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Interim Report
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QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES
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FURTHER STUDIES ON REDUCTION OF X-IRRADIATION MORTALITY OF GUINEA PIGS BY PLANT MATERIALS

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by

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FURTHER STUDIES ON REDUCTION OF X-IRRADIATION MORTALITY OF GUINEA PIGS BY PLANT MATERIALS

Confirmation of the observation of Lourau and Lartigue (1950), that supplementation of a bran and oats diet with cabbage decreases the radiosensitivity of guinea pigs, was reported by Duplan (1953) and Spector and Calloway (1959). The latter workers showed that the protective effect was not due to ascorbic acid and that supplementation with broccoli was also effective. Duplan stated that if the basal bran and oats diet were replaced by a balanced synthetic diet, identical results were obtained. However, examination of guinea pigs fed the bran-oats diet led Calloway and Munson (1961) to conclude that at least part of the observed response was due to improved nutritional status. Accordingly, a series of tests were conducted in which various improvements of the dietary were investigated. The data to be presented indicate that the radioprotective quality cannot be demonstrated in the presence of a purified casein diet; that the response is not attributable to a variety of chemically-defined materials; and that the protective agent is a water-soluble substance.

PROCEDURE

The standardized assay procedure described below was followed except as otherwise noted. Young, male guinea pigs of the Aristocratic or Hartley strain, weighing 250 to 350 gm, were maintained for 10 to 14 days on a basal diet consisting of 50 percent of whole field oats and 50 percent of wheat bran, supplemented with vitamin C by addition of 0.24 percent of sodium ascorbate to the dry diet or in the drinking water as 1.5 gm ascorbic acid plus 1.0 gm sodium bicarbonate per liter.[3] At the end of this standardization period, the animals were divided at random into experimental groups of approximately 20 each and given the various supplements for two weeks prior to radiation. This same dietary regime was followed for 20 to 30 days after irradiation.

The purified diet used was patterned after Reid and Briggs (1953) diet nr. 13 and was composed of, in gm per 100 gm: vitamin-free casein, 30; corn oil, 6.3; sucrose, 9.3; "alphacel", 15.0" "cerelose", 7.8; potassium acetate, 2.5; magnesium oxide, 0.5; USP Salts XIV, 5.998; zinc carbonate, 0.002; cornstarch, 19.4; glycerine, 1.0; B vitamins in cornstarch, 1.0[4]; fat-soluble vitamins in corn oil, 1.0[4]; and ascorbic acid, 0.2. Analyzed composition of this diet, together with that of the bran-oats diet and of broccoli, is given in Table 1. The composition of other supplements was based upon the composition determined by these analyses.

Broccoli was purchased locally and held under refrigeration (40°F.) for not more than five days. Fifty grams of fresh material were provided in a separate food cup. All alfalfa was from a single lot of high-protein, high-vitamin A, dehydrated leaf meal, not steam-treated and held under inert gas by the supplier (Consolidated Blendors, Fremont, Nebraska).

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3. The adequacy of vitamin C supplementation was established by measurement of whole blood levels. Values obtained in 24 animals ranged from 0.88 to 2.68 mg per 100 ml, average 1.6 mg.

4. Vitamins added per 100 gm diet: (in mg) thiamine HCl, 1.6; riboflavin, 1.6; pyridoxine HCl, 1.6; Ca pantothenate, 4.0; niacin , 20.0; folic acid, 1.0; inositol, 200.0; choline Cl, 200.0; alpha tocopherol, 1.82; 2 methyl-1,4-napthoquinone, 0.20; (in mcg) biotin, 60.0; vitamin B_{12}, 4.0; (in I.U.) vitamin A palmitate, 1776; and vitamin D_3, 160.

Table 1

Composition of basal diets and raw broccoli

Per 100 gm	Purified diet*	Bran-oats	Broccoli, raw
Moisture, gm	8.85	10.55	91.8
Nitrogen, gm	4.02	2.08	0.37
Amino acids, gm			
Arginine	1.10	1.03	0.23
Cystine	0.082	0.208	0.030
Methionine	0.732	0.192	0.037
Cysteine	0.264	0.176	0.067
Histidine	0.820	0.320	0.078
Isoleucine	1.59	0.52	0.11
Leucine	2.76	0.94	0.19
Lysine	2.46	0.65	0.20
Phenylalanine	1.40	0.54	0.11
Tyrosine	1.39	0.42	0.08
Threonine	1.26	0.79	0.13
Tryptophane	0.369	0.275	0.045
Valine	2.12	0.79	0.20
Crude fat, gm	7.61	2.74	0.113
Fiber, gm	(15)	12.6	1.3
Ash, gm	6.95	4.90	0.766
Silicon, mg	5.15	347.0	1.71
Boron, mg	<0.5	0.5	2.0
Sulfur, mg			
Total	206	156	111
Organic	179	156	70
Arsenic (as As_2O_3), µg	6	36	12
Nickel, mg	1.7	1.7	8.3
Para-aminobenzoic acid, mg	1.0	NF***	NF

