animals were housed individually in a controlled environment (26 ± 2°C), and all reasonable precautions were taken to assure a clean environment. However, upper respiratory infection was a prominent complication.

Tissues were obtained for histologic and biochemical examination following sodium pentobarbital anesthesia (50 mg per kg) and exsanguination.[3] All histologic and hematologic methods conformed to usual laboratory practices.

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[2] Irradiation was carried out at the Argonne National Laboratories.

[3] Histologic studies were conducted by the Hektoen Institute for Medical Research.

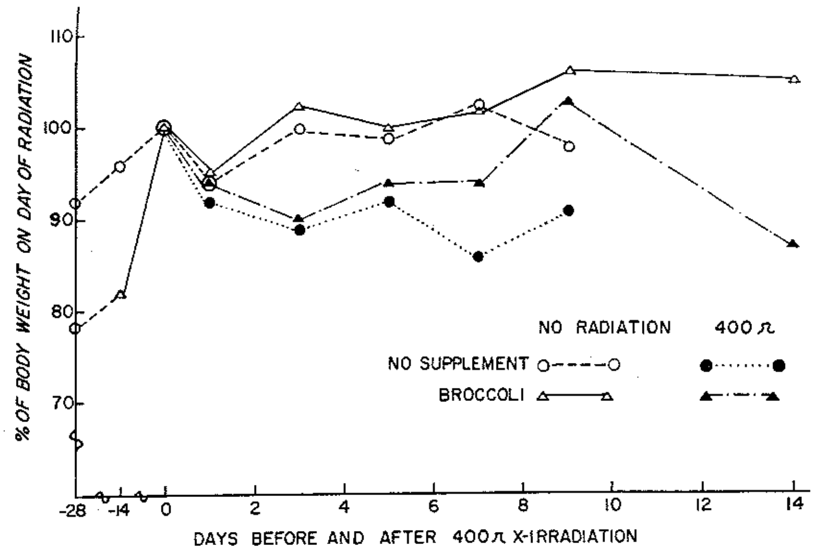

Fig. 1 Body weight of guinea pigs, expressed as percentage of weight on the day of irradiation. Mean body weights on that day were: no supplement, 318 gm (1); broccoli supplement, 367 gm (2).

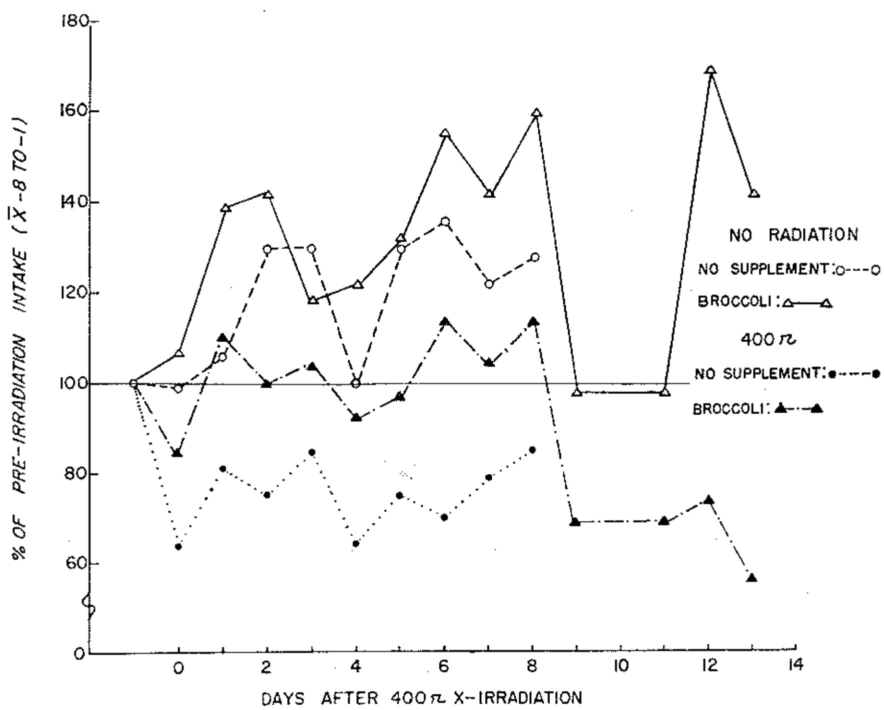

Fig. 2 Ad libitum food intake following irradiation, expressed as percentage of the average eaten daily for one week prior to exposure. These amounts were: basal 17.4 gm, no supplement (1); basal 13.8 gm plus broccoli 49 gm (2).

