# Supplemental Vitamin A Prevents the Tumor-induced Defect in Wound Healing

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**To test our hypothesis that supplemental vitamin A would mitigate the impaired healing that occurs in tumor-bearing animals, six groups of C3H mice, eight per group, eating a standard commercial mouse chow *ad libitum* that supports normal growth, reproduction, and longevity were innoculated with 200,000 C3HBA cells. When tumors measured approximately 6 mm in diameter, the mice were anesthesized and wounded (dorsal skin incisions and subcutaneous polyvinyl alcohol sponges). Twenty-four hours later, two groups (one continued on the chow and the other started on the chow supplemented with 150,000 IU vitamin A/kg chow) underwent local tumor irradiation; two groups, one ingesting the chow, the other the vitamin A supplemented chow, were started on cyclophophimide therapy; two groups, one ingesting the chow, the other the vitamin A supplemented chow, received neither local tumor irradiation nor cyclophosphamide therapy. An additional two groups ingesting the chow, one group neither innoculated with tumor nor wounded, the other wounded by not innoculated, served as controls. Wound breaking strength and sponge reparative collagen accumulation (assessed by hydroxyproline proline measurement) were used as indicators of wound healing. The mice were killed 12 days after wounding. Tumor presence decreased wound breaking strength and sponge hydroxyproline content; these effects were largely negated by supplemental vitamin A. Local tumor irradiation diminished the adverse effect of tumor on sponge reparative collagen content but to a lesser extent than the supplemental vitamin A. Supplemental vitamin A added to the irradiation effect on healing but irradiation did not add to the vitamin A effect. Cyclophosphamide, a systemic radiomimetic anti-tumor agent, did not alter the impaired wound healing of the tumor-bearing mice. Supplemental vitamin A mitigated the impaired wound healing in the cyclophosphamide-treated tumor-bearing mice. Supplemental vitamin A also moderated the effects of wounding, tumor, and tumor therapies (local irradiation and cyclophosphamid) on the increase in adrenal size, leukopenia, thrombocytopenia, and thymic involution (except the last was not moderated in the cyclophosphamide-treated tumor-bearing rats). The splenic enlargement in the untreated tumor-bearing wounded rats and in those treated with cyclophosphamide was lessened by supplemental vitamin A. We hypothesize that these anti-stress effects of vitamin A underlie, in part, its action in mitigating the impaired wound healing of tumor-bearing mice, including those treated by local irradiation or cyclophosphamide. These findings have implications for the care of patients with malignant tumors.**

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TUMOR DIAGNOSIS AND therapy frequently involve the creation of incisional wounds (*e.g.*, during biopsies or resections). The healing of dermal wounds in rats is impaired by the presence of tumors, even when distant from the wound site.[1] In addition, cancer patients are commonly treated with chemotherapeutic agents (*e.g.*, cyclophosphamide, doxorubicin) and/or irradiation to the site of tumor resection. These tumor therapeutic measures inhibit the healing of the incisional wounds in rodents[2–4] and cause systemic toxicity characterized by thymic involution, adrenal hypertrophy, leukopenia, thrombocytopenia, gastrointestinal ulceration, and immune depression.[5] All of these effects reduce the efficacy of the treatment. If these adjunctive therapies are instituted very shortly after tumor excisional surgery without compromising wound healing or host defense

Supported in part by Grant GM-35,768 to The Albert Einstein College of Medicine.

Presented in part at the 55th Annual Meeting of the American Society of Plastic and Reconstructive Surgery, Los Angeles, CA, October 26–31, 1986; the 71st Annual Meeting of the Federation of American Societies for Experimental Biology, Washington, DC, March 28–April 2, 1987; and the 39th Annual Meeting of the Surgical Forum of the American College of Surgeons, Chicago, IL, October 23–28, 1988.

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Accepted for publication August 7, 1989.

mechanisms, they will be even more valuable adjuncts in the care of patients with cancer than they are presently.

Previous studies in our laboratory investigating the role of supplemental dietary vitamin A as an adjunctive treatment in tumor-bearing animals receiving cyclophosphamide (CY) or radiation (local X or whole body) showed that the supplemental vitamin A significantly reduced the systemic toxicity resulting from these agents without compromising, and in fact, adding to their anti-tumor actions.[6,7] Additional studies with wounded non–tumor-bearing rodents receiving either CY or whole-body radiation have shown that vitamin A prevents both the CY- and radiation-induced wound-healing defects.[4,8]

In the present study the role of dietary supplemental vitamin A in preventing the tumor-induced wound-healing defect in otherwise untreated tumor-bearing mice and in tumor-bearing mice that received CY treatment or local irradiation to the site of the tumor was examined.