Per 100 gm**	Purified diet	Broccoli, raw
Calcium, gm	0.86	0.11
Phosphorus, gm	0.73	0.069
Sodium, mg	172	62
Potassium, mg	450	255
Magnesium, mg	330	46
Zinc, mg	1.2	0.3
Manganese, mg	0.8	0.3
Iron, mg	22.2	1.5
Copper, mg	0.4	0.3
Selenium, µg	<2	5
Molybdenum, µg	<10	25
Cobalt, µg	7	2
Aluminum, mg	3.3	0.4
Chlorine, mg	640	46
Fluorine, mg	2.1	0.1
Bromine, mg	15.7	2.4
Iodine, mg	0.57	0.24
Alpha tocopherol, mg	>2	2.0
Beta carotene, µg	-	820
Thiamine, mg	0.733	0.098
Riboflavin, mg	1.54	0.180
Niacine, mg	21.5	0.738
Pyridoxine, mg	1.59	0.18
Ca pantothenate, mg	6.52	1.33
Folic acid, µg	590	46
Biotin, µg	76	8
Vitamin B_{12}, µg	0.87	NF
Choline Cl, mg	212	44
Inositol, mg	207	40

* Modified Reid and Briggs diet Nr. 13 (1953).

** Data on the composition of bran-oats with respect to these constituents were presented previously (Calloway and Munson, (1961).

***NF = None found

Radiation was administered at the Argonne National Laboratories or at the USAF Radiation Laboratory, University of Chicago, using a General Electric Maximar X-ray machine. Radiation factors were: 180 KV; 15 ma; no filtration added, 21-22 r per minute; 105 cm target distance. Six animals were irradiated simultaneously in a horizontal beam and representatives from each treatment were included in each exposure. Except where noted, animals were exposed to 400 r, half of the dose applied to each side of the whole body.

Animals were individually housed in suspended, screen-bottomed cages in a controlled environment (26 ± 2°C.). Body weight and food intake were recorded intermittently throughout the experimental periods. Criteria from which the efficacy of any treatment was determined were survival time of decedents and percent mortality at 20 or 30 days after irradiation.

Experimental Series 1

Radiosensitivity of guinea pigs fed broccoli or alfalfa in conjunction with a purified casein diet. Because of the poor nutritional quality of a bran-oats diet, the relative radiosensitivity of animals fed a complete purified diet alone or with green plant materials was investigated. In order to accomplish this, 240 animals were purchased in the first trial; only 100 were judged satisfactory for irradiation (Exp. #24). Losses during standardization were heavy due to disease (pneumonia and cervical lymphadenitis) and to poor acceptance of the purified diet. Of the 100 irradiated, 40 received the usual bran-oats diet and 40, the purified diet; half of each group was also given broccoli. Due to unavailability of raw broccoli from any local source, frozen broccoli was substituted during about six days of the test period. A group of 20 animals, culled because of failure to eat the basal diet, but otherwise apparently healthy, was given Purina Guinea Pig Chow during the usual test periods.

Under these conditions, 30-day mortality was 60 percent in the group given broccoli with the bran-oats diet as shown in Table 2. Comparable results were obtained with the purified diet, with or without broccoli, and with Purina Guinea Pig Chow. Mortality was 100 percent in in the group receiving the basal diet alone.