For simplicity, the tables indicate a single mean value for all control animals over all time intervals. Statistical comparisons, however, were made between control and irradiated animals killed on any given day. Treatment effects were assessed by analysis of variance (Brownlee, '53).

RESULTS

Body weight and food intake data are presented graphically in figures 1 and 2, as percentages from a standard point. This scheme was used, because of the changing population at each time interval, to avoid artifacts induced by removing extreme values. Before irradiation, superior weight gain was achieved as a consequence of broccoli supplementation. Slight weight loss was recorded 24 hours after irradiation in both groups which reflected handling effects rather than irradiation, as the same change was apparent in the control population. Control animals regained this loss promptly and held weight constant for the remainder of the test. In the irradiated groups an additional small loss occurred by the third day, followed by an essentially steady state. Food intake was depressed in both irradiated groups, but to a lesser degree in group 2. At necropsy, a general absence of body fat and in some cases a gelatinous degeneration of the perirenal fat was seen most frequently in group 1.

Various manifestations of the radiation hemorrhagic syndrome were observed in animals from both groups sacrificed 5 days post-irradiation and thereafter (table 1). These consisted primarily of multiple diffuse intradermal hemorrhages, subserosal and mucosal hemorrhages of the gastrointestinal tract, and hemorrhages of the myocardium, the skeletal muscles, the meninges and the medullary contents of the long bones. These were observed to occur with about equal frequency and severity in both dietary groups, and presented no remarkable microscopic features. Patches of atelectasis and consolidation were seen in the lungs of some irradiated and nonirradiated animals of both dietary groups. No bacteriologic studies were conducted.

The nonirradiated controls of the bran and oats diet group presented a number of striking morphologic changes which were also evident in the irradiated members of this group. A frequent observation was the presence of a greatly distended gallbladder containing bile in which an amorphous sediment was present. Microscopic examination of the sediment indicated it to be composed of cellular detritus and mucus. This condition was seen only infrequently in the supplemented group.

Among the animals in group 2, both irradiated and nonirradiated, multiple subcapsular yellow nodules were seen on the surfaces of the liver. Microscopically these were seen as areas of focal fatty changes. The livers of group 2 animals were also significantly larger than those of group 1, both irradiated and control (table 2). Irradiation resulted in diminished liver weight in both groups, probably as a result of voluntray food restriction, since comparable changes were seen in the restricted controls. This weight loss was recovered by the 9th day in group 1 and by the 14th in group 2. Histopathologic studies revealed reduced liver glycogen content of animals in group 1 as compared with those in group 2. The group 1 animals had liver glycogen stores approximately the same as those of the initial controls. In addition, on microscopic examination moderate intralobular cholangiole proliferation and some areas of hydropic degeneration were seen in animals from both groups.

Liver vitamin A content was 4 times as great in controls of group 2 as in group 1, 17 I.U. as compared with 4 I.U. (table 2). Values recorded in irradiated animals did not differ significantly from their own diet controls. The low levels of group 1 reflect a marked drop from animals as received from the supplier.

In a number of animals in group 1 the adrenals developed a grayish color. In some, this condition was so marked as to make it impossible to distinguish between the cortical and medullary portions on transverse section. Histopathologic examination, however, failed to reveal any differences between the adrenals of the two groups. No significant difference in adrenal size was evident due to diet. Following irradiation, adrenal size dropped significantly in group 1 at day 5 but group 2 remained constant until day 14 when enlargement was noted.