## Materials and Methods

### *Animals and Housing*

When received in our animal institute, 5-week-old female C3H/HeN mice (The Jackson Laboratory, Bar Harbor, ME) were distributed randomly, 6 per cage, in plastic shoe box-type cages with wire mesh lids. They were kept in a windowless room at 25 C ± 1 C, 50% to 60% relative humidity with a 12-hour light/12-hour dark cycle. Purina Laboratory Chow 5001 (Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum* during the period of acclimatization.

### *Diets*

Purina Ground Laboratory Chow 5001 was used for the basal control chow. It contains approximately 15,000 IU vitamin A and 6.4 mg B carotene/kg diet (total = 18,000 IU vitamin A/kg diet), considerably more than the National Research Council's recommended daily allowance of vitamin A for normal mice and permits normal growth, pregnancy, lactation, and longevity. The basal control diet is therefore not deficient in vitamin A.

The experimental diet was prepared by the addition to the basal control chow of an alcoholic (ethanol) solution of vitamin A palmitate (150,000 IU/kg chow). The mixture was stirred for 1.5 hours in the dark. During this period most of the ethanol evaporated. Control chow was treated with equal volumes of ethanol. The diets were stored in the dark at 4 C. The food in the diet cups was changed every other day.

### *Tumors and Inoculation*

C3HBA breast adenocarcinoma was obtained from The Jackson Laboratory as a transplant in young female C3H/HeJ mice and maintained by passage in C3H mice. Inoculua were prepared from solid tumors 1.4 cm in diameter, as previously described.[9] Mice receiving tumor cells were inoculated subcutaneously with $2 \times 10^5$ cells in the mid-abdominal region.

### *Tumor Measurements*

Inoculum sites were palpated gently daily to determine the date of gross tumor appearance. After a tumor appeared, its size was estimated daily with the use of a caliper with millimeter divisions. Tumor size was recorded as the average of the major and minor diameters of the generally ovoid tumor.

### *Wounding*

When tumors measured 6.0 to 6.5 mm in diameter (7 to 10 days after inoculation) the mice were wounded; two mice of each group were operated on each of 4 successive days.

Operations (4 cm paravertebral dorsal skin incision with subcutaneous implantation of saline-moistened polyvinyl alcohol sponges) were performed aseptically on mice anesthetized lightly with intraperitoneal sodium pentobarbital (1 mg/20 g body weight) supplemented as necessary with light ether anesthesia. The dry weight of the sponges averaged 8 to 10 mg each. The incisions were closed with seven equidistant No. 36 stainless steel sutures. No dressings were applied.

### *Cyclophosphamide Chemotherapy*

Cyclophosphamide (Mead Johnson Laboratories, Evansville, IN) was administered intraperitoneally at a dosage of 24 mg CY/kg body weight in 1 ml normal saline 1, 3, and 5 days after wounding to some mice.

### *Tumor X-Irradiation*

Some mice were anesthetized lightly with sodium pentobarbital (1 mg/20 g body weight, i.p.) and were then irradiated with a Picker-Vanguard 280-kv peak x-ray therapy unit (half value layer = 0.5 mm copper) on the first postoperative day. The unit was calibrated to deliver 450 cGY per minute and was timed to deliver a single dose of 750 cGY to the mid-abdominal region bearing the tumor; the rest of the body was shielded by a 2 mm thick lead shield. The radiation was delivered through a port field 1 cm in diameter.

### *Blood Leukocyte and Platelet Counts*

Blood samples were obtained by heart puncture under anesthesia from two of the eight mice in each group just before animals were killed 12 days after wounding. For

total leukocyte counts, erythrocytes were lysed with 0.1 N HCl and the remaining leukocytes were counted in a Neubauer hemocytometer. Platelets were counted from blood samples obtained during heart puncture using the method of Tocantins.[10]

*Wound Breaking Strength Measurements, Assessment of Reparative Collagen Accumulation in Sponges*

Twelve days after wounding, mice were injected intraperitoneally with the described dose of sodium pentobarbital. The length of the skin incision was measured *in situ* and the scar was excised with generous margins of surrounding skin. The breaking strength was measured in the fresh state as described previously.[11] The subcutaneous polyvinylalcohol sponges were removed and their hydroxyproline content (OH-P) was measured by the method of Woessner[12] as an index of the collagen content of the sponge reparative tissue.

