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Effect of Dietary Supplementation with Broccoli on X-Irradiation-Induced Enzyme Changes in the Guinea Pig¹

RUSSELL HILF², RICHARD ADACHI, AND GLADYS ECKFELDT

*U. S. Army QM Food and Container Institute for the Armed Forces,
QM Research and Engineering Command, Chicago, Illinois*

INTRODUCTION

In a preliminary report, Spector and Calloway (1) demonstrated that feeding of cabbage or broccoli significantly reduced the mortality of X-irradiated guinea pigs, corroborating findings of Lourau and Lartigue (2) and of Duplan (3). Studies were performed to determine more precisely the differences existing between animals fed broccoli and those not receiving the supplement. Data concerning the nutritional aspects, as well as changes in organ weights and hematologic and histologic responses, were reported by Calloway and Munson (4). The data presented in this report are concerned with the effect of broccoli supplementation on certain tissue and serum enzyme changes induced by exposure to X-irradiation.

Several biochemical parameters have been studied as indicators of radiation-induced metabolic alterations. Disturbances in enzyme systems can alter functional activity, and these changes might occur prior to morphological changes. Most of this earlier work has been reviewed by DuBois and Petersen (5). These investigators demonstrated increases in adenosine triphosphatase (ATPase) and 5'-nucleotidase levels of hematopoietic tissues after X-irradiation (6). These changes could be correlated with exposure dose and were prevented when radioprotective compounds were employed (7). These authors suggest that these enzyme assays might be used for evaluating radioprotective agents.

Other reports have appeared in which serum glutamic-oxalacetic transaminase

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² Present address: Squibb Institute for Medical Research, New Brunswick, New Jersey.

levels were elevated after X-irradiation (8, 9). This serum enzyme, as well as glutamic-pyruvic transaminase and alkaline and acid phosphatase, was also measured in order to determine if differences existed in the broccoli-fed animals. The results presented here indicate that broccoli prevented some of the postirradiation changes in spleen and lymph node phosphatases.

MATERIALS AND METHODS

Male albino guinea pigs of the Aristocratic strain, weighing 250 to 300 gm, were given a diet of wheat bran and oats (50-50) ad libitum. After a 2-week adjustment period, the animals were divided in a random fashion, half of the animals receiving 50 gm of broccoli daily in addition to the bran and oats basal diet. Two weeks after initiation of the broccoli supplementation, the animals were exposed to 400 r of X-irradiation. Control animals were treated in an identical manner other than exposure to radiation. Randomly selected animals, 6 animals per group, were sacrificed under sodium pentobarbital anesthesia on days 1, 3, 5, 7, 9, and 14 after X-irradiation. Complete details of diet, radiation, anatomic, and histologic results are given in an earlier paper (4).

Blood was removed by heart puncture, and serum was obtained for enzyme assay. Spleens, lymph nodes, and adrenals were quickly removed, blotted, weighed, and quick-frozen until enzyme assays could be performed. The data for the control animals are presented as a single mean value calculated from all control animals at all time intervals.

Tissue ATPase activity was determined by the procedure of DuBois and Potter (10). 5'-Nucleotidase activity was determined by the method of Cochran *et al.* (11). The tissue concentration of the homogenates was decreased in some of the above procedures when necessary. However, these modifications were adjusted during the calculation of enzyme activity. Results of these enzyme determinations are expressed as micrograms of phosphorus liberated per milligram of tissue.

Serum acid and alkaline phosphatase measurements were performed by a micro modification of the procedure of Bessey *et al.* (12). One unit is defined as the amount necessary to liberate 1 mM of *p*-nitrophenol per liter per hour at 38°C. Serum glutamic oxalacetic and glutamic-pyruvic transaminases were measured by the procedure of Reitman and Frankel (13), and units are equivalent to that which will cause a decrease in optical density at 340 m μ of 0.001 per minute per milliliter of serum at 37°C, as defined under the conditions of Karmen *et al.* (14).

RESULTS

The response of spleen ATPase to irradiation is illustrated in Fig. 1. In the un-supplemented animals, ATPase levels increased significantly on day 1 ($p < 0.05$) and day 3 ($p < 0.01$) after irradiation as compared to the controls³ ($4.7 \pm 0.7 \mu\text{g}$

³ Control data presented as the mean \pm the standard error of the mean.

