Supplemental Vitamin A Prevents the Acute Radiation-induced Defect in Wound Healing

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Acute radiation injury leads to thymic involution, adrenal enlargement, leukopenia, thrombocytopenia, gastrointestinal ulceration, and impaired wound healing. The authors hypothesized that supplemental vitamin A would mitigate these adverse effects in rats exposed to acute whole-body radiation. This hypothesis was based on previous experiments in their laboratory that showed that supplemental vitamin A is thymotropic for normal rodents and lessens the thymic involution, lymphopenia, and adrenal enlargement that follows stress, trauma, and neoplasia, largely obviates the impaired wound healing induced by the radiomimetic drugs streptozotocin and cyclophosphamide, lessens the systemic response (thymic involution, adrenal enlargement, leukopenia, lymphocytopenia) to local radiation, and shifts the median lethal dose ($LD_{50/30}$) following whole-body radiation to the right. To test their hypothesis, dorsal skin incisions and subcutaneous implantation of polyvinyl alcohol sponges were performed in anesthetized Sprague–Dawley rats at varying times following sham radiation or varying doses of whole-body radiation (175–850 rad). In each experiment, the control diet [which contains about 18,000 IU vit. A/kg chow (3 × the NRC RDA for normal rats)] was supplemented with 150,000 IU vit. A/kg diet beginning at, before, or after sham radiation and wounding or radiation and wounding. The supplemental vitamin A prevented the impaired wound healing and lessened the weight loss, leukopenia, thrombocytopenia, thymic involution, adrenal enlargement, decrease in splenic weight, and gastric ulceration of the radiated (750–850 rad) wounded rats. This was true whether the supplemental vitamin A was begun before (2 or 4 days) or after (1–2 hours to 4 days) radiation and wounding; the supplemental vitamin A was more effective when started before or up to 2 days after radiation and wounding. The authors believe that prevention of the impaired wound healing following radiation by supplemental vitamin A is due to its 1) enhancing the early inflammatory reaction to wounding, including increasing the number of monocytes and macrophages at the wound site; 2) possible effect on modulating collagenase activity; 3) effect on epithelial cell (and possible mesenchymal cell) differentiation; 4) stimulation of immune responsiveness; and 5) lessening of the adverse effects of radiation.

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ACUTE RADIATION INJURY leads to thymic involution, adrenal enlargement, leukopenia, thrombocytopenia, gastrointestinal ulceration, and impaired wound healing. We hypothesized that supplemental vitamin A would mitigate these adverse effects in rats exposed to acute whole-body radiation. This hypothesis was based on previous experiments in our laboratory that showed that supplemental vitamin A is thymotropic for normal rodents and lessens the thymic involution, lymphopenia, and adrenal enlargement that follows stress, trauma,[1,2] and neoplasia[3] and speeds allograft rejection,[4] as had been shown by Jurin and Tannock.[5] We showed also that supplemental vitamin A ameliorates the impaired wound healing of rats with femoral fracture[6]; Ehrlich and Hunt had shown that supplemental vitamin A will mitigate the adverse effects of glucocorticoids on healing.[7,8] Other experiments in our laboratory demonstrated that supplemental vitamin A largely obviates the impaired wound healing induced by the radiomimetic drugs streptozotocin[9] and cyclophosphamide,[10] lessens the systemic response (thymic involution, adrenal enlargement, leukopenia, lymphocytopenia) to local radiation,[11] and lessens the leukopenia, lymphocytopenia, thrombocytopenia, thymic involution, and adrenal enlargement following whole-body radiation and shifts the median lethal dose ($LD_{50/30}$) to the right by approximately 100 rad.[12–14]

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A series of experiments carried out with rats to test our hypothesis is reported in this paper; the results support the hypothesis.

Methods

Rats, Housing, Diets

The rats used in these experiments were male Sprague–Dawley weighing about 350 g obtained from two suppliers (Charles River Breeding Laboratories, Wilmington, MA and Camm Research Laboratories, Wayne, NJ). The rats were maintained for acclimatization in our laboratory for 1 week prior to use in any experiment. The rats were housed singly in two mesh stainless steel cages at a room temperature of 29° C and relative humidity of 40% and a 12-hour day:night cycle. They ate a commercial rat chow (Purina #5001, Ralston Purina Company, Checkerboard Square, St. Louis, MO) which contains 15,000 IU vitamin A and 6.4 mg of β carotene per kg chow, a level approximately three times the National Research Council's Recommended Daily Allowance of vitamin A for normal rats and which sustains normal growth, reproduction, lactation, and longevity of normal rats. This control diet is, therefore, not deficient in vitamin A. The rats in each experiment were divided by closely matched weights into groups, some of which continued to ingest the control rat chow while the others ingested the chow to which 150,000 IU of vitamin A (retinyl palmitate or retinyl acetate) was added per kilogram chow. The vitamin A-supplemented diet was made by dissolving the vitamin A [retinyl palmitate (1000 IU/mg) or retinyl acetate] (ICN Pharmaceuticals, Cleveland, OH) in 30 ml of absolute ethanol. The control chow was ground to a coarse powder in our laboratory. While the powdered feed was being mixed, the 30 ml of vitamin A solution was treated with 200 ml of tap water, causing a fine insoluble film precipitate of vitamin A. The suspension was added to 6 kg of powdered chow in small portions with constant mixing. Finally, 15 ml of absolute alcohol were used to wash the residue of vitamin A into the mixture. The diets were mixed for 2 or more hours. This level of vitamin A supplementation is not toxic for mice; we have maintained mice in good health on this type of diet for well over 1 year[15] and have used it in a number of investigations including studies of wound healing,[6] local x-ray-radiation,[11] and whole body gamma radiation.[12–14]

All mice ate and drank tap water *ad libitum* throughout the experiments; food intake was measured in three experiments. The rats were weighed serially in each experiment.

Table 1 outlines the ten experiments conducted, listing the times relating to radiation (and dosage), wounding, and vitamin A supplementation.

Radiation and Sham Radiation

In groups of eight to ten, the rats received whole-body gamma radiation in a ^{137}Cesium (Cs) small animal irradiation (Gammacell-40, Atomic Energy Ltd., Ottawa, Canada) chamber. This unit contains two ^{137}Cs sources of approximately 1800 curies each: one source was positioned above and the other below the exposure cavity so as to produce a uniformity of ±5% for the 0.66 Mev gamma radiation within the 30-cm diameter and 10-cm high sample chamber. The dose rate within the chamber, calibrated using thermoluminescent dosimeters (TLD), was 130 rad/minute. Forced ventilation was supplied to the exposure cavity during irradiation. Control rats were sham radiated, that is, they were placed in the chamber for the same lengths of time and conditions as the irradiated rats, but not radiated.

Wounding, Breaking-strength Measurements, and Reparative Collagen Assessment

Operations (7-cm paravertebral dorsal skin incision and, in some experiments, subcutaneous implantation of polyvinyl alcohol sponges) were performed aseptically as previously described by us[16,17] on rats anesthetized lightly with intraperitoneal sodium pentobarbital supplemented as necessary with light ether anesthesia. The dry weight of the sponges averaged 13 to 17 mg. The incisions were closed with seven equidistant No. 36 stainless steel sutures. No dressings were applied.

At various times after wounding, all rats were injected intraperitoneally with an anesthetic dose of sodium pentobarbital and blood samples obtained by heart puncture. The length of the skin incision was measured *in situ,* and then the scar was excised with generous margins of surrounding skin. The breaking strength was measured in the fresh state and after formalin fixation as described previously.[16,17] The increased breaking strength of 10% buffered formalin-fixed specimens is due to cross-linking of the reparative collagen *in vitro* by the formalin. The sponges were then dissected and their hydroxyproline content (OHP) measured by the method of Woessner[18]; this reflects the collagen content of the reparative granuloma.

In a number of experiments, the incisions and subcutaneous polyvinyl alcohol sponge implants were prepared for histologic examination after fixation in 10% buffered formalin. Hematoxylin-eosin and van Gieson stains were used. The slides will be examined under a blinded code and the data reported later.

TABLE 1. *List of Experiments*

Experiment No.	Radiation		Vitamin A Supplement		Wounding		Days Postoperative
	Rad	Time in Relation to Wounding (days)	Time in Relation to Radiation (days)	Time in Relation to Wounding	Skin Incision	Subcutaneous Sponge	
1	Sham	Immediately before	Immediately after	Immediately after	+	0	14
	175	Immediately before	Immediately after	Immediately after	+	0	14
	350	Immediately before	Immediately after	Immediately after	+	0	14
2	600	Immediately before	Immediately after	Immediately after	+	0	9
3	Sham	−2	−2	−4	+	0	5
	750	−2	−2	−4	+	0	5
	850	−2	−2	−4	+	0	5
4	Sham	−2	−2	−4	+	0	7
	750	−2	−2	−4	+	0	7
5	Sham	−2	−2	−4	+	+	10
	750	−2	−2	−4	+	+	10
6	Sham	Immediately before	Immediately after	Immediately after	+	0	7
	750	Immediately before	Immediately after	Immediately after	+	0	7
7	Sham	Immediately before	+1	+1	+	+	7
	750	Immediately before	+1	+1	+	+	7
8	Sham	Immediately before	+2	+2	+	+	10
	750	Immediately before	+2	+2	+	+	10
9	Sham	Immediately before	+4	+4	+	+	10
	850	Immediately before	+4	+4	+	+	10
10	Sham	Immediately before	+4	+4	+	+	14
	750	Immediately before	+4	+4	+	+	14

In each radiated subgroup there were two groups, one receiving the Vitamin A supplement, the other not. In Experiments 1 and 3, half the sham-radiated rats received the Vitamin A supplement, the other half did not; in the other experiments, none of the sham-radiated rats received the Vitamin A supplement.

Blood Counts

Peripheral blood was obtained from the tail vein; smears were made immediately and then stained with Wright's stain for differential leukocyte counts. For total leukocyte counts, erythrocytes were lysed with 0.1 N HCL and the remaining leukocytes counted in a Neubauer hemocytometer. Platelets were counted using the method of Tocantins.[19]

Thymus and Adrenals, Stomach

At the times of measurements of wound strength, the adrenal glands and thymus were dissected and weighed. The stomachs were removed and examined, especially for the presence of ulcers.

Statistical Treatment

The data of each experiment were analyzed by the Fisher LSD test.

Results

I. Experiments using 175, 350, and 600 rad total-body radiation

IA. Effect of supplemental vitamin A begun promptly (hours) after whole body irradiation and wounding: Experiments 1 and 2. In these experiments, rats eating the control rat chow were exposed to 175, 350, and 600 rad whole-body irradiation and then immediately subjected to wounding. They were then immediately divided into two groups, one of which continued on the control chow while the other was begun on the vitamin A-supplemented chow. Sham-radiated wounded rats were studied concurrently.

There were no statistically significant differences between the control chow sham-radiated and sham-radiated vitamin A-supplemented rats in terms of body-weight change, thymic weight, skin incision-breaking strength, both fresh and after formalin fixation during the 2 weeks

TABLE 2. *Effect of Supplemental Vitamin A on Wound Healing* of Total-Body Radiated Rats: Experiment 1 (14-day Skin Incisions)*

Radiation dosage	Group	Supplement	Body wt. at Operation (g)	Body wt. at Sacrifice (g)	Thymus wt (g)	Breaking Strength (g) Fresh	Breaking Strength (g) Formalin-fixed
Sham	I (8)†	None	379 ± 9‡	433 ± 10	0.40 ± 0.04	418 ± 36	1613 ± 162
	II (8)	Vitamin A§	379 ± 11	437 ± 11	0.40 ± 0.03	455 ± 47	1712 ± 170
175 rad	III (10)	None	379 ± 16	412 ± 13	0.31 ± 0.02	348 ± 33	1295 ± 142
	IV (10)	Vitamin A	385 ± 8	420 ± 10	0.35 ± 0.02	379 ± 35	1464 ± 103
350 rad	V (10)	None	384 ± 8	405 ± 7	0.31 ± 0.01	357 ± 26	1488 ± 81
	VI (10)	Vitamin A	384 ± 8	418 ± 9	0.34 ± 0.02	388 ± 20	1529 ± 38

* Wounding right after radiation.