*Adrenals, Thymus, and Spleen*

At the time of wound strength measurement, the adrenal glands, thymus, and spleen were dissected from each animal and weighed.

*Statistical Treatment*

All data, other than the blood leukocyte and platelet counts, were analyzed by ANOVA and then by the Tukey Method.[13]

*Experimental Design*

Eight groups of eight female C3H/HeN mice were established. One group served as the control and was neither wounded nor inoculated with tumor cells, nor treated with radiation, CY, or vitamin A. Six of the remaining seven groups were inoculated subcutaneously in the mid-abdominal region with $2 \times 10^5$ C3HBA tumor cells. When tumors averaged 6.0 to 6.5 mm in diameter, mice were wounded as described. Those animals that were wounded but not inoculated with tumor cells and did not receive any additional treatment served as wounded controls. Twenty-four hours after wounding, two of the six C3HBA-inoculated groups were irradiated, another two received CY (both as described previously), and the remaining two received no additional therapy (*i.e.*, neither irradiation nor CY). One group of each pair described continued on the basal control chow while the other group of each pair received the vitamin A supplemented chow at this time and for the duration of the experimental period. All mice ate and drank tap water *ad libitum*.

Twelve days after wounding, wound breaking strengths (WBS) and hydroxyproline content (OH-P) of implanted sponge reparative tissue were determined, total leukocytes and platelets counted (in two of eight mice in each group), and selected organs (adrenals, thymus, and spleen) were weighed.

## Results

*Wound Breaking Strength and Hydroxyproline Measurement (Table 1)*

Wound breaking strengths of skin incisions and accumulation of reparative collagen in the subcutaneously implanted polyvinyl alcohol sponges were less in the tumor-bearing wounded animals (group 3) compared to the non–tumor-bearing wounded animals (group 2) measured 12 days after wounding ($p < 0.001$ in each case). Supplemental dietary vitamin A (group 4) prevented the decreases ($p < 0.001$ in each case). WBS and OH-P measurements were similar in the vitamin A-supplemented, wounded, tumor-bearing animals (group 4) and wounded animals that had not been inoculated with tumor cells

TABLE 1. *Wound Breaking Strength and Hydroxyproline Measurements*

| Group | Treatment | WBS (g) | OH-P (μg/100 mg dry sponge) |
|---|---|---|---|
| 1 | Control | — | — |
| 2 | Wounded | 328 ± 22* | 541 ± 32* |
| 3 | WD + C3HBA (BA) | 196 ± 19 | 203 ± 19 |
| 4 | WD + BA + VA | 319 ± 29 | 547 ± 18 |
| 5 | WD + BA + 750 cGy | 260 ± 20 | 277 ± 14 |
| 6 | WD + BA + 750 cGy + VA | 381 ± 23 | 519 ± 20 |
| 7 | WD + BA + CY | 201 ± 20 | 181 ± 12 |
| 8 | WD + BA + CY + VA | 308 ± 18 | 503 ± 24 |
| Groups | 2 *vs.* 3 | <0.001 | <0.001 |
| | 2 *vs.* 4 | NS | NS |
| | 2 *vs.* 5 | NS | <0.001 |
| | 2 *vs.* 6 | NS | NS |
| | 2 *vs.* 7 | <0.001 | <0.001 |
| | 2 *vs.* 8 | NS | NS |
| | 3 *vs.* 4 | <0.001 | <0.001 |
| | 3 *vs.* 5 | NS | <0.005 |
| | 3 *vs.* 6 | <0.001 | <0.001 |
| | 3 *vs.* 7 | NS | NS |
| | 3 *vs.* 8 | <0.01 | <0.001 |
| | 4 *vs.* 5 | NS | <0.001 |
| | 4 *vs.* 6 | NS | NS |
| | 4 *vs.* 7 | <0.01 | <0.001 |
| | 4 *vs.* 8 | NS | NS |
| | 5 *vs.* 6 | <0.005 | <0.001 |
| | 5 *vs.* 7 | NS | <0.001 |
| | 5 *vs.* 8 | NS | <0.001 |
| | 6 *vs.* 7 | <0.001 | <0.001 |
| | 6 *vs.* 8 | NS | NS |
| | 7 *vs.* 8 | <0.01 | <0.001 |

NS, not significant.
WD, wounded.
BA, C3HBA.
VA, vitamin A.
CY, cyclophosphamide.
* Mean ± SEM.

nor given vitamin A (*i.e.*, wounded control animals (group 2).