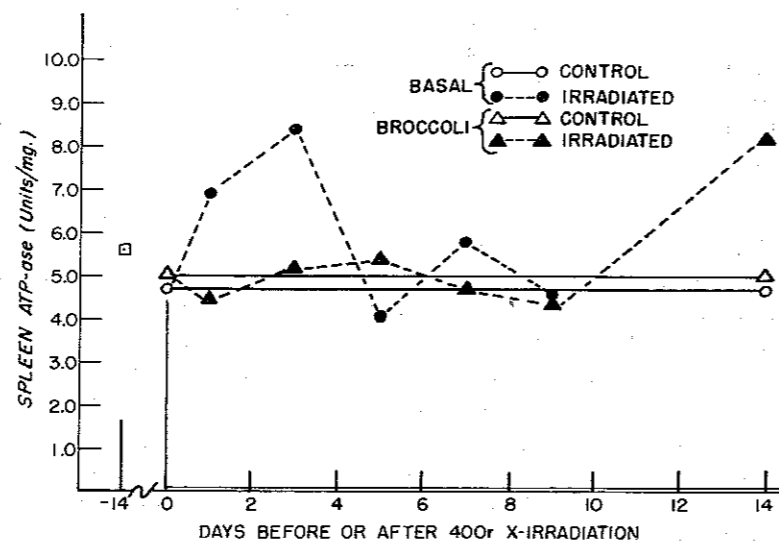


FIG. 1. Effect of broccoli supplementation on radiation-induced spleen ATPase activity. The data representing the control animals are the means obtained from duplicate assays of tissues from 14 animals. Each experimental point represents the mean obtained from duplicate assays of tissues from 6 animals, except day 14 in the broccoli-supplemented animals ($n = 7$).

of P per milligram). This radiation-induced increase was prevented in the broccoli-supplemented animals (control,³ 5.0 ± 0.3 μg of P per milligram), spleen ATPase activity being significantly lower on day 1 and day 3 as compared to the corresponding unsupplemented animals. An elevated ATPase level was observed in the broccoli-supplemented animals on day 14 ($p < 0.05$), at a time when all unsupplemented animals were dead.

Figure 2 shows the effect of broccoli supplementation on spleen 5'-nucleotidase. A result similar to that obtained with spleen ATPase was observed, 5'-nucleotidase activity being elevated (control,³ 4.2 ± 0.6 μg of P per milligram) in the unsupplemented animals on the third day post irradiation ($p < 0.01$). A second rise on day 9 after irradiation was noted prior to death of these animals. Broccoli prevented the early postirradiation increase in 5'-nucleotidase activity (control,³ 6.0 ± 0.9 μg of P per milligram), the values being significantly lower ($p < 0.05$) than the corresponding unsupplemented animals. However, a rise was obtained on day 14 in these animals.

Lymphatic tissue and adrenals were examined for changes in ATPase and 5'-nucleotidase activities. These data are recorded in Table I. Lymph node 5'-nucleotidase activity in the unsupplemented animals increased after irradiation, showing a peak on day 1 after exposure. This was followed by a decrease, and, finally, a secondary increase occurred, values prior to death of these animals being twice