† No. rats, Sprague–Dawley, male, Charles River Breeding Laboratories.

‡ Mean ± SEM.

§ 150,000 IU vitamin A (retinyl acetate) added per kg control diet.

P values: I *vs* III, <0.01; I *vs* V, <0.01; II *vs* IV, NS; II *vs* VI, <0.05; all other comparisons, NS.

after surgery, or peripheral leukocyte counts (postoperative days 9–10) (Tables 2 and 3).

Among rats exposed to 175 and 350 rad, the control unsupplemented chow rats had significantly smaller thymuses after 2 weeks than those of the sham-radiated control chow and sham-radiated vitamin A-supplemented rats. The thymuses of the radiated vitamin A-supplemented rats were somewhat larger than those of the control chow radiated rats but these differences were not statistically significant. Among the vitamin A-supplemented groups, there was no statistically significant difference in thymus weight between the sham-radiated and 175-rad groups, but the thymuses of the 350-rad group were smaller ($p < 0.05$) than those of the sham-radiated group. There were no statistically significant differences among any of the groups in terms of body-weight change or wound-healing strength, both tested in the fresh state and after formalin fixation (14 days after surgery), although the wounds of the radiated rats were somewhat weaker than those of the sham-radiated rats. Among the radiated rats, the breaking strengths of the incisions of the vitamin A-supplemented rats were slightly higher in each case than those of the control chow rats, but none of these differences were statistically significant.

The peripheral leukocyte counts of the control chow radiated rats, both 175 and 350 rad, were significantly lower at post-radiation and postoperative days 3, 7, and 10 ($p < 0.001$, <0.001, and <0.005, respectively) than those of the sham-radiated, control chow rats. This was also the case for the vitamin A-supplemented, radiated

TABLE 3. *Effect of Supplemental Vitamin A on the Leukocytes (LC) of Total-body Radiated and Wounded* Rats: Experiment 1*

Radiation Dosage	Group	Supplement	Days After Irradiation and Wounding: 0 LC $10^3/mm^3$	3 LC $10^3/mm^3$	7 LC $10^3/mm^3$	10 LC $10^3/mm^3$
Sham	I (N = 8)†	None	17.0 ± 0.58‡	12.2 ± 0.74	15.0 ± 0.96	15.8 ± 1.37
	II (N = 8)	Vit. A§	18.4 ± 1.48	13.6 ± 0.84	16.1 ± 0.95	16.5 ± 0.94
		P values I *vs* II	NS	NS	NS	NS
175 rad	III (N = 10)	None	16.8 ± 0.95	5.8 ± 0.62	6.2 ± 0.72	10.1 ± 1.12
	IV (N = 10)	Vit. A	17.7 ± 1.00	8.1 ± 0.84	10.6 ± 0.92	13.4 ± 1.27
		P values III *vs* IV	NS	<0.05	<0.05	NS
350 rad	V (N = 10)	None	17.7 ± 0.73	4.7 ± 0.51	6.3 ± 0.46	10.9 ± 0.96
	VI (N = 10)	Vit. A	17.4 ± 0.52	4.3 ± 0.39	6.5 ± 0.62	11.1 ± 0.74
		P values V *vs* VI	NS	NS	NS	NS
		P values I *vs* III	NS	<0.001	<0.001	<0.005
		I *vs* V	NS	<0.001	<0.001	<0.01
		III *vs* V	NS	NS	NS	NS
		II *vs* IV	NS	<0.001	<0.001	<0.05
		II *vs* VI	NS	<0.001	<0.001	<0.005
		IV *vs* VI	NS	<0.005	<0.005	NS

* Wounding right after radiation.

† Sprague-Dawley, male, Charles River Breeding Laboratories.

‡ Mean ± SEM.

§ 150,000 IU vitamin A (retinyl acetate) added per kg control diet.

TABLE 4. *Effect of Supplemental Vitamin A on Wound Healing* and the Peripheral Leukocytes of Total-body Radiated Rats (Experiment 2)*

Days after 600 rad and wounding		Supplemental Vitamin A	
		Group 1: None (N = 8)	Group 2: Vitamin A Acetate‡ 150,000 IU/Kg Diet (N = 7)
0	Body weight (g)	249 ± 3§	246 ± 3
	Leukocyte count ($10^3/mm^3$)	17.7 ± 1.2	16.3 ± 1.0
2	Body weight (g)	212 ± 4	207 ± 3
	Leukocyte count ($10^3/mm^3$)	3.7 ± 0.51	3.6 ± 0.48
5	Body weight (g)	238 ± 5	232 ± 6
	Leukocyte count ($10^3/mm^3$)	3.5 ± 0.32	3.1 ± 0.22
9	Body weight (g)	269 ± 4	260 ± 5
	Leukocyte count ($10^3/mm^3$)	8.3 ± 0.94	7.5 ± 0.67
	Thymus (mg)	260 ± 0.02	250 ± 0.62
	Wound-breaking strength (g)		
	Fresh	194 ± 17	223 ± 23
	Formalin-fixed	729 ± 40	777 ± 65

* Wounding done right after radiation.
† Sprague-Dawley male, Charles River Breeding Laboratories.
‡ Begun immediately after radiation and wounding.
§ Mean ± SEM.
None of the differences between Groups 1 and 2 is statistically significant.

rats compared with the vitamin A-supplemented, sham-radiated rats at 350 rad ($p < 0.001$, <0.001, and <0.005, respectively); and at 175 rad ($p < 0.001$, <0.001, and <0.05, respectively), although the leukopenia was less after 175 rad than after 350 rad. The peripheral leukocyte count of the 175-rad, vitamin A-supplemented rats was significantly higher ($p < 0.05$) than that of the control chow, 175-rad, radiated rats at days 3 and 7 after radiation and wounding; there were no statistically significant differences between the 350-rad groups whether or not supplemental vitamin A was given (Table 3).

After 600-rad radiation, there were no statistically significant differences between the vitamin A-supplemented and the control chow groups in terms of body-weight changes, peripheral leukocyte counts, thymus weights, and wound-breaking strengths, both fresh and after formalin fixation (9 days after surgery), although the incisions of the vitamin A-supplemented rats were somewhat stronger than those receiving the control chow (Table 4).

II. Experiments Using 750 and 850 Rad Total-body Radiation

IIA. Effect of supplemental vitamin A begun prior to radiation and wounding: Experiments 3, 4, and 5. In these three experiments, the supplemental vitamin A was begun 2 days before radiation; wounding was carried out 2 days after radiation.

The results of experiment 3 are summarized in Table 5. In the sham-radiated wounded rats, vitamin A supplementation did not affect the change in body weight, wound-breaking strength, or adrenal weight during the 5-day postoperative period. However, thymus weight and peripheral leukocyte counts were considerably greater in the vitamin A-supplemented rats ($p < 0.001$ in each case).

Whole-body radiation (750 and 850 rad) and wounding of the control chow rats led to decreases in body weight ($p < 0.001$ in each case), wound-breaking strength ($p < 0.05$ and <0.2, respectively), thymus weight ($p < 0.001$ in each case), and leukocyte count ($p < 0.001$ in each case) and increases in adrenal weight ($p < 0.02$ and <0.2, respectively); there were no statistically significant differences in these parameters between those exposed to 750 rad and those exposed to 850 rad, except that the body-weight loss was greater in the latter group ($p < 0.05$). Vitamin A supplementation of the 750- and 850-rad radiated wounded rats led to lesser body-weight losses and lesser decreases in peripheral leukocyte count as compared with control chow, radiated, wounded rats, but these differences were not statistically significant. There was a statistically significant lesser decrease in thymus weight ($p < 0.001$) and wound-breaking strength was higher ($p < 0.1$, 750 rad; $p < 0.005$, 850 rad) in the vitamin A-supplemented group. There were some punctate hemorrhages adjacent to the skin incision sites in the radiated control chow rats; these were not present in the vitamin A-supplemented group; this difference was present also in experiments 6 to 10.

In similar experiments, rats sham radiated and radiated (750 rad) wounded rats were killed at postoperative day 7 (experiment 4) and day 10 (experiment 5), respectively (Tables 6 and 7, Figs. 1 and 2). Weight loss of the radiated wounded rats was significantly less in the vitamin A-supplemented rats in each of these experiments as compared with the control chow, radiated, wounded rats ($p < 0.005$ and <0.001, respectively). The marked decrease in wound-breaking strength of the

TABLE 5. *Effect of Supplemental Vitamin A on Wound Healing of Whole-body Radiated Rats:* Experiment 3 (5-day Wounds)*

Group	#Rats	Radiation Dosage†	Supplement‡	Body Weight (g) At Rad.	Body Weight (g) At Sac.	Body Weight (g) Change	Thymus weight (mg)	Adrenals weight (mg)	Leukocyte Count ($10^3/mm^3$)	Breaking Strength (g) Fresh	Breaking Strength (g) Fixed
I	4	None	None	356 ± 5§	390 ± 5	33 ± 7	438 ± 20	68 ± 1	16.5 ± 1.5	120 ± 18	768 ± 43
II	4	None	Vit. A	353 ± 8	387 ± 11	29 ± 6	568 ± 11	68 ± 1	23.7 ± 1	107 ± 9	841 ± 37
III	8	750 rad	None	344 ± 5	342 ± 5	−2 ± 4	118 ± 3	72 ± 1	1.5 ± 0.1	81 ± 8	493 ± 33
IV	8	750 rad	Vit. A	362 ± 6	364 ± 5	3 ± 3	211 ± 5	70 ± 1	2.6 ± 0.2	111 ± 8	545 ± 34
V	8	850 rad		348 ± 5	328 ± 6	−20 ± 7	113 ± 2	70 ± 1	1.3 ± 0.2	92 ± 8	446 ± 32
VI	8	850 rad		355 ± 3	343 ± 4	−12 ± 4	207 ± 6	69 ± 1	1.8 ± 0.2	146 ± 14	531 ± 54
			P values I *vs* II	NS	NS	NS	<0.001	NS	<0.001	NS	NS
			I *vs* III	NS	<0.001	<0.001	<0.001	<0.02	<0.001	<0.05	<0.001
			I *vs* V	NS	<0.001	<0.001	<0.001	NS	<0.001	NS	<0.001
			II *vs* IV	NS	<0.02	<0.005	<0.001	NS	<0.001	NS	<0.001
			II *vs* VI	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.05	<0.001
			III *vs* IV	<0.05	<0.02	NS	<0.001	NS	NS	NS	NS
			III *vs* V	NS	NS	<0.05	NS	NS	NS	NS	NS
			IV *vs* VI	NS	<0.02	NS	NS	NS	NS	NS	NS
			V *vs* VI	NS	NS	NS	<0.001	NS	NS	<0.005	NS

* Male Sprague-Dawley rats, Charles River Breeding Laboratories.
† Radiation 2 days before operation.
‡ Vitamin A supplement began 2 days before radation, 4 days before operation.
§ Mean ± SEM.

radiated rats eating the control chow diet ($p < 0.001$ in each experiment) was prevented by the vitamin A supplementation as was the decrease in accumulation of reparative collagen in the subcutaneously implanted polyvinyl alcohol sponges (measured in experiment 5, $p < 0.001$). The sponge granulomas were hemorrhagic in all radiated control chow rats but not in about 30% of the vitamin A-supplemented rats. This difference was found also in experiments 7 to 10. The decrease in thymus weight ($p < 0.001$) and increase in adrenal weight ($p < 0.001$) were significantly ameliorated in the vitamin A-supplemented rats in both experiments ($p < 0.001$).