Sponge OH-P but not WBS was significantly higher in tumor-bearing animals ingesting the basal control chow that had received 750 cGY local irradiation to the tumor site (group 5) compared with unirradiated, tumor-bearing animals (group 3) ($p < 0.005$), but the latter was still significantly less than in the wounded control animals (group 2) ($p < 0.001$). No increases in WBS or sponge OH-P were seen in the CY-treated tumor-bearing group ingesting the basal control chow (group 7) compared with the wounded tumor-bearing mice (group 3).

Vitamin A supplementation increased WBS and sponge OH-P in the tumor-irradiated (group 6) and CY-treated (group 8) groups to levels that were similar to those of the wounded non–tumor-bearing control animals (group 2). There were no significant differences in WBS or sponge OH-P content between vitamin A-supplemented, irradiated (group 6), and vitamin A-supplemented CY-treated animals (group 8).

### *Adrenal Gland Size (Table 2)*

Adrenal gland weights in wounded control animals compared with unwounded controls were similar. Statistically significant increase in adrenal weight was observed in the tumor-bearing wounded animals ingesting the basal chow (group 3) compared with the wounded controls (group 2) ($p < 0.001$). This effect was prevented by vitamin A supplementation (group 4) ($p < 0.001$).

Adrenal gland weights were increased to a similar extent in the irradiated (group 5) and unirradiated (group 3) tumor-bearing animals ingesting the basal chow. An additional statistically significant increase in adrenal weight was seen in CY-treated tumor-bearing mice (group 7) compared with untreated (group 3) or irradiated (group 5) tumor-bearing animals, $p < 0.001$, in each case. Adrenal gland weight in irradiated or CY-treated animals given supplemental vitamin A was lowered to values similar to those of unwounded (group 1) and wounded (group 2) controls.

### *Thymus Gland Size (Table 2)*

Thymus gland weight was less in wounded control mice (group 2) than in unwounded controls (group 1) 12 days after wounding ($p < 0.005$ to 0.001). This decrease was not altered in the tumor-bearing wounded mice ingesting the basal chow (group 3). Supplemental vitamin A (group 4) prevented the thymic involution of the latter group ($p < 0.01$–$0.005$).

Local irradiation of the tumor in wounded rats led to an additional modest decrease in thymic weight, but this was not statistically significant. Vitamin A supplementation did not lead to an increase in thymic weight in such mice. In contrast, wounded tumor-bearing animals treated with CY (group 7) ingesting the basal chow had thymuses that were significantly smaller than wounded tumor-bearing animals that were untreated (group 3) or that received local irradiation (group 5), ($p < 0.001$ and $p < 0.05$ to 0.01, respectively). Vitamin A supplementation (group 8) moderated ($p < 0.001$) the thymic involution that resulted from CY treatment and returned thymus size to values comparable to those of wounded controls (group 2).