Table 2

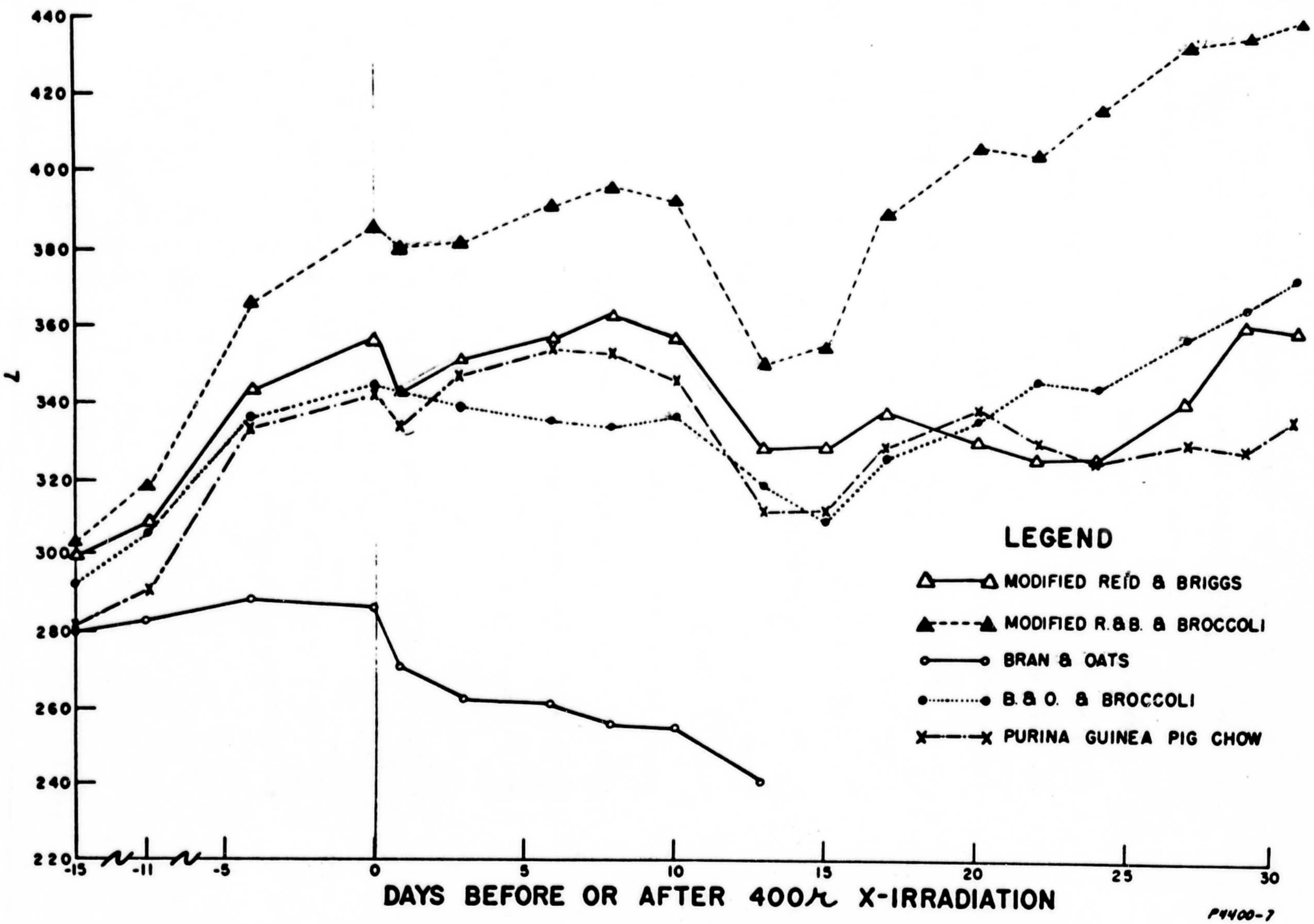
Response of guinea pigs fed basal diets of varying quality (to 400 r X-irradiation)

Basal diet	Supplement	Nr. irradiated	Mortality % at 30 days	Survival time of decedents, days
Exp. #24, OB Hartley Strain				
Bran-oats-C	None	16	100	10.2
Bran-oats-C	Raw broccoli	20	60	13.3
Purified diet	None	16	62	13.8
Purified diet	Raw broccoli	16	56	14.2
Purina Chow	None	20	50	16.6

Growth of the animals fell in three patterns seen in Figure 1: the unsupplemented bran-oats group inferior; broccoli-supplemented bran and oats diet, purified basal diet and Purina satisfactory and equal;and and broccoli-supplemented, purified diet superior. Caloric intake followed the same pattern.

FIGURE I

BODY WEIGHT OF GUINEA PIGS FED BROCCOLI IN CONJUNCTION WITH A BRAN AND OATS OR PURIFIED BASAL DIET. EXP. #24, OB HARTLEY STRAIN



The modified Reid and Briggs diet nr. 13 served as basal for all animals during the standardization period of the second test. Groups of 40 guinea pigs were then assigned to continue with this control, unsupplemented diet, or received, in addition, 50 gm of raw broccoli daily or 20 percent of alfalfa leaf meal incorporated into the basal diet at the expense of cornstarch. All broccoli was procured from a single producer in California and was shipped by air twice weekly with ice to assure freshness. Eighteen animals from each diet group were exposed to either 400 or 500 r of x-irradiation.