TABLE 1

Gross pathologic observations

	Percentage incidence on days after irradiation as noted[1]												
	No supplement						Broccoli supplement						
	Control	1	3	5	7	9	Control	1	3	5	7	9	14
Radiation injury													
Multiple hemorrhages in													
Myocardium	—	—	—	—	—	17	—	—	—	—	—	33	—
GI tract (mucosa-serosa)	—	—	—	—	50	100	—	—	—	—	17	83	—
Intradermal	—	—	—	—	—	83	—	—	—	—	—	67	14
Parenchyma of spleen	—	—	—	—	—	—	—	—	—	—	—	17	—
Lymph nodes	4	—	—	—	—	50	4	—	—	—	—	67	57
Meninges	—	—	—	—	—	17	—	—	—	—	—	—	29
Lungs	18	—	—	—	17	50	8	—	—	—	33	50	72
Epididymites	—	—	—	—	33	17	—	—	—	—	33	50	57
Skeletal muscles	—	—	—	—	—	17	—	—	—	—	33	50	—
Epiphyses of bones	—	—	—	—	—	—	—	—	—	—	—	17	—
Medullary cavities	—	—	—	—	—	33	—	—	—	—	—	33	—
Edema	—	—	17	—	17	—	—	—	—	—	—	—	—
Infection													
Pneumonia	23	—	17	17	17	17	15	—	—	33	17	33	14
Lymphadenitis	4	—	—	—	—	—	—	—	—	—	—	—	—
Multiple liver abscesses	—	—	—	—	—	—	—	—	—	—	—	—	29
Nutritional or metabolic pathology													
Distention of gallbladder with sediment in bile	13	—	—	33	67	17	—	—	17	—	17	17	—
Gross fatty changes (liver)	4	17	17	—	—	—	40	50	33	50	50	33	29
Gelatinous fat degeneration	9	—	—	—	17	50	—	—	—	—	17	—	—
Congenital defects													
Rectal prolapse	4	—	—	—	—	—	—	—	—	—	—	—	—
Gallbladder absent	—	—	—	—	17	—	—	—	—	—	—	—	—
Unknown etiology													
Gray discoloration adrenals	9	—	—	17	50	17	—	—	—	—	—	—	—

[1] Total N: diet 1 controls=22; diet 2 controls=25; days 1, 3, 5, 7 and 9 both diets=6; day 14=7.

TABLE 2

Tissue characteristics of control and irradiated guinea pigs

	Initial control	Standardized control	Diet	Controls		Days after irradiation					
				Fed ad lib.	Restricted-fed	1	3	5	7	9	14
Number	4	4	1	14	8	6	6	6	6	6	
			2	15	10	6	6	6	6	6	6–7
Liver weight, gm/100			1	3.94	3.43^{c}	3.63	3.42	3.40	3.98	4.00	
gm body weight	3.72	3.20	2	4.36^{a}	4.23^{a,c}	4.32^{a}	4.26^{a}	3.97^{a}	3.87	4.19	4.92
Liver vitamin A			1	5	4	4	5	4	4	4	
content, I.U./gm	26	10	2	19^{a}	14^{a}	15^{a}	24^{a}	21^{a}	16^{a}	15^{a}	26
Adrenal weight, mg/100			1	64	54^{c}	64	63	53^{b}	68	61	
gm body weight	48	44	2	55	56	53	54^{a}	55	56	56	69^{b}
Testes weight, mg/100			1	82	108	113	151	106^{b}	89	107	
gm body weight	178	143	2	238^{a}	214^{a}	204^{a}	210	240^{a}	150^{a}	173	164^{b}
Spleen weight, mg/100			1	137	125^{c}	104^{b}	112	99^{b}	102	91^{b}	
gm body weight	194	123	2	151	143^{c}	119^{a,b}	110	103^{b}	120^{a}	93^{b}	110^{b}
Heart weight, mg/100			1	331	351	358	332	326	378	319	
gm body weight	455	369	2	371	390	388	381	352	351	343	442
Kidney weight, gm/100			1	0.97	0.95	1.00	0.98	0.96	1.10^{b}	1.05	
gm body weight	0.96	0.98	2	0.92^{a}	0.92	0.89	0.93	0.89	0.86^{a}	0.89^{a}	1.13^{b}
Serum nonprotein N,			1	45	41	59	49	41	48	45	
mg/100 ml	42	40	2	41	38	53	40	38	46	43	48
Total leukocytes/mm^3			1	2471	3641	1700	1080	467^{b}	508^{b}	546^{b}	
			2	3680	3920	1567^{b}	1200^{b}	438^{b}	434^{b}	500^{b}	300^{b}
Erythrocytes, million/mm^3			1	4.90	4.78	4.94	4.99	4.65^{b}	4.29	4.18^{b}	
			2	4.90	4.98	5.06	4.62	3.74^{b}	4.18^{b}	3.27^{b}	1.73^{b}
Hematocrit, %			1	42	43	42	42	41	40	40	
			2	43	45	42	40	39	41	30^{b}	17^{b}
Hemoglobin, gm/100 ml			1	14.1	14.7	13.9	15.1	14.1	13.6	14.2	
			2	14.1	14.5	14.0	13.7	13.0^{b}	13.4	10.3^{a,b}	5.8^{b}

[1] Letters refer to statistically significant differences ($P < 0.05$): a indicates diet 1 vs. diet 2 on any given day, i.e., diet effect; b indicates irradiated vs. nonirradiated of a given diet on a given day, i.e., radiation effect; c indicates ad libitum-fed vs. restricted-fed controls of a given diet, i.e., food restriction effect.