TABLE 2. *Adrenal, Thymic, and Splenic Weights in Tumor-Bearing Wounded Mice*

| Group | Treatment | Adrenals (mg) | Thymus (mg) | Spleen (mg) |
|---|---|---|---|---|
| 1 | Control | 4.8 ± 0.3* | 40.6 ± 1.4* | 170 ± 6* |
| 2 | Wounded | 4.6 ± 0.2 | 31.0 ± 1.2 | 163 ± 10 |
| 3 | WD + C3HBA | 6.4 ± 0.1 | 31.9 ± 1.2 | 375 ± 12 |
| 4 | WD + BA + VA | 4.8 ± 0.2 | 39.5 ± 1.1 | 285 ± 9 |
| 5 | WD + BA + 750 cGy | 6.5 ± 0.3 | 26.0 ± 3.1 | 396 ± 24 |
| 6 | WD + BA + 750 cGy + VA | 4.7 ± 0.2 | 28.1 ± 0.8 | 322 ± 13 |
| 7 | WD + BA + CY | 8.1 ± 0.2 | 19.0 ± 0.7 | 397 ± 24 |
| 8 | WD + BA + CY + VA | 4.9 ± 0.2 | 30.8 ± 1.1 | 300 ± 18 |
| Groups | 1 *vs.* 2 | NS | 0.005–0.001 | NS |
| | 1 *vs.* 3 | <0.001 | 0.01–0.005 | <0.001 |
| | 1 *vs.* 4 | NS | NS | 0.01–0.005 |
| | 1 *vs.* 5 | <0.001 | <0.001 | <0.001 |
| | 1 *vs.* 6 | NS | <0.001 | <0.001 |
| | 1 *vs.* 7 | <0.001 | <0.001 | <0.001 |
| | 1 *vs.* 8 | NS | 0.005–0.001 | 0.005–0.001 |
| | 2 *vs.* 3 | <0.001 | NS | <0.001 |
| | 2 *vs.* 4 | NS | 0.005–0.001 | <0.001 |
| | 2 *vs.* 5 | <0.001 | NS | <0.001 |
| | 2 *vs.* 6 | NS | NS | <0.001 |
| | 2 *vs.* 7 | <0.001 | <0.001 | <0.001 |
| | 2 *vs.* 8 | NS | <0.001 | <0.001 |
| | 3 *vs.* 4 | <0.001 | 0.01–0.005 | <0.01 |
| | 3 *vs.* 5 | NS | NS | NS |
| | 3 *vs.* 6 | <0.001 | NS | NS |
| | 3 *vs.* 7 | <0.001 | <0.001 | NS |
| | 3 *vs.* 8 | <0.001 | NS | 0.05–0.025 |
| | 4 *vs.* 5 | <0.001 | <0.001 | <0.005 |
| | 4 *vs.* 6 | NS | <0.001 | NS |
| | 4 *vs.* 7 | <0.001 | <0.001 | <0.001 |
| | 4 *vs.* 8 | NS | 0.005–0.001 | NS |
| | 5 *vs.* 6 | <0.001 | NS | NS |
| | 5 *vs.* 7 | <0.001 | 0.05–0.01 | NS |
| | 5 *vs.* 8 | <0.001 | NS | <0.01 |
| | 6 *vs.* 7 | <0.001 | <0.001 | <0.05 |
| | 6 *vs.* 8 | NS | NS | NS |
| | 7 *vs.* 8 | <0.001 | <0.001 | <0.005 |

NS, not significant.
WD, wounded.
BA, C3HBA.
VA, vitamin A.
CY, cyclophosphamide.
* Mean ± SEM.

### *Spleen Size (Table 2)*

Spleen weight was similar in unwounded and wounded non–tumor-bearing mice. Statistically significant increases in splenic weight were seen in the tumor-bearing, wounded

animals ingesting the basal chow (group 3) compared with the unwounded (group 1) and wounded controls (group 2) ($p < 0.001$ in each case). This effect was moderated by vitamin A supplementation (group 4) ($p < 0.01$), but the spleens of these animals were still significantly enlarged compared with those of unwounded (group 1) and wounded controls (group 2) (p 0.01–0.005 and $p < 0.001$, respectively).

Irradiation (group 5) and CY treatment (group 7) resulted in slight additional increases in spleen weight in tumor-bearing animals, but these increases were not statistically significant. Supplemental vitamin A moderated these decreases, but the effect was significant ($p < 0.005$) only in the case of the CY-treated tumor-bearing mice.

TABLE 3. *White Blood Cell and Platelet Counts (Heart Blood)*

| Group | Treatment | WBC, $10^3/mm^3$ | Platelets, $10^3/mm^3$ |
|---|---|---|---|
| 1 | Control | 13.4;12.3 | 528;522 |
| 2 | Wounded | 14.1;12.8 | 438;485 |
| 3 | WD + C3HBA | 10.0;9.1 | 214;287 |
| 4 | WD + BA + VA | 16.9;15.7 | 388;324 |
| 5 | WD + BA + 750 cGy | 9.6;8.0 | 300;264 |
| 6 | WD + BA + 750 cGy + VA | 14.7;16.5 | 382;343 |
| 7 | WD + BA + Cy | 8.1;7.5 | 95;147 |
| 8 | WD + BA + Cy + VA | 14.8;13.4 | 267;307 |

Values for 2 mice from each group.
WD, wounded.
BA, C3HBA.
VA, vitamin A.
CY, cyclophosphamide.