The data in Table 3 show that animals given the basal diet alone or with alfalfa grew at an equal, slow rate of about 15 gm per week prior to irradiation, whereas the weight increment of the broccoli group was about double that. Following irradiation at either dose level, growth of both of the supplemented groups was superior to that of the control group. Food intake followed roughly the same pattern. It may be noted that growth was not necessarily associated with resistance to radiation as the highest mortality was sustained by the broccoli group at both radiation levels. Neither supplement appreciably modified mortality, the supplemented groups falling on either side of the control value. The mortality percentages and survival times following 400 r irradiation are within the range observed among bran-oats fed groups given effective supplements.

Table 3

Mortality, body weight, and food intake of X-irradiated guinea pigs fed a purified diet with or without supplements of alfalfa or broccoli

Radiation dose, r supplement	400 None	Broccoli Basal	 Broc.	Alfalfa	500 None	Broccoli Basal	 Broc.	Alfalfa
Mortality, % at 30 days	22	39		11	44	61		28
Survival time, days	14.8	11.9		15.0	15.4	13.7		11.0
Body weight, gm on day[1]								
-12	301	297		298	295	303		308
0	329	360		328	323	359		334
+2	312	356		322	306	355		323
+9	337	379(17)[2]		354	330	376(17)		355
+16	317(16)	343(12)		338(17)	293(13)	296(12)		348(13)
+23	334(14)	391(11)		351(16)	317(10)	356 (7)		353(13)
+30	347(14)	418(11)		374(16)	352(10)	376(7)		374(13)
Food intake, gm per days		Basal	Broc.			Basal	Broc.	
-12 to -1	16.0	15.4	48	17.0	15.6	15.5	50	16.8
0 to +2	15.2	15.6	49	16.0	16.0	14.6	49	16.0
+ 3 to +9	19.6	18.3	50	20.8	19.0	18.6	49	20.8
+10 to +16	12.0	9.8	38	13.4	9.7	7.7	26	13.9
+17 to +23	17.4	16.3	49	17.8	16.3	15.9	43	17.7
+24 to +30	19.9	20.7	50	23.3	21.5	16.9	50	21.3

1. Before (-) or after (+) irradiation.

2. Nr. surviving and included in mean; same N applies to food intake data.

Experimental Series 2

Comparison of radiosensitivity of cereal-fed guinea pigs given broccoli or a mixture of pure materials with the same composition. The next experiment was designed to determine whether the radioprotective agent was a recognized substance, by providing in chemically-defined form all of the constituents of broccoli shown in Table 1. Forty-eight ingredients made up the "synthetic broccoli" which matched the natural composition, except that only one-third of the sulfur was derived from organic sources and chloride was in excess due to unavoidable additions from vitamins and amino acids (1.788% of dry solids instead of 0.56%). The l-form of amino acids was used except for isoleucine, phenylalanine, threonine, and valine where racemic mixtures were employed. Total nitrogen content was made up with glutamic acid and monosodium glutamate. Since accurate data on the composition of broccoli lipids and carbohydrates were not available, another plant oil, cottonseed, was selected as the source of the small amount of crude fat, and glucose ("Cerelose") and cellulose ("Alphacel") as carbohydrates.

The basal bran-oats diet was supplemented with one percent of vitamin A-fortified corn oil (1776 I.U. per 100 gm as palmitate) and 0.24 percent of sodium ascorbate. This diet was fed to all animals during the standardization period and to the dietary control group thereafter. Vitamin A palmitate was omitted from all diets to which other supplements were added, as each of these provided sufficient amounts of pro-vitamin A. Dry materials were substituted for the basal diet on a weight basis. Supplements consisted of: (1) raw broccoli, 50 gm per day; (2) 20 percent of alfalfa; (3) 10 percent of alfalfa plus raw broccoli, 25 gm per day; or (4) 20 percent of synthetic broccoli. A group fed ground Purina Guinea Pig Chow fortified with 0.24 percent of sodium ascorbate was included.

Each radiation group consisted of ten male and ten female guinea pigs and five or six animals fed the same diets served as non-irradiated controls. Since there was no discernible difference between sexes in radiation response, the data were pooled.

Body weight and food intake of irradiated groups, seen in Table 4, showed customary and parallel changes. The group given only vitamin A gained the least weight before irradiation and sustained the most severe losses afterward. The 75 percent mortality and average survival time of 16.7 days was improved over that usually experienced in the absence of vitamin A. The diet containing synthetic broccoli was accepted well and weight gain was improved over that of the control group. Both mortality (95 percent) and survival time (14.7 days) were poorer than in the control group, suggesting some mildly toxic property of the synthetic mixture in the irradiated animal.