IIB. Effect of supplemental vitamin A begun immediately after radiation (750 rad) and wounding: Experiment 6. When the supplemented vitamin A chow was offered immediately after radiation (750 rad) and wounding, the markedly impaired ($p < 0.001$) wound healing (7 days after surgery) seen in the radiated wounded rats eating the control chow was prevented completely and the weight loss ($p < 0.001$), thrombocytopenia ($p < 0.025$), thymic involution ($p < 0.001$), adrenal enlargement ($p < 0.001$), and gastric ulceration ($p < 0.001$) seen in the control chow, radiated, wounded rats were lessened significantly (Table 8, Figs. 3–7).

IIC. Effect of supplemental vitamin A begun 1 day after radiation (750 rad) and wounding: Experiment 7. When the supplemental vitamin A was not started until 1 day after radiation and wounding, there were no statistically significant differences in the changes in body weight of the radiated wounded rats eating the control diet and those eating the vitamin A-supplemented diet. However, the breaking strengths of the skin incisions (7 days after surgery) of the vitamin A-supplemented radiated rats were considerably (about 54%) and significantly ($p < 0.001$) stronger than those of the control

TABLE 6. *Effect of Supplemental Vitamin A on Wound Healing* of Total-body Radiated Rats:† Experiment 4 (7-day Wounds)*

Group	Radiation Dosage‡	# Rats	Supplementation‡	Body Weight (g) At Rad. (Day-2)	Body Weight (g) At Op. (Day-0)	Body Weight (g) At Sac. (Day + 7)	Body Weight (g) Change	Thymus Weight (mg)	Adrenal Weight (mg)	Breaking Strength (g) Fresh	Breaking Strength (g) Fixed
I	None	9	None	280 ± 3§	285 ± 4	319 ± 5	40 ± 3	392 ± 10	62.7 ± 0.9	153 ± 14	764 ± 49
II	750 rad	10	None	285 ± 2	243 ± 2	269 ± 3	−15 ± 2	115 ± 3	72.7 ± 0.7	65 ± 5	339 ± 10
III	750 rad	10	Vit. A	287 ± 3	251 ± 3	284 ± 4	−3 ± 2	199 ± 4	69.0 ± 0.3	134 ± 13	491 ± 30
			P values I *vs* II	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
			I *vs* III	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001
			II *vs* III	NS	NS	<0.02	<0.005	<0.001	<0.001	<0.001	<0.001

* Wounding done 2 days after radiation.
† Sprague-Dawley males, from Camm Research Laboratories.
‡ Vitamin A supplement begun 4 days before operation and 2 days before radiation.
§ Mean ± SEM.

TABLE 7. *Effect of Supplemental Vitamin A on Wound Healing of Total-body Radiated Rats:* Experiment 5 (10-day Wounds)*

Group	# Rats	Radiation Dosage	Supplement‡		Body Weight (g) Start Vit. A (Day −4)	At Rad (Day −2)	At Op (Day 0)	At Sac. (Day +10)	Change Rad. Sac.	Thymus weight (mg)	Adrenal weight (mg)	Hydroxy-proline μg/100 mg dry sponge	Breaking Strength (g) Fresh	Fixed
I	9	None	None		264 ± 3§	273 ± 4	276 ± 4	304 ± 5	31 ± 2	306 ± 7	61.40 ± 0.5	1,828 ± 179	248 ± 16	1251 ± 40
II	8	750 rad	None		272 ± 2	283 ± 2	240 ± 2	271 ± 3	−12 ± 3	111 ± 2	69.2 ± 0.4	846 ± 73	126 ± 11	857 ± 46
III	10	750 rad	Vit. A		270 ± 2	280 ± 2	240 ± 3	287 ± 5	7 ± 3	195 ± 2	65.7 ± 0.6	1,619 ± 92	216 ± 12	977 ± 52
			P values	I *vs* II	<0.05	<0.025	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
				II *vs* III	NS	NS	NS	<0.02	<0.001	<0.001	<0.001	<0.001	<0.001	NS
				I *vs* III	NS	NS	<0.001	<0.02	<0.001	<0.001	<0.001	NS	NS	<0.001

* Sprague–Dawley male rats from Camm Research Laboratories.
† Radiation 2 days before operation.
‡ Purina Lab Chow or Chow with 150,000 IU Vitamin A supplement begun 4 days before operation, 2 days before radiation.
§ Mean ± SEM.

diet radiated rats, though somewhat less (about 22%, p < 0.001) than those of the sham-radiated rats. Reparative collagen accumulation in the subcutaneous polyvinyl alcohol sponges was about four times greater (p < 0.001) in the vitamin A-supplemented radiated rats than that in the control radiated rats and significantly higher (p < 0.001) than that of the sham-radiated rats eating the control diet. The decrease in thymus weight was significantly less in the vitamin A-supplemented, radiated, wounded rats (p < 0.02) than in the control chow radiated rats and the increase in adrenal size was prevented (p < 0.001). Also, gastric ulceration, substantial in the control diet, radiated, wounded rats, was almost completely prevented in the vitamin A-supplemented, radiated, wounded group (Table 9, Fig. 8).

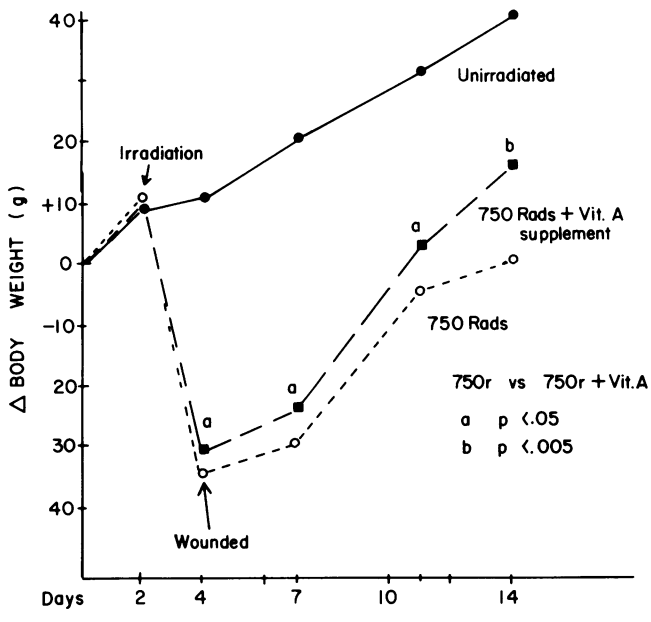

FIG. 1. Experiment 5: Effect of supplemental Vitamin A on body weight of radiated (750 rad) and wounded rats. The supplemental vitamin A was begun 4 days prior to wounding; wounding was done 2 days after radiation, p values listed.

IIID. Effect of supplemental vitamin A begun 2 days after radiation (750 rad) and wounding: Experiment 8. In this experiment, the supplemental vitamin A was not begun until 2 days after radiation (750 rad) and wounding, and the rats were killed on the tenth postoperative day. There were no statistically significant differences in the food intake or body-weight changes of the radiated, wounded, vitamin A-supplemented rats when compared to those of their counterparts eating the control chow. Both radiated wounded groups ate considerably less (about 32%) during days 2 to 5 after radiation and wounding (p < 0.001 in each case) and lost considerably more weight than the sham-radiated wounded rats (p < 0.001 in each case) (Table 10).

The decrease in wound-breaking strengths (10 days after surgery) (p < 0.05) and accumulation of reparative collagen in the subcutaneous implanted polyvinyl alcohol sponges (p < 0.005) seen in the control diet radiated rats when compared to the sham-radiated rats were

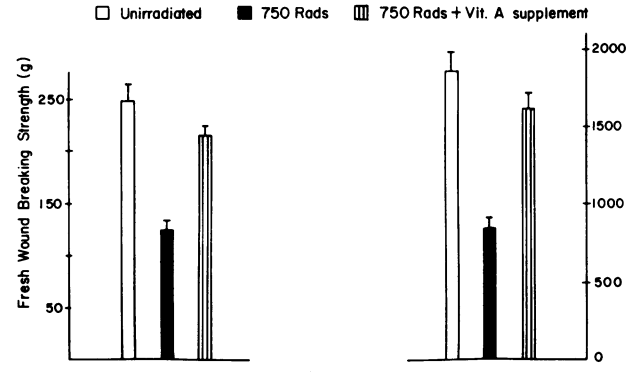

FIG. 2. Experiment 5: Effect of supplemental Vitamin A on wound-breaking strength and accumulation of reparative collagen in subcutaneous polyvinyl alcohol sponges. The supplemental vitamin A was begun 2 days before wounding and 4 days before radiation. Ten days postoperative, sham-radiated, wounded *vs* control chow, radiated, wounded, p < 0.001 for each; supplemental vitamin A, radiated wound *vs* control chow, radiated, wounded, p < 0.001 for each; sham-radiated *vs* vitamin A-supplemented, NS for each.

TABLE 8. *Effect of Supplemental Vitamin A* on Wound Healing of Radiated Rats:† (Dorsal Skin Incisions, 7 Days After Surgery Experiment 6*

Group	# Rats	Radiation and Supplement	Body Wt. (g)			Wound-breaking Strength, (g) (7 Days)		Thymus weight (mg)	Adrenal weight (mg)	Platelets (10^3/mm^3)	Gastric Ulcers	
			At Op.	At Sac.	Change	Fresh	Fixed				# Rats with Ulcers	Total # Ulcers
I	10	None	294 ± 4§	304 ± 5	10 ± 2	146 ± 7	816 ± 39	428 ± 18	61.9 ± 0.6	163.3 ± 13.6	0/11	0
II	10	750 rad‡	295 ± 3	270 ± 3	−24 ± 2	83 ± 5	411 ± 26	100 ± 3	76.3 ± 0.3	47.3 ± 2.1	9/11	32
III	10	750 rad† Vit. A	297 ± 4	283 ± 4	−14 ± 2	144 ± 8	469 ± 26	197 ± 7	67.0 ± 0.7	77.5 ± 4.8	2/11	3
		P values I *vs* II	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		II *vs* III	NS	<0.01	<0.001	<0.001	NS	<0.001	<0.001	<0.025	<0.001	<0.001
		I *vs* III	NS	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001	NS	NS

* The supplemental vitamin A was begun right *after* radiation and wounding.
† Male Sprague-Dawley rats, Camm Research Laboratories.
‡ Radiation just before wounding.
§ Mean ± SEM.

prevented in the vitamin A-supplemented radiated rats ($p < 0.05$ and <0.001, respectively). In fact, the accumulation of reparative collagen in the sponges was significantly greater in the latter rats than in the sham-radiated rats ingesting the control diet ($p < 0.02$) (Fig. 9). The marked drop in the peripheral leukocyte count 6 days after radiation and wounding was not significantly different in the control chow and vitamin A-supplemented groups, but the vitamin A-supplementation lessened the thrombocytopenia significantly ($p < 0.01$) and the decreases in thymus ($p < 0.001$) and spleen ($p < 0.001$) weights and increase in adrenal weight ($p < 0.001$), each measured 10 days after radiation and wounding (Table 11 and Fig. 10).