*White Blood Cell and Platelet Counts (Table 3)*

These data are on two of eight mice in each group. White blood cell and platelet counts in control (unwounded, non–tumor-bearing, group 1) and wounded non–tumor-bearing control mice (group 2) were similar 12 days after wounding. Tumor-bearing, wounded mice (group 3) were leukopenic and thrombocytopenic compared with wounded controls; both effects were mitigated by vitamin A supplementation (group 4).

WBC counts were somewhat lower in the tumor-bearing mice that received local irradiation (group 5) or CY (group 7) compared with the untreated tumor-bearing mice (group 3). Platelet counts were decreased comparably in irradiated (group 5) and unirradiated (group 3) tumor-bearing mice, while the thrombocytopenia was accentuated in the CY-treated mice (group 7). Supplemental vitamin A (groups 6 and 8) mitigated the reduced WBC and platelet counts in both the irradiated and CY-treated tumor-bearing mice (groups 5 and 7).

## Discussion

*The Tumor-Induced Wound Healing Defect*

Healing of skin incisions and the formation of reparative tissue in subcutaneously implanted sponges involves a complex series of events including bleeding, coagulation, the deposition and activation of platelets, deposition of fibrin, and influx of inflammatory cells (at first polymorphonuclear cells and then mononuclear cells) at the site of the injury in the early phases of wound repair. After several days, endothelial cell proliferation and new capillary formation, along with fibroplasia and collagen and proteoglycan production, begin with subsequent increase in wound strength. Epithelialization over the surface of the skin incision occurs rapidly and involves mainly cell migration and proliferation. Injury, disease, metabolic, physiologic, nutritional alterations, and some therapeutic agents can affect wound healing by interfering with one or more of these steps and result in impaired wound strength.

Devereux and associates[1] have shown that wound healing in rats bearing tumors is severely impaired as assessed by measurements of the breaking strength of healing skin incisions and the hydroxyproline content of subcutaneous polyvinyl alcohol sponge reparative tissue. The tumor-induced healing defect was accompanied by body weight loss, hypoproteinemia, and cachexia, factors that themselves may impair wound healing. This is supported by a clinical study by Irwin and colleagues[14] in which an increase in incisional hernias was observed in patients with malignancies.

Our experimental data presented in this report also show that dorsal skin incision breaking strength and accumulation of reparative collagen in subcutaneous implanted sponge granulomas were reduced significantly in mice bearing C3HBA tumors. The tumor-induced thrombocytopenia observed in the representative mice studied may have caused deficient blood clotting after wounding and may have resulted in diminished levels of platelet-derived growth factors (PDGF), polypeptides released on platelet activation, which play a key role in wound repair. PDGF is chemotactic for leukocytes and fibroblasts *in vitro*, accelerates endothelial cell and fibroblast proliferation *in vitro*, and accelerates wound healing *in vivo*.[15–18]

*Irradiation-Induced Wound Healing Defect*

Local irradiation, whole-body irradiation, and cyclophosphamide administration all weaken wounds of non–tumor-bearing animals. As shown by Devereux and associates[1] and confirmed in the present work, the presence of tumor decreases wound strength, although the wound is distant from the tumor site. Our data suggest that the depression of wound strength by tumor is greater than the decrease due to the level of local x-radiation or

cyclophosphamide therapy used in these experiments. In earlier experiments in our laboratory, C3HBA tumors were shown to be stressful and immunosuppressive to the host. Surgery (sham excision) was shown also to be stressful and immunosuppressive in non–tumor-bearing controls, as was surgery (local tumor excision) in tumor-bearing mice. However after 5 days the stressful and immunosuppressive effects of the operation had largely disappeared and these mice had recovered to a level approximating that of unoperated non–tumor-bearing mice.