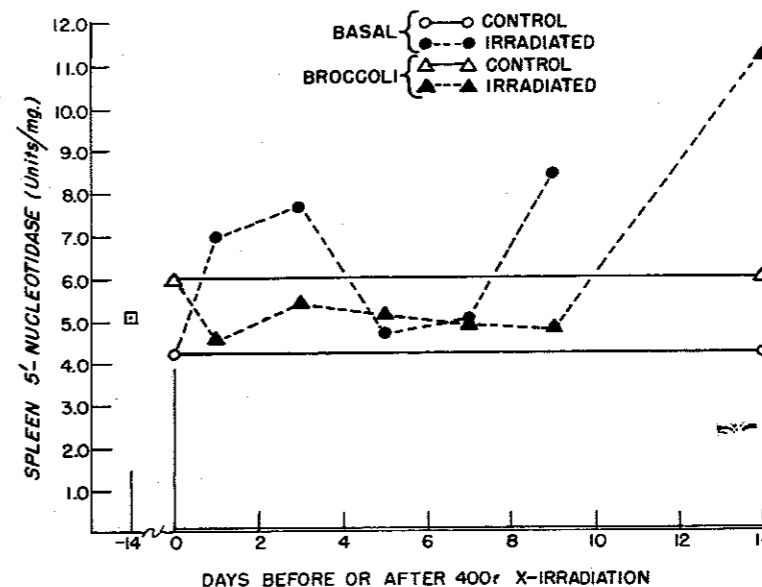


FIG. 2. Effect of broccoli supplementation on radiation-induced spleen 5'-nucleotidase activity. The data representing the control animals are the means obtained from duplicate assays of tissues from 14 animals. Each experimental point represents the mean obtained from duplicate assays of tissues from 6 animals, except day 14 in the broccoli-supplemented animals ($n = 7$).

as high as for the controls. On the other hand, the animals receiving broccoli showed a very gradual increase in lymph node 5'-nucleotidase activity throughout the entire experimental period.

Lymph node ATPase assays resulted in both unsupplemented and broccoli-supplemented animals exhibiting a postirradiation increase on day 1. However, the ATPase levels in broccoli-supplemented animals decreased and remained at control levels from day 3 to the end of the experimental period. The unsupplemented animals, after a decrease from the initial peak, increased again on days 7 to 9. Adrenal ATPase and 5'-nucleotidase activities exhibited no definite changes after irradiation and showed no differences between unsupplemented and broccoli-supplemented animals.

Serum enzyme data are recorded in Table II. In general, serum enzyme levels decreased after exposure to irradiation in the unsupplemented animals. Broccoli supplementation did not alter these responses significantly, except when serum acid phosphatase was measured. With this serum enzyme, broccoli prevented the radiation-induced decrease obtained in the unsupplemented animals. Some differences were obtained when the glutamic-pyruvic transaminase levels were compared. A significant decrease was observed on the first day postirradiation in the

TABLE I
CHANGES IN LYMPH NODE AND ADRENAL ENZYMES AFTER
X-IRRADIATION IN GUINEA PIGS^a

Days before or after X-irradiation	Lymph node				Adrenal			
	5'-Nucleotidase ^b		ATPase ^c		5'-Nucleotidase ^b		ATPase ^c	
	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli
-14	1.7	—	1.6	—	10.8	—	3.9	—
0 (control)	3.0 ± 0.3 ^d	2.0 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	7.6 ± 0.5	8.3 ± 0.7	2.6 ± 0.3	1.9 ± 0.2
1	5.1 ^e	2.4 ^f	2.3	2.2	7.3	7.7	1.3 ^e	1.1
3	4.2 ^e	2.6 ^{e,f}	1.8	1.2	6.6	9.8	1.8	2.0
5	3.9	3.0 ^e	1.5	1.6	6.8	5.8	1.8	1.1
7	5.9	3.2 ^e	2.4	1.5 ^f	5.9 ^b	6.8	1.3 ^e	1.7
9	6.4 ^e	3.0 ^f	2.2	1.5	10.0	7.6	2.8	1.8
14	—	4.1	—	1.3	—	7.3	—	1.0

^a Data represent numerical means of duplicate enzyme assays. Control; $n = 14$, days -14, 1, 3, 5, 7, 9; $n = 6$ and day 14; $n = 7$.

^b Activity expressed as micrograms of phosphorus liberated per milligram of tissue per 30 minutes at 38°C.

^c Activity expressed as micrograms of phosphorus liberated per milligram of tissue per 15 minutes at 38°C.

^d Control data given as numerical mean ± standard error of the mean.

^e Differs significantly from the corresponding control (sacrificed at the same time interval) at probability level of 0.05.

^f Differs significantly from the corresponding dietary control (bran and oats) at probability level of 0.05.

unsupplemented animals, whereas a significant decrease occurred in the fifth day after irradiation in the broccoli-supplemented animals.