IIE. Effect of supplemental vitamin A begun 4 days after radiation (850 rad) and wounding: Experiment 9. In this experiment, the vitamin A supplementation was begun 4 days after radiation (850 rad) and wounding; the rats were killed on the tenth postoperative day. Food intake, measured starting on day 4 and continuing until killing, was not statistically different in the sham-radiated and radiated control chow groups. Food intake was less in the vitamin A-supplemented radiated rats, and significantly so ($p < 0.01$) as compared with the sham-radiated group on postoperative days 7 to 9. However, the body weights of the vitamin A-supplemented radiated and control chow radiated rats were similar; both had lost weight, so that by the 4th day after radiation and wounding they weighed about 10% less than the sham-

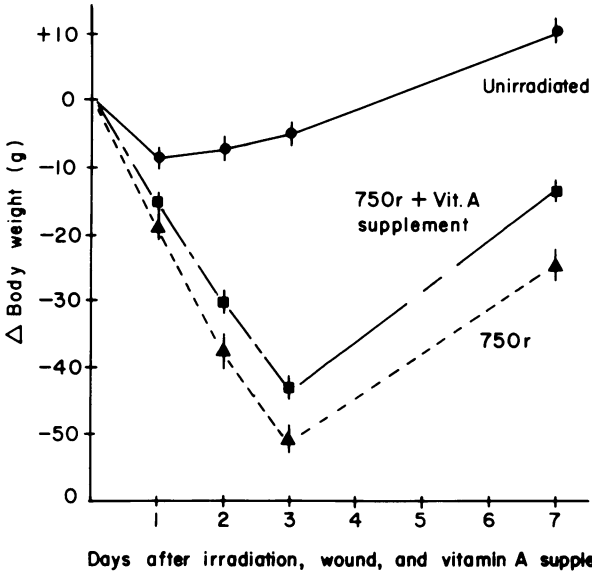

FIG. 3. Experiment 6: Effect of supplemental vitamin A on body weight of radiated (750 rad) and wounded rats. Wounding was done right after radiation. Vitamin A supplementation started right after radiation and wounding. Sham-radiated wounded *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$.

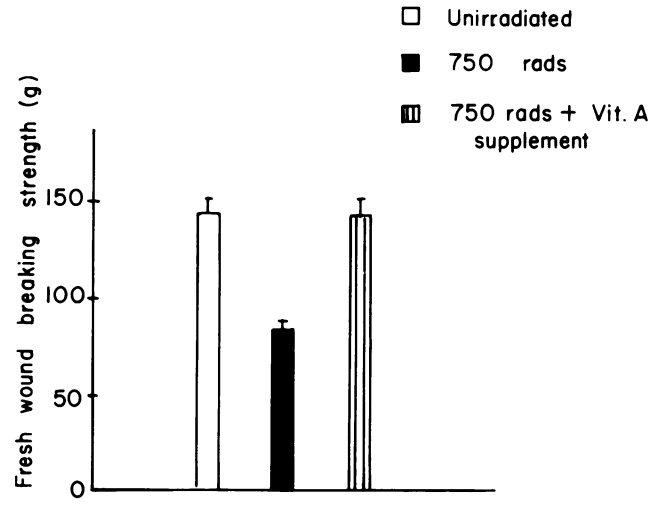

FIG. 4. Experiment 6: Effect of supplemental vitamin A on wound-breaking strength of radiated (750 rad) rats, 7 days postoperative. The supplemental vitamin A begun right after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, NS.

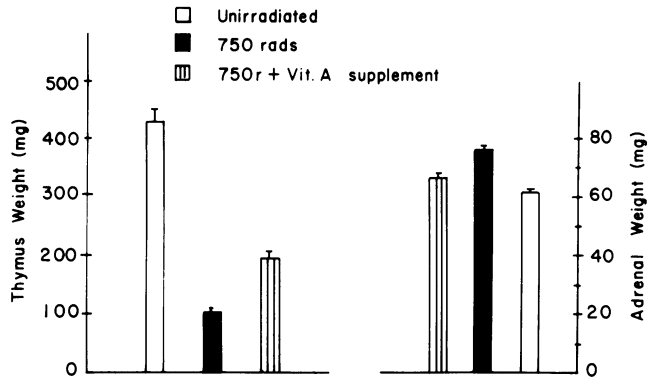

FIG. 5. Experiment 6: Effect of supplemental vitamin A on thymus and adrenal weights of radiated (750 rad) rats, 7 days postoperative. Supplemental vitamin A begun right after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$ for each. Vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$ in each case.

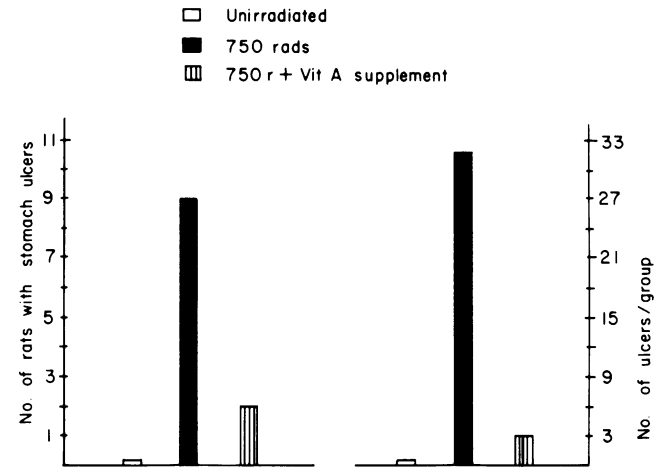

FIG. 7. Experiment 6: Effect of supplemental vitamin A on occurrence of gastric ulcers in radiated (750 rad) rats. Supplemental vitamin A begun right after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, NS.

radiated rats ($p < 0.001$ in each case) and these differences continued until killing ($p < 0.001$ in each case) (Table 12). On the 7th day after radiation and wounding, the control chow radiated rats had become severely leukopenic ($p < 0.001$) and thrombocytopenic ($p < 0.001$). The vitamin A-supplemented, radiated, wounded rats had by this time received the supplemental vitamin A for only 3 days: their peripheral leukocyte and platelet counts were higher than those of the control chow, radiated, wounded rats (1600 *vs* 950/mm^3 and 45,000 *vs* 27,000/mm^3), but the differences were not statistically significant. The decrease in thymus weight ($p < 0.001$) and increase in adrenal weight ($p < 0.001$) of the control chow radiated rats as compared with the sham-radiated rats were lessened significantly ($p < 0.001$ and <0.005, respectively) in the vitamin A-supplemented radiated rats. All radiated rats had gastric ulcers, but the severity was less in the vitamin A-supplemented group (Table 13).

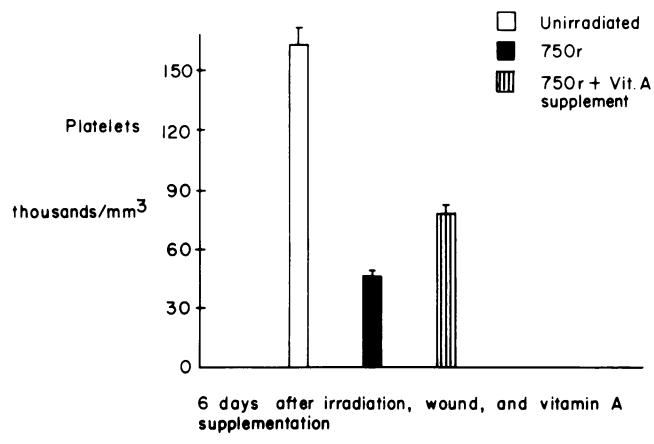

FIG. 6. Experiment 6: Effect of supplemental vitamin A on peripheral blood platelets of radiated (750 rad) rats. Supplemental vitamin A begun right after radiation and wounding. Blood platelets measured 6 days after radiation and wounding. Sham-radiated wounded rats *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.025$.

The wound-breaking strength was decreased markedly in the control chow radiated rats as compared with the sham-radiated rats (a factor of 2.25, $p < 0.001$); this was significantly ameliorated in the vitamin A-supplemented group (274 *vs* 163 g, $p < 0.005$) but not entirely corrected (274 *vs* 374 g, $p < 0.005$) (Table 13, Fig. 11).

Experiment 10. In this experiment, the start of vitamin A supplementation was delayed until 4 days after radiation (750 rad) and wounding and the rats were killed on the 14th postoperative day. There was no statistically significant difference in food intake among the groups of rats as measured on days 4 to 14, the period during which vitamin A supplementation was given to one of the two radiated and wounded groups. Both radiated wounded groups lost about 10% of their body weight during the first 4 days, while the sham-radiated rats gained about two per cent ($p < 0.001$ in each case). During the next 10 days, all three groups gained weight, but at killing the control chow radiated and vitamin A-supplemented radiated groups had lost two to three per cent of their weight just before radiation and wounding, while the sham-radiated group had gained seven per cent ($p < 0.001$ in each case) (Table 14).

Peripheral leukocyte and platelet counts done on the 11th day after radiation and wounding, 7 days after the start of vitamin A supplementation, showed a marked leukopenia ($p < 0.001$) and thrombocytopenia (p

TABLE 9. *Effect of Supplemental Vitamin A on Wound Healing of Total Body Radiated Rats: Experiment 7 (7-day Wounds)*

Group	# Rats	Radiation Dosage†	Supplement‡	Body Weight (g) At Op.	Body Weight (g) At Sac.	Body Weight (g) Change	Thymus weight (mg)	Adrenal weight (mg)	Hydroxyproline μg/100 mg dry sponge	Breaking Strength (g) Fresh	Breaking Strength (g) Fixed
I	10	None	None	305 ± 2§	322 ± 3	17 ± 2	453 ± 29	65 ± 3	557 ± 28	187 ± 12	874 ± 39
II	10	750 rad	None	300 ± 3	288 ± 4	−12 ± 3	86 ± 5	78 ± 2	224 ± 9	94 ± 10	460 ± 34
III	10	750 rad	Vit. A	302 ± 2	289 ± 2	−12 ± 2	151 ± 10	58 ± 3	812 ± 61	145 ± 13	561 ± 27
			P values I *vs* II	NS	<0.001	<0.001	<0.001	<0.005	<0.001	<0.001	<0.001
			I *vs* III	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
			II *vs* III	NS	NS	NS	<0.02	<0.001	<0.001	<0.001	<0.05

* Male Sprague-Dawley rats, Camm Research Laboratories.
† Radiation just before wounding.
‡ Vitamin A supplement begun 1 day after radiation and operation.
§ Mean ± SEM.

< 0.001) in the radiated rats eating the control chow. The thrombocytopenia was lessened significantly ($p < 0.05$) in the radiated, wounded, vitamin A-supplemented rats; the leukocyte count was higher in the latter group ($2.5 \times 10^3/mm^3$ *vs* $1.3 \times 10^3/mm^3$) but the difference was not statistically significant (Table 15).

At sacrifice on the 14th postoperative day, the decreases in thymus ($p < 0.001$) and spleen ($p < 0.001$) weights and increase in adrenal weight ($p < 0.001$) in the control, radiated, wounded rats were significantly less in the vitamin A-supplemented radiated rats as compared to the control chow radiated rats ($p < 0.001$, <0.01, <0.05, respectively), but the amelioration was not to the levels found in the sham-radiated group (Fig. 12). Four of nine rats in the radiated, wounded, control chow and two of nine rats in the radiated, wounded, vitamin A-supplemented group had small gastric ulcers.

There was a slight decrease (7%) in skin incision-breaking strength in the radiated control chow group compared with the sham-radiated group. The wound-breaking strength of the vitamin A-supplemented radiated group was somewhat higher (12%) than that of the control chow radiated group and minimally higher (4%) than that of the sham-radiated, control chow group but these differences were not statistically significant (Fig. 13).

There was a marked diminution (44%) in the accumulation of reparative collagen in the subcutaneous polyvinyl alcohol sponges of the radiated control chow rats as compared to that of the control chow sham-radiated rats ($p < 0.001$). This was completely prevented in the vitamin A-supplemented radiated rats ($p < 0.001$); in fact, the accumulation of sponge reparative collagen was greater in the vitamin A-supplemented rats than in the control chow sham-irradiated rats ($p < 0.001$) (Fig. 13).