Most striking of the changes observed was the atrophic appearance of the testes and secondary sex organs of the animals in group 1. Histopathology revealed a decrease in the size of the semiferous tubules as compared with initial and standardized controls, but without differences in spermatogenic activity. Testes weight was significantly greater in group 2 animals, both irradiated and control. Following irradiation, testes weight was diminished significantly by the 9th day in group 1 but not until the 14th day in group 2. Spermatogenesis was depressed in both groups after irradiation.

Spleen weight did not vary significantly between controls of the two diet groups. Although the weight of this organ was reduced in restricted controls, a much more pronounced diminution was apparent in irradiated animals, a phenomenon described by others (Bloom and Bloom, '54). Morphologic changes also followed the typical pattern of lymphoid degeneration.

Although heart weight was greater and kidney weight less on the average in group 2 than in group 1, the differences were of marginal significance. No demonstrable change in heart mass occurred following irradiation. In group 1, kidney size remained essentially constant until the 7th day, when an increase was noted; on days 7 and 9 kidney size was significantly greater in group 1 than in 2. A comparable increase was observed in group 2 by the 14th day. No analytical data bearing on the composition of the increased weight were obtained. There was a transient increase in serum nonprotein nitrogen content (Δ 15 mg/100 ml) 24 hours after irradiation but whether this is related to kidney function is unknown.

Leukocyte counts[4] showed enormous variation in the control population and no significant effect of diet. A precipitous fall in white cells was seen at 24 hours after irradiation and values were maximally reduced by the 5th day. No recovery was evident in the period of study in either group. Histologically, the lymph nodes of the irradiated animals in group 1 appeared to have suffered more extensive injury and undergone a greater degree of disorganization than those in group 2. The regenerative process in the lymph nodes of animals in group 2 was confined to the germinal centers of the cortex where it proceeded in an orderly diffuse pattern from the 5th day post-irradiation onward. In group 1 the regenerative processes were most active in the medullary cords rather than the cortex.

Although the pathologist reported depression of bone marrow in some group 1 controls, erythrocyte count, hematocrit and hemoglobin values were identical with those in group 2. Hypoplasia was seen in the bone marrow as early as day 1 and was marked at days 3, 5 and 7. Red cell counts began to fall about the 5th day, attaining lower levels in group 2 than in 1. The lowest values recorded were those of day 14 when counts ranged from 1:30 to 2.58 million per mm^3, packed cell volume from 9 to 28%, and hemoglobin from 3.6 to 8.5 gm per 100 ml.

DISCUSSION

The radiation dose administered is, in our experience, about $LD_{60/30}$ for groups of animals fed commercial guinea pig feed. Thirty-day mortality in guinea pigs given bran and oats plus broccoli is normally 30 to 40% and in those given bran and oats alone is 95 to 100% (Spector and Calloway, '59). Neither gross nor microscopic features of the radiation-induced changes reported here are unusual for acute exposure in this dose range.

The profound differences between the nonirradiated animals of the two groups require comment. Several nutritional imbalances exist in the bran and oats basal diet (see table 3). The diet is devoid of vitamin A and remarkably low in protein, riboflavin, calcium, sodium and the halogens. Pantothenic acid, folic acid and iron levels are marginal and phosphorus content is excessive. Supplementation with broccoli improves the dietary with respect to each of these factors and corrects for all vitamin insufficiences. The sediment observed in the gallbladders of group 1 animals is a recognized manifestation of

[4] All blood components were influenced to an unknown degree as a consequence of pentobarbital administration. If one accepts the assumption that both groups were affected to the same degree, then the data have comparative but not absolute value. No evidence bearing on the validity of this assumption is at hand.