DISCUSSION

An increase in spleen ATPase and 5'-nucleotidase activity was observed after X-irradiation. These results agree with those reported by DuBois and Petersen (6) in the mouse and rat. The data also indicate that some of these changes were prevented by dietary supplementation with broccoli. These results suggest that spleen ATPase and 5'-nucleotidase assays might serve as one type of measurement to determine the efficacy of a substance to protect against radiation-induced death. The results in the guinea pig thus agree with the results obtained by Petersen and DuBois (7) with other radioprotective agents. More data would be required to determine the validity of these enzyme changes for use as an assay procedure. Fur-

TABLE II
CHANGES IN SERUM ENZYMES AFTER X-IRRADIATION IN GUINEA PIGS^a

Days before or after X-irradiation	Glutamic-pyruvic transaminase ^b		Glutamic-oxalacetic transaminase ^b		Alkaline phosphatase ^c		Acid phosphatase ^c	
	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli	Bran and oats	Bran and oats + broccoli
	-14	42.2	—	67.3	—	4.4	—	1.6
0 (control)	47.0	41.0	54.0	49.0	4.2	4.9	1.3	1.5
1	29.8 ^d	29.0	41.0	41.2	3.0 ^d	3.6 ^d	1.0 ^d	1.4 ^e
3	21.9	20.9	28.9 ^d	32.0	3.4	4.4	1.3	1.5 ^e
5	43.7	20.7 ^{d,e}	37.6	32.9	4.3	4.0	1.2	1.4
7	38.9	30.8	36.2	36.7	2.3	4.7 ^e	0.8 ^d	1.1 ^e
9	25.6	22.2	22.0	35.0	2.4	3.4	0.9 ^d	0.8 ^d
14	—	17.7	—	76.5	—	1.6 ^d	—	1.2

^a Data represent numerical means of duplicate enzyme assays. Control; $n = 14$, days -14, 1, 3, 5, 7, 9; $n = 6$ and day 14; $n = 7$.

^b Units equivalent to that causing a decrease in optical density at 340 m μ of 0.001 per milliliter of serum at 37°C.

^c Units equivalent to amount liberating 1 mM of *p*-nitrophenol per liter per hour at 38°C.

^d Differs significantly from the corresponding control (sacrificed at the same time interval) at probability level of 0.05.

^e Differs significantly from the corresponding dietary control (bran and oats) at probability level of 0.05.

ther studies are necessary to determine the mechanism of the prevention of these enzyme changes.

There has been some question as to the validity of the changes of these two enzymes in the spleen, because spleen weight usually decreases after X-irradiation (15, 16). The increases in enzyme activity reported here were greater than could be accounted for simply from loss in tissue weight. The spleen size decreased to the same degree in both dietary groups (4), but the broccoli-supplemented animals did not exhibit the postirradiation increase in enzyme activity which was observed in the unsupplemented group.

Differences between the two dietary groups were also obtained when lymph node 5'-nucleotidase was measured. Broccoli appeared to prevent the immediate postirradiation rise in enzyme activity. However, as the time after irradiation progressed, a gradual increase in 5'-nucleotidase activity occurred, and by day 14, 5'-nucleotidase activity was double that of the initial values. This was a similar relative increase as obtained in the unsupplemented animals on the ninth day after irradiation.

A different result was obtained when lymph node ATPase was measured. Broccoli did not prevent the immediate postirradiation response. However, ATPase

levels in the broccoli-supplemented animals returned to initial levels and remained there until the end of the experimental period. The data suggest that a secondary rise in ATPase activity occurred in the lymph nodes of animals receiving the basal diet alone.

In general, a decrease in the serum enzymes studied was observed under the conditions of this experiment. These results differ from those reported by others for serum alkaline phosphatase and glutamic-oxalacetic transaminase (5, 8, 9). The difference in results appears to depend on species used, the magnitude and type of irradiation, and the time after exposure (5). In these experiments, only minor differences were observed between the unsupplemented and broccoli-supplemented groups. The serum acid phosphatase changes were of interest, because the broccoli appeared to postpone the changes postirradiation. The decrease in acid phosphatase might be correlated with the atrophic appearance of the testes in the unsupplemented animals (4). Nevertheless, the spleen ATPase and 5'-nucleotidase assays appeared to be more suitable as a screening procedure for radioprotective compounds.

SUMMARY

The radioprotective effect of broccoli supplementation was investigated in guinea pigs. ATPase and 5'-nucleotidase activities in spleen, lymph nodes, and adrenals were measured at various intervals after exposure to X-rays. Broccoli prevented the postirradiation rise in these enzymes in the spleen. Serum acid and alkaline phosphatase, glutamic-oxalacetic transaminase, and glutamic-pyruvic transaminase activity decreased after irradiation, and no differences were observed between the two dietary groups. It would appear that the two spleen phosphatases studied could be used as an assay system for radioprotective compounds.

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