Discussion

Wound healing following acute whole-body radiation is impaired; the degree of impairment varies with the

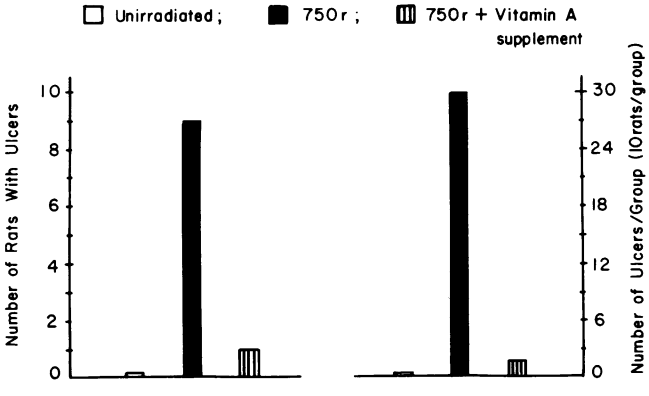

FIG. 8. Experiment 7: Effect of supplemental vitamin A on occurrence of gastric ulcers in radiated (750 rad) rats. Supplemental vitamin A begun 1 day after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, NS.

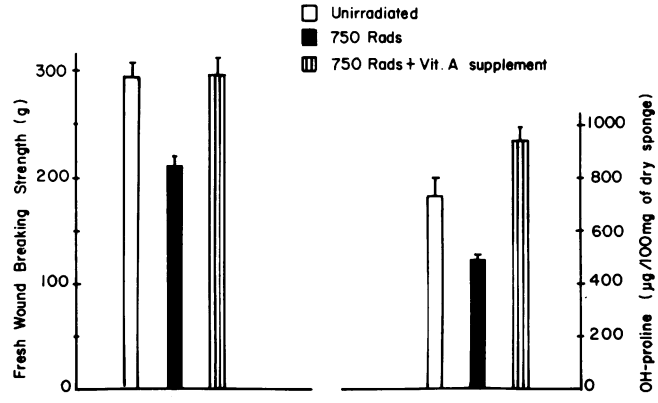

FIG. 9. Experiment 8: Effect of supplemental vitamin A on wound-breaking strength (WBS) and accumulation of reparative collagen (OHP) in subcutaneous polyvinyl alcohol sponges of radiated (750 rad) rats; 10 days postoperative. Supplemental vitamin A begun 2 days after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, WBS $p < 0.05$, OHP $p < 0.005$. Vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, WBS $p < 0.05$, OHP $p < 0.001$. Vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, WBS NS, OHP $p < 0.02$ (vitamin A-supplemented the greater).

TABLE 10. *Effect of Supplemental Vitamin A on Food Consumption and Body Weight of Total-body Radiated Rats:* Experiment 8*

Group	# Rats	Radiation Dosage†	Supplement‡	Food Consumed/Period (g)§ Days After Rad. & Wounding 2	3–5	6–7	8–9	Body Wt. (g) A‖	B¶	C**	Change A–C
I	8	None	None	28 ± 3	89 ± 4	47 ± 2	51 ± 2	334 ± 6	333 ± 7	350 ± 6	16 ± 3
II	8	750 rad	None	17 ± 4	64 ± 4	49 ± 2	53 ± 2	335 ± 8	313 ± 7	328 ± 6	−5 ± 4
III	8	750 rad	Vit. A	13 ± 2	65 ± 4	52 ± 1	58 ± 2	331 ± 5	309 ± 6	318 ± 5	−14 ± 1
			P values I *vs* II	<0.02	<0.001	NS	NS	NS	<0.05	<0.05	<0.001
			I *vs* III	<0.005	<0.001	<0.05	<0.05	NS	<0.02	<0.001	<0.001
			II *vs* III	NS	NS	NS	NS	NS	NS	NS	NS

* Male Sprague-Dawley rats, Camm Research Laboratories.
† Radiation just before wounding.
‡ Vitamin A supplementation started 2 days after wounding.
§ Food consumption was measured for 8 days.
‖ Body weight morning of radiation and wounding.
¶ Body weight on day vitamin A supplementation begun (2 days after radiation and wounding).
** Body weight at sacrifice (10 days after radiation and wounding).

dosage of whole-body radiation, the time of wounding in relation to radiation exposure, the nature and extent of the wound and other injuries, the degree and type of wound microbial contamination, the preexisting health of the individual, and the quality of medical care.[20–29]

The precise mechanisms underlying the delay in wound healing following whole-body radiation have not been delineated. Various hematologic, metabolic, physiologic, and immunologic alterations consequent to acute whole-body radiation have been considered. Stromberg and his associates[23] have shown that shielding part of the bone marrow during acute whole-body x-irradiation lowered mortality and lessened the decrease in the closure of open dorsal skin wounds of rats made 4 days after radiation. Donati, a co-author of the preceeding paper, reported a few years later (1971)[24] using the same experimental model system that while administration of tetracycline and streptomycin, begun 1 day prior to radiation and 5 days prior to making the open skin wounds, diminished mortality, these drugs (and also chloramphenicol) did not ameliorate the impaired wound healing of the radiated rats. In a subsequent study, Donati, McLaughlin, and Stromberg[25] found in similar experimental radiation-wounding experiments carried out with germ-free and conventional rats that, as anticipated from earlier studies,[30] mortality was much less in the unwounded, germ-free, radiated (800 rad) rats than in the unwounded conventional rats (0% *vs* 55%). Mortality increased in both germ-free and conventional radiated rats when wounding (open skin wounds of the back) was carried out 4 days after radiation. The previously observed retardation in healing of the open skin wounds of radiated conventional rats was observed again, but no such slowing was seen in the radiated germ-free rats. The authors interpret their findings as suggesting" . . . that wound healing abnormalities which occur following radiation in rats result, in part, from bacterial contamination and entry of more virulent organisms due to impaired immune capability secondary to hematologic radiation injury." We suggest that the possibility should be borne in mind, however, that the dose of 800 rad that caused 55% mortality in the conventional rats and 0% mortality in the germ-free might have affected certain key systems (*e.g.*, metabolic, physiologic, immunologic, hematologic) to a significantly less extent in the germ-free rats, independently of adverse secondary effects due to possible infection in the conventional rats.

Radakovich, Dutton, and Schilling[26] found about 30 years ago that there was no delay in the closure of open

TABLE 11. *Effect of supplemental Vitamin A on Wound Healing of Total-body Radiated Rats:* Experiment 8 (10-day Wounds)*

Group	# Rats	Radiation Dosage†	Supplement‡	Leukocytes§ (10^3/mm^3)	Platelets§ (10^3/mm^3)	Thymus weight (mg)	Adrenal weight (mg)	Spleen weight (mg)	Rats with Ulcer‖	Fresh Breaking Strength (g)	Hydroxyproline μg/100 mg dry sponge
I	8	None	None	16.6 ± 0.9	537 ± 55	359 ± 4	62 ± 1	764 ± 17	0	293 ± 23	735 ± 70
II	8	750 rad	None	1.4 ± 0.2	131 ± 10	89 ± 3	81 ± 1	267 ± 9	8	220 ± 22	489 ± 24
III	8	750 rad	Vit. A	2.3 ± 0.3	236 ± 32	173 ± 10	73 ± 2	326 ± 10	8	295 ± 26	941 ± 52
			P values I *vs* II	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.05	<0.005
			I *vs* III	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.02
			II *vs* III	NS	<0.01	<0.001	<0.001	<0.001	NS	<0.05	<0.001

* Sprague-Dawley, male, from Camm Labs. †Radiation just before wounding.
‡ Vitamin A supplementation begun 2 days after radiation and operation.
§ Done 6 days after radiation and wounding, 4 days after vitamin A supplementation.
‖ Many very small ulcers in each radiated and wounded rat.

skin wounds of rats exposed to 150, 450, and 650 rad whole-body radiation; the wounds were made on the backs of the rats on the day of radiation or 1, 2, 4, or 8 weeks after radiation. The wounds of surviving rats exposed to 650 rad ($LD_{20/30}$ or $LD_{50/30}$) closed faster than those of the unirradiated controls. We know of no subsequent study that has attempted to confirm this latter observation, although the finding is consistent with the co-carcinogenic action of ultraviolet radiation.

It is noteworthy that a small open skin wound made one day before whole-body radiation shifts the $LD_{50/30}$ to the right, resulting, *e.g.*, in mice, in a dose reduction factor of 1.2.[31,32] Ledney and his colleagues[32] found that ". . . wounding before irradiation provoked an increase in marrow and splenic clonogenic cells that was earlier and greater than that noted for irradiated mice." They believe that the increased survival likely reflects the increases in colony-forming cells, which would presumably enhance myeloproliferative recovery.

Stromberg, McLaughin, and Donati[33] have found that when beta mercaptoethylamine, serotonin, and S-2-amino propyl (amino ethyl) phosphoric acid (Walter Reed 2721-C) were injected separately into rats 15 minutes before whole-body x-ray-radiation (800 rad in air), mortality was decreased (from 63% to 25%–40%), and the slowing of the rate of closure of open skin wounds on the back made 4 days after radiation was less in the drug-treated survivors than in the surviving rats injected with saline.

The data we have reported in this article support our hypothesis that, in rats, supplemental vitamin A would lessen the adverse effects of whole-body radiation (750 and 850 rad) on wound healing. This was so whether the supplemental vitamin A was begun before or after (hours to 4 days, the latter the longest time interval we have studied so far); the earlier the supplemental vitamin A is started, the more protective it is likely to be. It is especially noteworthy, however, that it is effective when started after acute, whole-body radiation and not only when given prior to radiation. This has important clinical and therapeutic implications.

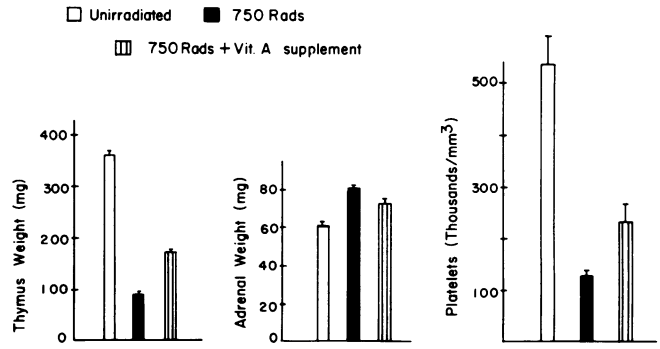

FIG. 10. Experiment 8: Effect of supplemental vitamin A on thymus and adrenal weights and peripheral blood platelets of radiated (750 rad) rats. Supplemental vitamin A begun 2 days after radiation and wounding. Platelets measured 4 days after start of vitamin A supplementation. Control chow, radiated, wounded, *vs* sham-radiated wounded, $p < 0.001$ for each; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$, $p < 0.001$, $p < 0.01$, respectively; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$ in each case.

Our data regarding the ameliorating effects of supplemental vitamin A on thymus, adrenal and spleen weights, peripheral leukocytes, platelets, and gastric ulceration are in keeping with previous findings in our laboratory of A) the thymotropic effect (thymus weight, number of thymic lymphocytes, acceleration of skin allograft rejection) of supplemental dietary vitamin A in normal mice and rats, in wounded rats, in rats with femoral fracture, and in tumor-bearing mice[1–4]; B) the changes in peripheral leukocytes (especially lymphocytes) that follow vitamin A supplementation of normal mice,[34] and the amelioration of the drop in peripheral leukocytes (especially lymphocytes) following local x-ray irradiation of

TABLE 12. *Effect of Supplemental Vitamin A on Food Consumption and Body Weight of Total-body Radiated Rats:* Experiment 9*

Group	# Rats	Radiation Dosage†	Supplements‡	Food Consumed/ Period (g)§ Days After Radiation and Wounding		Body Weights (g)			
				4–6	7–9	A‖	B¶	C**	Change A–C
I	10	None	None	82 ± 4***	90 ± 3	355 ± 3	355 ± 3	356 ± 3	1 ± 2
II	10	850 rad	None	80 ± 3	84 ± 3	352 ± 3	319 ± 3	319 ± 3	−34 ± 4
III	10	850 rad	Vit. A	70 ± 7	78 ± 3	356 ± 3	320 ± 3	326 ± 3	−30 ± 2
			P values I *vs* II	NS	NS	NS	<0.001	<0.001	<0.001
			I *vs* III	NS	<0.01	NS	<0.001	<0.001	<0.001
			II *vs* III	NS	NS	NS	NS	NS	NS

* Male Sprague–Dawley rats, Camm Research Laboratories.
† Radiation just before wounding.
‡ Vit. A Supplement begun 4 days after radiation and wounding.
§ Food Consumption was measured for six days.
‖ Body weight morning of radiation and wounding.
¶ Body weight on day Vitamin A supplementation begun.
** Body weight at sacrifice (10 days after radiation and wounding).
***Mean ± SEM.