Food intake and weight gains of the three groups given green plant supplements were approximately equal, but mortality was lower in the presence of alfalfa. Mortality was 50 percent in the broccoli group, 15 percent in the alfalfa and 10 percent with the combined supplement. Superior weight gain and food intake, both before and after irradiation, occurred in the Purina group. However, mortality was the same as with broccoli supplementation and survival time was somewhat shortened, 12.9 days as compared with 15.4 days.

Table 4

Mortality, body weight, and food intake of 400 r X-irradiated guinea pigs fed natural and synthetic broccoli and alfalfa

	Bran and oats + ascorbate +							Purina + ascorbate
	Vitamin A	Synthetic broccoli	Broccoli		Alfalfa	Alfalfa + broccoli		
Mortality, % at 30 days	75	95	50		15	10		45
Survival time, days	16.7	14.7	15.4		16.0	19.0		12.9
Body weight, gm on day[1]								
-12	313	316	334		299	328		323
-1	328	350	378		356	382		394
+2	302	333	361		350	369		383
+9	295	334(19)	352		366	384		399
+16	242(12)[2]	326(3)	320(12)		342(19)	352		421(11)
+23	263(6)	384(1)	358(10)		374(17)	383(18)		441(11)
+30	281(5)	466(1)	393(10)		419(17)	425(18)		481(11)
Food intake, gm per days			Basal	Broc.		Basal	Broc.	
-10 to -1	14.7	17.6	15.7	49	21.4	18.9	25	25.8
0 to +7	10.6	15.6	13.7	48	18.1	15.2	25	23.5
+8 to +14	9.1	11.4	10.6	44	16.4	16.4	24	19.7
+15 to +21	6.4	9.5	9.3	40	17.9	14.4	23	24.1
+22 to +28	11.7	28.1	16.5	50	26.9	25.1	25	27.9

1. Before (-) or after (+) irradiation.

2. Nr. surviving and included in mean; same N applies to food intake data.

Experimental Series 3

Radiosensitivity of guinea pigs fed a bran-oats diet supplemented with pure substances to match the composition of the purified diet.

Further attempts to define the protective agent were carried out, in which supplements were added to the bran-oats diet to equate its contribution of nutrients with that of the purified diet. The possibility existed that the slightly toxic property of the synthetic broccoli might have been due to an acid-base imbalance, a factor shown to adversely affect blood formation, growth and skeletal development in the rabbit (Thacker, 1959). The bran-oats diet is, itself, an acid-ash mixture. Therefore, two mineral supplements[5/] were devised in which the more acid contained 20 mcg more titratable acidity per 100 gm of diet. Protein differences were made up by the use of 18 percent of soy protein (Drackett) supplemented with dl-methionine, l-leucine, l-lysine HCl, l-tyrosine, and dl-valine, to equate the total contribution of essential amino acids with that of the purified diet. Vitamins[6/] were added, as necessary, to raise the levels to those of the purified diet.

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5. Mineral supplements included Na from Na ascorbate, Co from vitamin B_{12}, and the following C.P. salts, in gm, per 100 gm of diet.

	Both acidic and basic		Acidic	Basic
NaF	0.0037	NaCl	0.2814	-
$NaBrO_3$	0.0283	Na_2CO_3	-	0.2551
MgO	0.1459	$CaCO_3$	2.1580	2.9072
$FeSO_4 \cdot 7H_2O$	0.0697	$CaCl_2$	0.8320	-
$Al_2(SO_4)_3 \cdot 18H_2O$	0.0123			
KI	0.0007			

Since P was in excess of purified diet, Ca/P ratio was equated rather than total Ca content.

6. Vitamin supplement per 100 gm of diet: in mg Na ascorbate, 240.0; thiamine HCl; 1.0; riboflavin, 1.5; pyridoxine HCl, 0.7; folic acid, 0.8; para-aminobenzoic acid, 1.0; 2 methyl-1, 4-napthoquinone, 0.2; in mcg biotin, 16.0; vitamin B_{12}, 4.0; in I.U. vitamin A palmitate, 1720 and vitamin D_3, 160.

In the first test, all animals were fed the bran-oats diet with 20 percent of alfalfa added to improve vitamin A status during the standardization period. They were then distributed according to body weight in groups of 24 each among the dietary groups shown in Table 5. Acceptance of the purified diet was very poor and the conditions of this group deteriorated as a consequence, with several deaths occurring during the two weeks prior to irradiation. Adjustment to the diet containing the acidic mineral mixture was equally difficult. Only twelve animals from each of these dietary groups were irradiated and six others were reserved as non-irradiated controls. Substantial improvement in body weight was seen only in the group given the supplement of 20 percent of alfalfa (see Table 5). However, all other groups maintained or gained slightly in body weight prior to irradiation of 18 randomly selected animals per group.