TABLE 3

Comparison of nutrient composition of the diets fed with commercial guinea pig chow

		Commercial feed	Bran-oats	Bran-oats + broccoli[1]
	per 100 gm			
Nitrogen	gm	3.34	2.08	2.58
Lipid	gm	5.29	2.74	2.47
Ash	gm	7.61	4.90	5.79
α-Tocopherol	mg	3.2	3.5	7.7
β-Carotene	μg	390	tr.	2000
Thiamine	mg	0.676	0.585	0.707
Riboflavin	mg	0.747	0.155	0.563
Niacin	mg	6.6	13.0	12.2
Pyridoxine	mg	0.74	0.79	1.07
Ca-pantothenate	mg	4.31	2.34	5.11
Folic acid	μg	290	128	214
Biotin	μg	42	45	56
Vitamin B_{12}	μg	2.8	0.2	< 0.2
Choline Cl	mg	241	222	285
Inositol	mg	245	459	465
Calcium	gm	1.52	0.30	0.51
Phosphorus	gm	0.57	1.07	1.02
Sodium	mg	177	5[2]	155
Potassium	gm	1.70	1.07	1.48
Magnesium	mg	310	320	368
Zinc	mg	4.7	3.6	3.6
Manganese	mg	23	21	18
Iron	mg	17	8	10
Copper	mg	0.7	0.7	1.3
Selenium	μg	< 2	< 2	*ca.* 12
Molybdenum	μg	150	100	141
Cobalt	μg	5	5	9
Aluminum	mg	1.5	2.3	2.8
Chlorine	mg	330	26	133
Fluorine	mg	1.59	< 0.2	*ca.* 0.24
Bromine	mg	7.5	< 0.4	*ca.* 5.9
Iodine	mg	0.09	0	0.59
Sulfur	mg	248	156	396

[1] Calculated on the basis of 20% of broccoli dry solids with 80% of the bran-oats mixture. This ratio is the best estimate of proportional consumption based on 7 separate experiments.

[2] The drinking solution of sodium ascorbate provides 27 mg/100 ml in addition.

avitaminosis A in this species (Erspamer, '38) and vitamin A insufficiency is also reflected in the reduced content of vitamin A in the livers of this group. Whether the other differences noted in the biliary system, i.e., increased liver size and minimal fatty changes in group 2, are a consequence of nutritional status or due to specific positive effects of broccoli feeding cannot be determined from the data at hand.

The involution of the gonads and accessory sex organs, the retardation of growth, reduced liver glycogen content and the lack of body fat seen in group 1 indicate a hormonal derangement of dietary etiology. These changes closely resemble those of the pseudohypophysectomy described by Mulinos and Pomerantz ('40), induced by chronic food restriction. In the present experiment, total dry solids intake differed by about one gram per day, on the average, prior to irradiation. Therefore, the developmental differences may be ascribable primarily to qualitative restriction, as well as to marginal quantitative limitation. The rapid appearance of these changes indicates the sensitivity of the guinea pig to nutritional factors. The gonads and particularly the accessory sex organs are sensitive indicators of endocrine function. It has been well established that a number of interrelationships exist between nutrients and endocrine glands, their secretions, and the responses of target organs or tissues to hormonal stimulation. Evidence exists that certain nutritional requirements are increased during conditions of stress. The changes observed in the nonirradiated, ad libitum-fed animals of group 1 indicate them to be poorly equipped to respond to stress of any kind.

An attractive hypothesis for the greater radiosensitivity of the unsupplemented group, then, lies simply in nutritional deficiency. Both chronic caloric restriction[5] and vitamin A deficiency (Ershoff, '52) have been shown to lower resistance to X-irradiation. However, preliminary studies have been conducted in which the quality of the bran-oats diet was improved with respect to protein, vitamins and the major minerals, alone or in consonance. Beneficial effects were noted in growth but radiosensitivity was not consistently reduced. Studies are in progress to determine what contributions to the bran-oats diet made by the broccoli supplement enable guinea pigs to withstand radiation insult.

SUMMARY

Young, male, guinea pigs were fed a basal diet of bran and oats, with or without a daily supplement of 50 gm of raw broccoli, for two weeks before and after exposure to 400 r whole body x-radiation. Animals were killed sequentially for histologic and hematologic examination. Provision of broccoli resulted in improved nutriture, larger livers with higher stores of vitamin A and mimimal fatty changes, and superior gonadal development. Following irradiation in both groups, decrements in body weight and food intake as well as the characteristic events of the radiation syndrome were seen. Broccoli supplementation of the cereal diet generally reduced the degree of distortion or delayed its appearance.

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