TABLE 13. *Effect of Supplemental Vitamin A on Wound Healing of Total-body Radiated Rats:* Experiment 9 (10-day Wounds)*

Group	#Rats	Radiation Dosage†	Supplement‡	Leukocytes§ (10^3/mm^3)	Platelets§ 10^3/mm^3	Thymus weight (mg)	Adrenal weight (mg)	Rats with Ulcers‖	Fresh Breaking Strength (g)
I	10	None	None	12.8 ± 0.6	660 ± 72	371 ± 11	72 ± 0.9	0	374 ± 29
II	10	850 rad	None	1.0 ± 0.2	27 ± 3	96 ± 4	81 ± 0.4	10	163 ± 17
III	10	850 rad	Vit. A	1.6 ± 0.1	45 ± 8	183 ± 4	78 ± 0.3	10	274 ± 24
			P values I *vs* II	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
			I *vs* III	<0.001	<0.001	<0.001	<0.001	<0.001	<0.005
			II *vs* III	NS	NS	<0.001	<0.005	NS	<0.005

* Male Sprague-Dawley rats, Camm Research Laboratories.
† Radiation just before wounding.
‡ Vit. A Supplement begun 4 days after radiation and wounding.
§ Done 7 days after radiation and wounding, 3 days after Vitamin A supplementation.
‖ Many small ulcers in each radiated and wounded rat. The rats in group III have noticeably fewer ulcers.

a hind limb of mice,[11] C) the alleviation of gastrointestinal ulceration following physical stress[35] and administration of toxic chemicals[36]; and D) the alleviation of the thrombocytopenia by supplemental vitamin A of unwounded mice exposed to whole-body irradiation reported by Shen, Mendecki, Rettura, et al.[37]

It is our view that multiple actions of vitamin A are involved in these effects. There is evidence for an increased requirement for vitamin A after injury and infections under conditions where preexisting vitamin A deficiency does not exist. For example, Kagan and Kaiser[38] have found that rats with subcutaneous abscesses, as the result of repeated injections of turpentine and sweet almond oil, showed sustained decreases in serum vitamin A concentration and liver vitamin A, whereas kidney concentration and urinary excretion of vitamin A were increased. Rae and Courtemanche[39] and Hunt (personal communication, TK Hunt) reported substantial decreases in plasma vitamin A following burns, and Moore[40] and Hunt (personal communication) have found decreases at autopsy in liver vitamin A in injured patients.

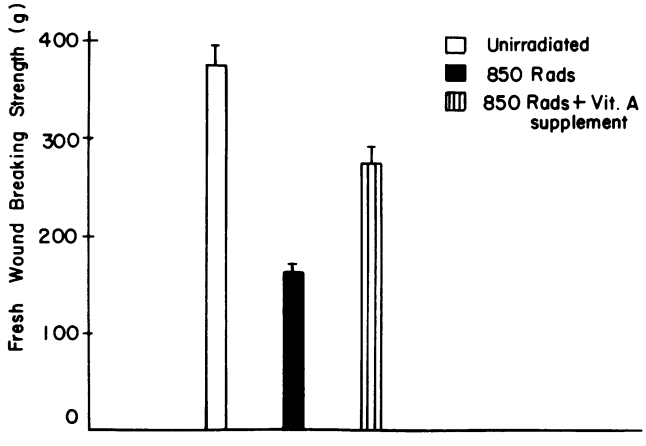

FIG. 11. Experiment 9: Effect of supplemental vitamin A on wound-breaking strength of radiated (850 rad) rats, postoperative day 10. Supplemental vitamin A begun 4 days after radiation and wounding, 6 days before sacrifice. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$. Vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.005$; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, $p < 0.005$.

Hunt, Ehrlich, and associates[7,8] have shown that supplemental vitamin A restored toward normal the breaking strength of incisions and the closure of open skin wounds in cortisone-treated rats, but the vitamin A had no accelerating effect on the healing of wounds in rats not receiving cortisone. On the other hand, Herrmann and Woodward[41] found that vitamin A given to "non-deficient" normal rats resulted in increased fibroplasia, as judged histologically and by the hydroxyproline content of polyvinyl alcohol sponge granulomas.

Martin and his associates[42], Chernov and his colleagues[43], and we[35] have shown that supplemental vitamin A lessens the occurrence of stress ulcers.

We have found that supplemental vitamin A given to healthy rats ingesting a commercial rat chow containing more vitamin A than the National Research Council Recommended Daily Allowance has a number of beneficial effects.

1. Lessens the weight loss due to anesthesia, operation, and injury.
2. Promotes the early inflammatory reaction of wound healing (1–3 days after surgery) and increases vascularization of reparative tissue (3–7 days after surgery).
3. Increases the incidence and severity of experimental intra-abdominal postoperative adhesions following ligation of a small fold of peritoneum. Citral, a vitamin A antagonist, decreases the incidence and severity of such postoperative intra-abdominal adhesions and antagonizes the adhesion potentiating effect of vitamin A.[44]
4. Increases reparative collagen in subcutaneously implanted polyvinyl alcohol sponges of otherwise healthy, uninjured rats.
5. Ameliorates the otherwise impaired wound healing of injured animals (unilateral or bilateral femoral fractures) by increasing the gain of strength of skin incisions

TABLE 14. *Effect of Supplemental Vitamin A on Food Consumption and Body Weight of Total-body Radiated Rats: Experiment 10 (14-day Wounds)*

Group	# Rats	Radiation Dosage	Supplement*	Food Consumed/Period (g)† Days After Operation 4–5	6–7	8–10	11–13	Body Wt (g) A‡	B§	C‖	Change A–C
I	9	None	None	52 ± 2	57 ± 2	95 ± 4	78 ± 4	333 ± 9	340 ± 8	355 ± 7	22 ± 3
II	9	750 rad	None	46 ± 3	52 ± 3	83 ± 4	70 ± 5	341 ± 7	310 ± 6	333 ± 8	−7 ± 4
III	9	750 rad	Vit. A	50 ± 4	50 ± 3	87 ± 5	67 ± 3	334 ± 7	306 ± 5	326 ± 6	−9 ± 3
			P values I *vs* II	NS	NS	NS	NS	NS	<0.005	<0.05	<0.001
			I *vs* III	NS	NS	NS	NS	NS	<0.005	<0.005	<0.001
			II *vs* III	NS	NS	NS	NS	NS	NS	NS	NS

* Vitamin A supplementation started 4 days after wounding. †Food consumption was measured for 10 days starting the day vitamin A supplementation begun.

‡ Body weight on morning of radition and wounding.

§ Body weight on day vitamin A supplementation begun 4 days after wounding.

‖ Body weight at sacrifice, day 14.

and accumulation of reparative collagen in subcutaneously implanted sponges.

6. Serum vitamin A concentrations are lower in wounded rats with fracture than in those rats only wounded at various levels of vitamin A supplementation. The level of vitamin A supplementation has little effect on serum vitamin A levels despite differences in liver vitamin A contents.

7. Increases the rate of gain of strength and accumulation of reparative collagen in colon anastomoses.[45]

8. Affects the changes that occur after injury in peripheral leukocytes, particularly the polymorphonuclear cells, lymphocytes, and monocytes.

9. Increases the influx of macrophages into the wound site, a finding suggested previously by others.

10. Increases thymic size and number of thymic lymphocytes in uninjured, intact rats and mice. This thymotropic effect is independent of the adrenals, since it is seen in adrenalectomized rats.

11. Lessens substantially the abrupt decrease in the size of the thymus and the number of thymic lymphocytes and the increase in adrenal size, which characteristically occur following injury.

12. Lessens the adrenal enlargement and hemorrhage, gastrointestinal ulceration, and thymolytic effects of toxic chemicals such as 7, 12 dimethyl(α)benzanthracene. Ameliorates the ulcerogenic action of aspirin.[46]

13. Increases cell-mediated immunity, *e.g.*, it accelerates skin allograft rejection. This was also reported by Jurin and Tannock.[5] Cohen and his associates[47] have shown also that supplemental vitamin A given to patients increases the *in vitro* mitogenic responsiveness of peripheral blood lymphocytes tested after elective operations.

14. Increases the resistance to experimental peritonitis following perforation of ligated cecum.[48]

15. Minimizes gastric and duodenal ulceration and death of rats following Noble–Collip Drum shock.[49]

16. Prevents the impaired wound healing and thymic involution (weight and number of thymic lymphocytes) of streptozotocin diabetic rats, an effect that is independent of any effect of supplemental vitamin A on the hyperglycemia, polydypsia, glycosuria, and polyuria.

17. Prevents the impaired wound healing and thymic involution of rats given cyclophosphamide; ameliorates the decreased resistance of injured rats to it and decreases mortality of rats subjected to wounding and cyclophosphamide.

TABLE 15. *Effect of Supplemental Vitamin A on Wound Healing of Total-body Radiated Rats:* Experiment 10 (14-day Wounds)*

Group	# Rats	Radiation Dosage†	Supplement‡	Leukocyte§ (10^3/mm^3)	Platelets§ (10^3/mm^3)	Thymus weight (mg)	Adrenal weight (mg)	Spleen weight (mg)	Rats with Gastric Ulcers	Fresh Breaking Strength	Hydroxyproline μg/100 mg dry sponge
I	9	None	None	13.8 ± 1.0	734 ± 102	373 ± 13	68 ± 1	798 ± 10	None	565 ± 32	1,534 ± 68
II	9	750 rad	None	1.3 ± 0.2	74 ± 3	137 ± 7	79 ± 1	392 ± 27	4/9	525 ± 46	856 ± 63
III	9	750 rad	Vit. A	2.5 ± 0.4	157 ± 15	258 ± 9	76 ± 2	471 ± 12	2/9	587 ± 37	1,980 ± 102
			P values I *vs* II	<0.001	<0.001	<0.001	<0.001	<0.001	<0.025	NS	<0.001
			I *vs* III	<0.001	<0.001	<0.001	<0.001	<0.001	NS	NS	<0.001
			II *vs* III	NS	<0.05	<0.001	<0.05	<0.01	NS	NS	<0.001

* Sprague-Dawley, male, from Camm Labs. †Radiation done just before wounding.

‡ Vitamin A palmitate supplemented chow begun 4 days after radiation and operation.

§ Done 11 days after radiation and wounding and 7 days after vitamin A supplementation begun.

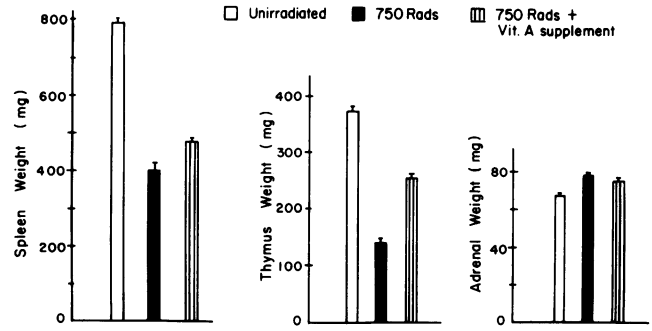

FIG. 12. Experiment 10: Effect of supplemental vitamin A on spleen, thymus, and adrenal weights of radiated (750 rad) rats. Supplemental vitamin A begun 4 days after radiation and wounding. Control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$ in each case; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.01$, $p < 0.001$, $p < 0.05$, respectively; vitamin A-supplemented, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$ in each case.

18. Decreases weight loss, leukopenia (principally lymphopenia), thymic involution, and adrenal enlargement of rats following x-ray-radiation of an extremity.