Mortality of the group given the unsupplemented bran-oats diet was 94 percent with the usual average survival time of decedents of 10.9 days. The 67 percent mortality experienced in the purified-diet group was essentially the same as that noted earlier in the face of poor eating behavior. All other groups showed equivalent low mortalities of 33 to 39 percent.

Studies of the non-irradiated animals revealed that homeostatic mechanisms were sufficient to maintain a normal blood pH level of about 7.5 in spite of the differences in dietaries (see Table 5). Urine pH was low in the unsupplemented group and in groups given the full supplement of protein, vitamins, and minerals, whether these minerals were acidic or basic. Apparently, the basic mineral supplement was

Table 5

Mortality and body weight of 400 r X-irradiated guinea pigs fed bran and oats with pure supplements to equate composition with a purified diet, and tissue characteristics of non-irradiated controls

Basal Diet	Bran and oats + vitamin C					Purified
Supplement	None	Protein & vitamins + acid minerals	Protein & vitamins + basic minerals	Basic minerals	Alfalfa	
N	18	12	18	18	18	12
Mortality, % at 30 days	94	33	39	39	39	67
Survival time, days	10.9	7.0	11.3	14.3	12.1	11.8
Body weight, gm on day						
-9	303	307	306	303	308	301
0	309	276	308	314	356	293
+3	293(16)	266(10)	296	305	348	285
+10	282(10)	290	313	317(17)	276	305(11)
+17	284(3)	275(8)	289(11)	297(13)	374(11)	285(4)
+28	249(1)	286	340	284	411	352
Tissues of non-irradiated controls (mean and standard deviation, N=6)						
Blood pH	7.43 ± 0.23	7.48 ± 0.16	7.37 ± 0.29	7.75 ± 0.04	7.50 ± 0.21	7.59 ± 0.13
Bladder Urine, pH	6.25 ± 0.50	5.86 ± 0.21	6.10 ± 0.31	7.72 ± 0.20	7.59 ± 0.31	7.41 ± 0.60
Weights in mg per 100 gm body weight						
Liver	3190 ± 270	3710 ± 600	3190 ± 110	4160 ± 190	4450 ± 900	3660 ± 670
Spleen	138 ± 51	162 ± 31	122 ± 17	148 ± 33	120 ± 23	107 ± 81
Kidneys	910 ± 170	1360 ± 290	1020 ± 110	1160 ± 310	920 ± 160	1010 ± 120
Adrenals	52 ± 7	80 ± 22	56 ± 7	52 ± 15	43 ± 6	53 ± 8
Testes	66 ± 25	283 ± 107	330 ± 73	126 ± 61	197 ± 79	173 ± 73

insufficient to overcome the acidotic effect of the protein mixture. With the basic mineral supplement alone, with alfalfa, or the purified diet the pH of urine was elevated, and equal to blood pH. Testes weight was improved in all supplemented groups, particularly where the diets contained added protein and vitamins. Adrenal weight was increased in the group given the acidic mineral mixture, perhaps a reflection of the stressful nature of this diet.

At this point it seemed reasonable to conclude that the protective agent was a mineral or that the effect was due to a balance of minerals. However, a second trial was carried out under the original test conditions, i.e., with animals fed the vitamin A-free basal bran-oats diet throughout the standardization and pre-irradiation periods, with or without broccoli. Separate groups were given the soy protein-amino acid-vitamin supplement described above to assess the influence of dietary improvement other than mineral. The animals used in this test arrived in poor condition due to inclement weather and shipping delays and the status of those given the unsupplemented diet or the mineral mixture alone did not improve. All other groups fared well and supplementation with raw broccoli evoked excellent weight gains, as shown in Table 6. Following irradiation, mortality was 100 percent in all groups to which no green plant supplement was given (see Table 6). Survival time averaged 4.5 to 5.5 days in the absence of vitamins A and 11.0 days in its presence; all animals which died within the first 10 days after irradiation displayed clinical signs of vitamin A deficiency which were confirmed on necropsy. Alfalfa supplementation was superior to broccoli, both with respect to mortality and to survival time, 19 versus 31 percent and 23.2 versus 15.8 days, respectively. In conjunction with the protein-vitamin supplement, broccoli was less effective, reducing mortality only to 60 percent, which could be due to an excessive intake of some component, most probably protein. These data demonstrate that mineral supplementation is without effect in the face of a supervening vitamin A deficiency, but that provision of vitamin A does not reduce mortality consequent to a single whole-body dose of radiation.