19. Increases the resistance ($LD_{50/30}$) of rats to whole-body x-ray-radiation; lessens leukopenia (principally lymphopenia), thymic involution, adrenal enlargement, and thrombocytopenia. These ameliorating effects are evident (though less marked) even when the supplemental vitamin A is begun 2 to 3 days after radiation. Little effect was seen when the vitamin A-supplementation was begun 6 days after radiation.

20. Is effective as a preventative and therapeutic agent in a number of experimental neoplasms.[50-54]

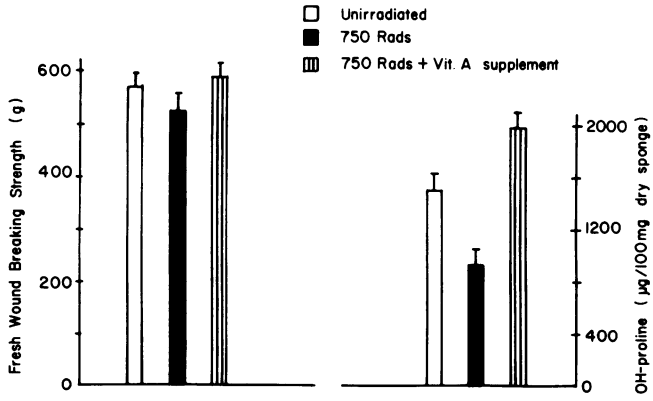

FIG. 13. Experiment 8: Effect of supplemental vitamin A on wound-breaking strength (WBS) and accumulation of reparative collagen (OHP) in subcutaneous polyvinyl alcohol sponges of radiated (750 rad) rats, 14 days postoperative. Supplemental vitamin A begun 4 days after radiation and wounding. No statistically significant differences in WBC among the three groups. In regard to sponge OHP, control chow, radiated, wounded *vs* sham-radiated wounded, $p < 0.001$; vitamin A-supplemented, radiated, wounded *vs* control chow, radiated, wounded, $p < 0.001$; vitamin A-supplemental, radiated, wounded *vs* sham-radiated wounded, OHP, $p < 0.001$ (vitamin A-supplemented higher).

21. Adds substantially to the antineoplastic action of local radiation therapy and also chemotherapy for certain experimental tumors.[15,55,58]

We believe that prevention of the impaired wound healing following radiation by supplemental vitamin A is due to its 1) enhancing the early inflammatory reaction to wounding, including increasing the number of monocytes and macrophages at the wound site; macrophages are relatively radio-resistant and survive for long periods of time *in situ;* 2) a possible effect on modulating collagenase activity (the accumulation of reparative collagen in the healing wound is the result of a net balance between collagen synthesis and collagenolysis); 3) effect on epithelial cell (and possibly mesenchymal cell) differentiation; 4) stimulating immune responsiveness; and 5) lessening the adverse effects of radiation.

The mechanisms whereby supplemental vitamin A protects against radiation injury have not been established definitively; we have discussed this matter elsewhere.[11,13,14] Data of the studies in our laboratory suggest that a major protective action of the supplemental vitamin A is due to prevention of the sequelae of radiation injury rather than an immediate direct radioprotective action. This is supported by our observation that supplemental vitamin A begun several hours after local radiation of an extremity of mice moderated the weight loss, adrenal hypertrophy, thymic involution, and leukopenia. Support for this view is also given by our finding that supplemental vitamin A begun 1 to 3 days after whole-body radiation shifted the $LD_{50/30}$ and $LD_{50/100}$ to the right.

However, this in no way rules out the concept that supplemental vitamin A may also have direct radioprotective action and a role in the repair of radiation damage. Beta carotene and other terpenes related to vitamin A (but not retinoic acid) exert protection against ultraviolet phototoxicity; this suggests that vitamin A could elicit radio-protective action against ionizing radiation. However, because of the very high energy content of x-radiation, vitamin A cannot act as a barrier to the radiation. This is in contrast to the way in which β carotene and vitamin A prevent the primary event damage in ultraviolet radiolysis.

Although vitamin A likely does not prevent the primary radiolytic reactions of x-radiation, it could prevent damage to cell constituents by products of x-radiolysis of the solvent. For example, x-radiation promotes the following reaction:

$$H_2O \rightarrow H_2O^+ + e^- \text{ (ionization of water)}$$
$$H_2O^+ + H_2O \rightarrow H_3O^+ (H^+ + H_2O) + OH^\cdot$$
$$\text{Sum: } 2H_2O \rightarrow H_3O^+ + OH^\cdot + e^-$$

The OH-free radical or its dimer H_2O_2 are responsible for some of the radiation-induced cell damage. Enzymes,

such as peroxidases and catalase, can decompose the H_2O_2 into $H_2O + \frac{1}{2}O_2$. The hydrated electron produced by radiolysis of water is sufficiently energized to cause radical formation in nucleic acids and thereby favor cross-linking of nucleic acids or cleavage of the link between the base and the deoxysugar. Detoxication of the hydrated electron can occur by metabolic utilization of the electron by enzymes involved in superoxide (O_2^-) metabolism. Theoretically, vitamin A could protect cells against the radiolytic products of water, $OH^\cdot$ and e^-, because vitamin A has a very high degree of conjugation of single and double bonds. The highly conjugated double bond system of vitamin A makes it possible for these molecules to "buffer" hydrated electrons until these are disposed of by chemical interactions with electrophilic agents or by metabolic utilization of hydrated electrons attached to oxygen (superoxide, O_2^-).

In this connection, the earlier work of Lucy and Lichti[56] shows that retinol (but not retinoic acid) reacts with electron acceptors such as iodine and can donate or share electrons with quinones. Also, the double bond of the B-ionine ring of vitamin A or retinoic acid is readily oxidized by $OH^\cdot$ or H_2O_2 to the epoxy derivatives as shown by DeLuca.[57] Oxidation of retinoic acid to the epoxy or hydroxyl derivatives may be a part of a natural mechanism for enzymatic and chemical detoxication of peroxides and hydroxyl-free radicals.

One of the authors (Eli Seifter) and Jacques Padawer of the Department of Anatomy, Albert Einstein College of Medicine, have put forth the view that vitamin A may protect against (or reverse) radiation damage by promoting cell differentiation as opposed to cell division. Actively dividing cells are more radio-sensitive than are nondividing or postmitotic cells. They speculate that vitamin A aldehyde promotes differentiation (and, therefore, decreases radiation toxicity) at the level of ribonucleoside diphosphate reductase, an enzyme that regulates DNA synthesis:

Nucleoside diphosphate +SH SH
(dithiol subunit of nucleoside diphosphate reductase) →
Deoxyribonucleoside diphosphate
+ S—S (oxidized form of enzyme)

In the presence of excess retinal $\left(\begin{matrix}H\\RCO\end{matrix}\right)$, the following reaction would occur readily:

$$\begin{matrix}H\\RC=O\end{matrix} + \begin{matrix}HS & SH\end{matrix} \rightarrow R-C\begin{matrix}H\\ \diagup\ \diagdown\\ S \quad S\end{matrix}.$$

The reaction is likely to have two important physiologic effects: A) conversion of the radiosensitive forms of the enzyme (the dithiol and disulfide) to a more radioresistant thioacetal, and B) regulation of DNA synthesis by inhibiting the biosynthesis of DNA precursor nucleotides from ribonucleotides. The latter reaction would favor RNA synthesis and cell differentiation rather than DNA synthesis and cell proliferation.

The possible direct radio-protective effects of supplemental vitamin A does not interfere with the antitumor action of local-tumor x-irradiation, but, in fact, we found that it adds substantially to the antitumor action.[14,15,55,58] We think that this added antitumor action by supplemental vitamin A is largely the result of its indirect radio-protective effects, as a result of which host resistance to neoplasms is increased. We have speculated also that tumor destruction by radiation may give rise to antigens and that the development of antitumor immune reactions to these antigens is stimulated by vitamin A supplementation.

Supplemental vitamin A also mitigates many of the adverse effects of cyclophosphamide (*e.g.*, impaired wound healing, weight loss, thrombocytopenia) without interfering with its antitumor action, but, in fact, adding to it.[10,14,58]

We consider the actions of vitamin A in increasing host resistance to be very important. Medawar and his associates[59,60] have pointed out that the immunity-promoting action of vitamin A is likely through its actions on the cellularity of thymus glands and the function of certain lymphocyte subsets. This view is similar to the one we had expressed earlier.[3] Similarly, Lotan and Dennert[61] and Lotan[62] have related the thymic effects of vitamin A to the enhancement of the tumor immunity by vitamin A. These are likely important in resistance to infection also, a factor of major importance following acute whole-body radiation.

We think that key to the action of dietary vitamin A in this regard is its transit route. When given via the gastrointestinal tract, dietary vitamin A is absorbed by small bowel mucosa and then is transported in the lymph draining these segments of the gut. In addition to thereby generating lymph that is high in vitamin A, the lamina propria and Peyer's patches provide the lymph with high concentrations of β lymphocytes and monocytes. The interactions between vitamin A and these leukocytes in the lymph likely lead to activation of these two cell types. The activated cells and vitamin A then enter the blood circulation via the subclavian vein from whence they are distributed.

It should be emphasized that the control chow diets that we have used in the various studies carred out in our laboratory during the past decade contain two to three times the NRC Recommended Daily Allowance

for normal rodents and is, therefore, not vitamin A deficient. The amount of supplemental vitamin A that we have added, 150,000 IU/kg control chow, did not lead to vitamin A toxicity when ingested daily for over 1 year by mice. This may be associated with the route by which the supplemental vitamin A was given, namely, with the diet. At the doses we have used, vitamin A is not cytotoxic nor does it labilize lysosomal membranes.

The findings that the adverse effects of whole-body radiation alone and whole-body radiation and wounding are mitigated to a substantial degree by supplemental vitamin A show that 1) it has prophylactic action when begun prior to radiation, as do other radio-protective agents; 2) it has therapeutic action when given up to several days after radiation, an action that differentiates it from other radio-protective agents; 3) even when radiation dosage was so high that supplemental vitamin A did not decrease mortality, it prolonged survival significantly.[13] This would furnish additional time for other therapeutic measures to be beneficial, *e.g.*, antimicrobial agents, transfusion of blood, platelets, leukocyte cells, or marrow cells. This contrasts with the effects of most other radio-protective agents, such as sulfhydryl compounds, which must be given prior to or during radiation to be protective. These data suggest that therapeutic supplemental vitamin A may ameliorate the impaired wound healing and lessen morbidity and mortality in humans following accidental or purposeful radiation exposure. In other experiments we have found that supplemental β carotene acts similarly to supplemental vitamin A under a number of experimental conditions, *e.g.*, radiation injury, tumor therapy, thymotropic effects.[13,14,53-55,58,63,64] Beta carotene may have some special advantages because of its very low toxicity.