Table 6

Mortality following 400 r X-irradiation of guinea pigs fed bran and oats with or without minerals, protein and vitamins, and green plant materials

	Weight gain before irrad. gm/wk	Mortality at 30 days %	Survival time of decedents days
1. Bran-oats plus vitamin C	0	100	4.5
1 + alfalfa, 20 %	10.5	19	23.2
1 + broccoli, raw, 50 gm	35.0	31	15.8
2. Bran-oats-vitamin C + basic minerals	4.0	100	5.5
2 + broccoli, raw, 50 gm	38.5	38	10.7
3. Bran-oats-vitamin C + protein and vitamins	14.0	100	11.0
3+ broccoli, raw, 50 gm	35.5	60	12.1

The efficacy of the mineral supplement has been evaluated by two collaborating laboratories using comparable test systems. No radioprotective quality was observed by these workers in the presence (Newell 1960) or absence (Darby 1960) of vitamin A. This leaves the earlier observation in doubt. Approximately 20 experiments have been conducted using this assay procedure. With the possible exception of the present case and one reported below, no false positive results were encountered. Unusually high mortality figures are not as difficult to explain as are the low figures, particularly when the behavior of the unsupplemented group is perfectly normal. The preferred hypothesis is that the observation was valid with the response attributable to some contaminant or a more readily available form of one of the salts. Groups given minerals have been included in subsequent experiments reported below, but the issue has not been clarified.

Experimental Series 4

Radioprotective effects of water-soluble or insoluble fractions of alfalfa in the cereal-fed guinea pig. Since the protective effect of broccoli could not be duplicated by the synthetic mixtures, fractionation of green plant material has been undertaken. Alfalfa was selected because it is at least as effective as broccoli and is easier to handle and store. Solubility in water was investigated first.[7]

Fractions were added to 80 parts of the basal diet of bran and oats supplemented with vitamins C and A, at levels representing the contribution of 20 parts by weight of alfalfa. Due to the addition of water in the extraction process, 20 gm of alfalfa yielded 49 gm of material, 38 gm of which was water-insoluble residue and 11 gm of water-soluble extract. An additional group was fed the bran-oats diet fortified with 5.7 percent casein (the protein contribution of 20 parts of alfalfa) and the basic mineral mix and vitamins as in series 3 above, except that vitamin D was omitted. Each dietary group consisted of 18 irradiated animals and five non-irradiated controls.

7. Fractionation was carried out at the Quartermaster Research and Engineering Center, Natick, Mass., under the direction of Drs. Louis Long and Torsten Hasselstrom of the Pioneering Research Div. Preparatory methods for these and related materials will be presented elsewhere.

The data assembled in Table 7 show clearly that the agent which promotes survival was present in the water-soluble portion of alfalfa. Mortality percentages of groups given this fraction alone, in combination with the water-insoluble residue, or as alfalfa were 28, 24, and 28, respectively, as compared with 67 percent mortality experienced in the unsupplemented group and with the residue alone. Mean survival time of decedents of the alfalfa group was 17.6 days; of the combined fractions group, 18.8 days; of the residue group, 16.1 days; and of the water-soluble group, 13.8 days. Thus, survival time may have been extended in the presence of the residue fraction but the number of decedents was too small to permit accurate assessment. Mortality was only six percent in the group given the pure supplements, or one death out of 18 animals irradiated. This is the second experiment in which pure supplements were effective in reducing mortality. However, the overwhelming body of evidence, obtained both in-house and by the two collaborating laboratories, shows that such supplements are usually ineffective in reducing mortality.

Neither body nor tissue weight revealed any differences which appeared to correlate with survival potential. Growth prior to irradiation was superior in the alfalfa-fed group but was essentially equal in all others. Following irradiation, animals fed alfalfa or the combined fractions recovered to their pre-irradiation growth curves whereas rate of gain was diminished in the group given the water-soluble fraction. Organ size varied among dietary controls but there was no characteristic common to the groups which showed superior radioresistance.

Table 7

Mortality and body weight of 400 r X-irradiated guinea pigs fed alfalfa fractions and tissue weights of comparable controls

	No supplements	Water-soluble fraction(I)	Water-insoluble residue(II)	I + II[1]	Alfalfa	Casein, vitamins, and minerals
Mortality at 30 days, %	67	28	67	24	28	6
Survival time, days	15.6	13.8	16.1	18.8	17.6	9.0
Body weight, gm on day[2]						
-10	295	293	299	306	293	298
- 1	315	313	323	326	329	317
+ 2	297	303	311	330	329	321
+ 9	299	319	322	361	359(17)	358(17)
+16	257(13)[3]	304(14)	290(11)	351(15)	352(17)	352(17)
+23	279(6)	325(13)	330(6)	366(14)	401(13)	381(17)
+30	283(6)	352(13)	345(6)	388(13)	433(13)	406(17)
Tissue weight of non-irradiated controls, mg per 100 gm body weight						
Liver	3550	4186	3870	3200	4120	3360
Kidney	851	838	756	711	753	838
Spleen	112	135	107	103	108	134
Testes	223	237	267	291	305	217
Adrenals	57	51	47	43	48	46