References

1. Seifter E, Rettura G, Seifter J, et al. Thymotropic action of vitamin A (Abstract #4904). Fed Proc 1973; 32:947.
2. Barbul A, Thysen B, Rettura G, et al. White cells involvement in the inflammatory, wound healing, and immune actions of vitamin A. Journal of Parenteral and Enteral Nutrition 1978; 2:129–140.
3. Seifter E, Rettura G, Levenson SM, et al. A mechanism of action of vitamin A in immunogenic tumor systems. *In* Siegenthaler W, Luthy R, eds. Current Chemotherapy. Washington, DC: The American Society for Microbiology, 1978; 1290–1291.
4. Rettura G, Levenson SM, Schittek A, et al. Vitamin A: actions in oncogenesis and skin graft rejection. Surg Forum 1975; 26:301–303.
5. Jurin M, Tannock IF. Influence of vitamin A on immunological response. Immunology 1972; 23:283–287.
6. Seifter E, Crowley LV, Rettura G, et al. Influence of vitamin A on wound healing in rats with femoral fracture. Ann Surg 1975; 181:836–841.
7. Ehrlich HP, Hunt TK. Effects of cortisone and vitamin A on wound healing. Ann Surg 1968; 167:324–328.
8. Hunt TK, Ehrlich HP, Garcia JA, et al. Effect of vitamin A on reversing the inhibitory effect of cortisone on healing of open wounds in animals and man. Ann Surg 1969; 170:633–641.
9. Seifter E, Rettura G, Padawer J, et al. Impaired wound healing in streptozotocin diabetes: prevention by supplemental vitamin A. Ann Surg 1981; 194:42–50.
10. Stratford F, Rettura G, Babyatsky M, et al. Impaired wound healing due to cyclophosphamide alleviated by supplemental vitamin A. Surg Forum 1980; 31:224–225.
11. Seifter E, Rettura G, Stratford F, et al. Vitamin A inhibits some aspects of systemic disease due to local x-radiation. Journal of Parenteral and Enteral Nutrition 1981; 5:288–294.
12. Rettura G, Seifter E, Stratford F, et al. Reduction of radiation sickness by vitamin A. 181st National Meeting, AGFD, American Chemical Society, Atlanta, Georgia, 1981, March 29–April 6.
13. Seifter E, Rettura G, Padawer J, et al. Supplemental vitamin A or beta carotene reduce morbidity and mortality in mice given whole body gamma radiation. Journal of the National Cancer Institute 1984, (in press).
14. Levenson SM, Rettura G, Seifter E. Effects of supplemental dietary vitamin A and beta carotene on experimental tumors, local tumor excision, chemotherapy, radiation injury, and radiotherapy. *In* Butterworth C, Hutchinson ML, eds. Nutrition Factors in the Induction and Maintenance of Malignancy. New York: Academic Press, 1983; 169–203.
15. Seifter E, Rettura G, Padawer J, et al. Regression of C3HBA mouse tumor due to x-ray therapy combined with supplemental beta carotene or vitamin A. Journal of the National Cancer Institute 1983; 71:409–417.
16. Levenson SM, Crowley LV, Geever EF, et al. Some studies on wound healing: experimental methods, effect of ascorbic acid and effect of deuterium oxide. J Trauma 1964; 4:543–566.
17. Levenson SM, Geever EF, Crowley LV, et al. The healing of rat skin wounds. Ann Surg 1965; 161:293–308.
18. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. Arch Biochem Biophys 1961; 93:440–447.
19. Tocantins LM. Technical methods for the study of blood platelets. Arch Pathol 1937; 23:850–879.
20. Conklin JJ, Walker RI, Hirsch EF. Current concepts in the management of radiation injuries and associates trauma. Surg Gynecol Obstet 1983; 156:809–829.
21. Messerschmidt O. Strahlenbelastung und offene Hautwunde. Archiv für klinische und experimentelle Dermatologie 1966; 227:329–335.
22. Levenson SM, Oates JF, Geever EF. Effects of whole body x-irradiation on wound healing. Surg Forum 1960; 11:840–844.
23. Stromberg LWR, Woodward KT, Mahin DT, et al. Altered wound healing in x-irradiated rats: the effect of bone marrow shielding. Experientia 1967; 23:1064–1065.
24. Donati RM. Combined surgical and radiation injury. IV. Effect of anti-microbials on the wound healing pattern of the x-irradiated rat. Arch Surg 1971; 102:132–135.
25. Donati RM, McLaughlin MM, Stromberg LWR. Combined surgical and radiation injury. VIII. The effect of the gnotobiotic state on wound closure. Experientia 1973; 29:1388–1390.
26. Radakovich M, Dutton AM, Schilling JA. The effect of total body irradiation on wound closure, Ann Surg 1954; 139:186–194.
27. Stromberg LWR, Woodward KT, Mahin DT, et al. Combined surgical and radiation injury. The effect of timing of wounding and whole body gamma irradiation on 30 day mortality and rate of wound contracture in the rodent. Ann Surg 1968; 167:18–22.
28. Zemljanoj AG. Heilung geschlossener frakturen der langen rohrerknochen bei strahlenkrankheit an versuchstieren. Med Radiol 1956; 5:72–74.
29. Dubos M, Neveux Y, Monpeyssin M, et al. Impact of ionizing radiation on response to thermal and surgical trauma. *In* Walker RI, Gruber DF, MacVittie TJ, et al., eds. The Pathophysiology of Combined Injury and Trauma. Bethesda, MD: Armed Forces Radiobiology Research Institute, 1983; 51–64.
30. McLaughlin MM, Dacquisto MP, Jacobus DP, et al. Effects of the germfree state on responses of mice to whole-body irradiation. Radiat Res 1964; 23:333–349.

31. Ledney GD, Exum ED, Sheehy PA. Survival enhanced by skin-wound trauma in mice exposed to ^{60}Co radiation. Experientia 1981; 37:193–194.
32. Ledney GD, Stewart DA, Exum ED, et al. Skin wound-enhanced survival and myelocytopoiesis in mice after whole-body irradiation. Acta Radiol Oncol 1981; 20:29–38.
33. Stromberg LWR, McLaughlin MM, Donati RM. Combined surgical and radiation injury. III. The effect of pre-irradiation radioprotective drug treatment (33270). PSEBM 1968; 129:140–143.
34. Rettura G, Barbul A, Padawer J, et al. Vitamin A induced lymphocytosis and monocytosis. 175th National Meeting, AGFD, American Chemical Society, Anaheim, California, 1978, March 13–17 (Abstract #44).
35. Rettura G, Stratford F, Levenson SM, et al. Prevention of ulceration and other stigmata in stressed rodents given supplemental beta carotene. American Society for Parenteral and Enteral Nutrition, New Orleans, Louisiana, 1981, February 2–5.
36. Levenson SM, Seifter E, Rettura G, et al. Vitamin A inhibits ulcerogenic action of toluenediamine (Abstract #3376). Fed Proc 1979; 38:869.
37. Shen RN, Mendecki J, Rettura R, et al. Partial prevention of radiation toxicity by supplemental vitamin A and beta carotene (Abstract #4877). Fed Proc 1983; 42:1124.
38. Kagan BM, Kaiser E. Vitamin A metabolism in infection. J Nutr 1955; 57:277–286.
39. Rae K, Courtemanche AD. Vitamin A assay in burned patients. J Trauma 1976; 15:419–424.
40. Moore T. Vitamin A. New York: Elsevier, 1957.
41. Herrmann JB, Woodward SC. An experimental study of wound healing accelerators. Am Surg 1972; 38:26–34.
42. Martin MS, Lambert R, Martin F, et al. Effet protecteur de la vitamin A sur l'ulcere de contrainte du rat. Comptes Rendus des Séances de la Société de Biologie et de ses filiales (Paris) 1967; 161:2527–2530.
43. Chernov MS, Hale HW, Jr, Wood M. Prevention of stress ulcers. Am J Surg 1971; 122:674–677.
44. Demetriou AA, Seifter E, Levenson SM. Effect of vitamin A and citral on peritoneal adhesion formation. J Surg Res 1974; 17:325–329.
45. Bark S, Rettura G, Goldman D, et al. Effect of supplemental vitamin A on the healing of colon anastomosis. J Surg Res 1984; 36:470–474.
46. Seifter E, Rettura G, Padawer J, et al. Reduction of high dose aspirin toxicity by dietary vitamin A. 183rd National Meeting, American Chemical Society, Agriculture and Food, Las Vegas, Nevada, 1982, March 29–April 3.
47. Cohen BF, Gill G, Cullen PR, et al. Reversal of postoperative immunosuppression in man by vitamin A. Surg Gynecol Obstet 1979; 149:658–662.
48. Demetriou AA, Franco I, Rettura G, et al. Effects of vitamin A and beta carotene on intraabdominal sepsis. Arch Surg 1984; 119:161–165.
49. Seifter E, Rettura G, Levenson SM, et al. Prevention of some sequelae of Noble-Collip drum shock by vitamin A. First Annual Conference on Shock, Airlie, Virginia, 1978, June 1–3.
50. Rettura G, Schittek A, Hardy M, et al. Brief communication: Anti-tumor action of vitamin A in mice inoculated with adenocarcinoma cells. Journal of the National Cancer Institute 1975; 54:1489–1491.
51. Rettura G, Barbul A, Levenson SM, et al. Increased survival in mice with C3H breast adenocarcinoma after tumor excision and vitamin A supplementation. Surg Forum 1978; 29:170–172.
52. Lan S, Rettura G, Levenson SM, et al. Paraneoplastic neutrophilia: response to local tumor excision and vitamin A. Surg Forum 1980; 31:420–421.
53. Seifter E, Rettura G, Levenson SM. Carotenoids and cell-mediated immune responses. *In* Charalambous G, Inglett G, eds. The Quality of Foods and Beverages–Chemistry and Technology. New York: Academic Press, 1981; 335–347.
54. Rettura G, Stratford F, Levenson SM, et al. Prophylactic and therapeutic actions of supplemental beta carotene in mice inoculated with C3HBA adenocarcinoma cells: lack of therapeutic action of supplemental ascorbic acid. Journal of the National Cancer Institute 1982; 69:73–77.
55. Seifter E, Padawer J, Rettura G, et al. Cancer control: x-ray induced C3HBA tumor regression and prevention of its regrowth by beta carotene and vitamin A. *In* Mettlin C, Murphy GP, eds. Progress in Cancer Control. IV. Research in the Cancer Center. New York: Alan R. Liss, Inc., 1983; 237–247.
56. Lucy JA, Lichti FU. Reactions of vitamin A with acceptors of electrons. Biochem J 1969; 112:231–241.
57. DeLuca HF. Retinoic acid metabolism. Fed Proc 1979; 38:2519–2523.
58. Seifter E, Rettura G, Stratford F, et al. Combination therapy with retinoids in animal models. *In* Meyskens FL, Mathe G, Schmahl D, eds, Proceedings, Spitzy KH, Karrer K, eds. Symposium Retinoids: A New Approach to Prevention and Therapy of Cancer. 1983; 201/5-201/9, SY 63-3, Part 201.
59. Medawar PB, Hunt R. Anti-cancer action of retinoids. Immunology 1981; 42:349–353.
60. Malkovsky M, Edwards AJ, Hunt R, et al. T-cell-mediated enhancement of host-versus-graft reactivity in mice fed a diet enriched in vitamin A acetate. Nature 1983; 302:338–340.
61. Lotan R, Dennert G. Stimulatory effects of vitamin A analogs on induction of cell-mediated cytotoxicity *in vivo*. Cancer Res 1979; 39:55–58.
62. Lotan R. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. Biochim Biophys Acta 1980; 605:31–91.
63. Seifter E, Rettura G, Stratford F, et al. C3HBA tumor prevention and treatment with beta carotene (Abstract #2413). Fed Proc 1981; 40:652.
64. Seifter E, Levenson SM, Rettura G. The lymphopoietic action of vitamin A and beta carotene. 181st National Meeting, AGFD, American Chemical Society, Atlanta, Georgia, 1981, March 29–April 6.

Discussion

DR. JOHN A. SCHILLING (Seattle, Washington): This is a splendid paper of Dr. Levenson's, another interesting step in a tortuous route to controlled wound healing, common to all of surgery.

As I listened to the paper, I could not help but think back 20 or 25 years ago when DeDuve described lysosomes. Vitamin A was one of the labilizing drugs, whereas cortisone was a stabilizer.

And then Tom Hunt, of this Association, some years ago described a salutary influence of vitamin A in a chronic leg ulcer.

And now this sophisticated continuation of original observations by Dr. Levenson is important. We have so many patients that we must operate on with immune suppressive drugs and irradiation therapy.

Finally, I would like to ask Dr. Levenson if he now recommends giving vitamin A to his patients in these categories? And if so, how much?

DR. CHARLES E. LUCAS (Detroit, Michigan): Did you monitor the breaking strength of the uncut skin? And if so, was the uncut skin altered by the radiation, and was that alteration ameliorated by the vitamin A?