1. Due to an accidental death, N of this group was 17 irradiated.

2. Before (-) or after (+) irradiation.

3. Nr. surviving and included in mean.

In a subsequent experiment the mineral mixture was fed separately with the vitamin A and C fortified bran-oats diet and a second group received casein and vitamins. Ash prepared from alfalfa by incineration in a muffle furnace was fed at a 3.5 percent level. Unfortunately, mortality was so low in all groups as to preclude accurate evaluation. The data given in Table 8 suggest a marginal improvement with the mineral supplement and alfalfa, but not with alfalfa ash or casein plus vitamins. Newell (1960) has reported that the ash fraction of alfalfa is ineffective. Thus, if the unknown material is a mineral it must be one which is destroyed upon incineration or is not physiologically available as the oxide.

Table 8

Mortality of guinea pigs fed pure substances, alfalfa, or its ash and exposed to 400 r X-irradiation

	Mortality at 30 days percent	Survival time of decedents days
1. Bran-oats plus vitamins A and C	36	15.8
as 1 plus minerals	13	13.0
as 1 plus casein and vitamins	25	12.5
as 1 plus alfalfa ash	25	16.3
2. Bran-oats plus vitamin C plus 20 percent of alfalfa	13	15.0

DISCUSSION

An attempt was made earlier to separate an active fraction from broccoli (Spector and Calloway, 1960). Most of the activity was lost and the marginal benefit derived from the saline-insoluble residue was most probably attributable to the beta-carotene retained in this fraction. Also, the fractionation procedure employed did not provide precise additivity of the resulting fractions, as any material which was soluble in water, insoluble in saline, and non-dialyzable would have been lost.

Two hypotheses can be offered to account for the responses observed. First, the bran-oats diet may create a requirement which does not exist in conjunction with the purified diet, either due to the presence of some detrimental substance or to a specific imbalance. Alternatively, an unrecognized essential substance may be provided by both green plant materials and some ingredient of the purified diet. Our findings suggest that the radioprotective agent may not be identical with the guinea pig growth-stimulating factors described by others (O'Dell et al., 1960; Briggs, 1961; Kohler et al., 1938; Cannon and Emerson, 1939; Ershoff, 1957) as the radioprotective activity is retained in a fraction which does not stimulate growth, and growth stimulation occurs in the absence of a radioprotective effect with the purified diet. The distribution of the radioprotective agent appears to parallel that of the factor from lettuce or grass juice which is also water-soluble. Ershoff, et al. (1959), have reported that alfalfa and alfalfa residue prolonged survival of thyrotoxic rats fed casein or soy protein-containing diets and that dried alfalfa juice was also effective with the soy protein diet but not with the casein. Our experience in a totally different system is remarkably similar. There is no clear indication as to whether the radioprotective activity differs from the increased resistance to Salmonellosis resulting from cabbage-feeding in the guinea pig, (O'Dell, et al., 1960). Certainly, an agent which promotes resistance to bacterial infection might be expected to increase survival following irradiation where bacterial invasion plays a prominent role. However, preliminary studies by O'Dell (1961), suggest that supplementation with alfalfa does not influence resistance to Salmonella infection. Either the disease- and radiation-resistance agents are different or alfalfa and the Brassica vegetables protect against radiation injury by different mechanisms. Subsequent investigation should reveal whether a second agent, which is effective in prolonging but not in promoting survival and has growth-stimulating or disease-resistance properties, may be present in the water-insoluble portion of alfalfa or in broccoli. In any case, solution of the problem appears to depend upon identification of the active agent from plant sources and further fractionation studies are in progress.

SUMMARY

1. Exposure to 400 r of whole-body X-irradiation resulted in 97 percent mortality in 10 to 15 days among young male guinea pigs fed a basal diet of bran and oats plus ascorbic acid. Supplementation with alfalfa or broccoli, for two weeks before irradiation and during 30 days after irradiation reduced mortality and extended survival time.

2. Although supplementation of an adequate purified diet with broccoli or alfalfa promoted superior weight gain, radiosensitivity was unaffected under these conditions.

3. The beneficial effect of green plant supplementation could not be duplicated by feeding a mixture of 48 chemically-defined ingredients patterned upon the composition of broccoli, in conjunction with the bran-oats diet.

4. The radioprotective agent(s) in alfalfa was water-soluble.

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