Local and whole-body irradiation of healthy animals result in decreased body weight gain, thymic involution, adrenal enlargement, lymphopenia, thrombocytopenia,[5,6] and impaired wound healing.[8] Radiation injury is, to a large extent, the result of damage to cell constituents by products of water radiolysis (*i.e.*, OH and e). The OH free radical formed and its dimer $H_2O_2$ are responsible for some of the radiation-induced cell damage. Removal of the OH or $H_2O_2$ may be accomplished by the use of some radioprotective agents such as aminoalkylthiols, which become oxidized to disulfides while reducing the OH of $H_2O_2$ to $H_2O + \frac{1}{2} O_2$. The hydrated electron produced by radiolysis of water is sufficiently energized to cause radical formation in nucleic acids and thereby favor crosslinking of nucleic acids or cleavage of the link between the base and the deoxy sugar. Undifferentiated, rapidly proliferating cells (*e.g.*, fibroblasts, bone marrow precursors, neoplastic cells) are especially sensitive to such damage.

Vitamin A and the carotenoids are molecules that have evolved as the active sites of radiant and electromagnetic energy systems in both plants and animals. Some carotenoids are also localized in structures that produce oxygen radicals and pro-oxidants *in situ*. These systems are capable of energy acceptance and either transference or transduction of that energy. Theoretically vitamin A could protect cells against the radiolysis of water 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 or quench hydrated electrons until these are disposed of by chemical interactions with electrophilic agents or by metabolic use of hydrated electrons attached to oxygen (superoxide $O_2^-$).[6,19,20]

### *Cyclophosphamide-Induced Wound Healing Defect*

Cyclophosphamide is a synthetic compound widely used in tumor chemotherapy, in organ transplantation as a immunosuppressive agent to prevent allograft organ rejection, and in the management of some disease entities having both severe immune and inflammatory aspects. It is a radiomimetic alkylating agent (*i.e.*, it produces some of the chemical changes and physiologic actions induced by radiation) that require activation by oxidative liver enzymes to form the cytotoxic compound. In addition to the anti-tumor metabolite, acrolein, a toxic and tumor-promoting agent is formed.

Cyclophosphamide inhibits the proliferation of undifferentiated cells, including mesenchymal cells, fibroblasts, bone marrow precursors, and neoplastic cells. As a result there is decreased wound neovascularization net accumulation of reparative collagen and wound strength, pancytopenia, thrombocytopenia, and diminished inflammatory reactions. In addition a host stress reaction occurs that causes an increase in glucocortical hormone secretion resulting in thymic involution, increase in adrenal weight, and body weight loss.[4]

### *Roles of Supplemental Dietary Vitamin A in the Healing of Wounds and the Prevention of the Tumor- and Tumor Therapy-Induced Healing Defects*

The basal control commercial chow used in our study is not deficient in vitamin A. In fact it contains considerably more vitamin A than the National Research Council recommendation for normal, actively growing mice. Thus the effects we have seen when the vitamin A-supplemented chow was given were not due to correction of a vitamin A-deficient diet.

Our data regarding the ameliorating effects of supplemental dietary vitamin A on wound breaking strength, reparative collagen deposition, thymus, adrenal, and spleen weights, and presumably on peripheral blood leukocyte and platelet counts in tumor-bearing wounded animals are in accord with previous findings in our laboratory of (1) the healing–accelerating actions of supplemental dietary vitamin A in wounded animals treated with whole-body irradiation[8] or cyclophosphamide;[4] (2) the influence of supplemental dietary vitamin A in preventing the thymic involution, adrenal weight increase, and splenic weight increase that occur in wounded rats, animals with femoral fracture, tumor-bearing mice, and rats subjected to whole-body irradiation[9,21,22]; and (3) the alleviation of the leukopenia and thrombocytopenia by supplemental dietary vitamin A in mice after local x-irradiation of a hind limb,[5] in unwounded mice exposed to whole-body irradiation,[19] and in wounded rats treated with cyclophosphamide.[4]

### *Stress and Wound Healing: The Phenomenology of Supplemental Vitamin A*

A strong correlation seems to exist between wound responses and the thymus gland. Agents that increase thymic weight and enhance thymic function, such as supplemental vitamin A[21,22] and supplemental arginine,[23] have a stimulatory effect on wound breaking strength and reparative collagen accumulation.[23–25] The mechanisms by

which vitamin A and arginine protect the thymus gland from the involuting effect of stress is not known. Conversely agents that depress thymic function, such as cytotoxic drugs, glucocorticoids, and various other stressors, impair wound healing.[4,26,27] On the other hand, wound healing is accelerated in thymectomized adult rats.[28]

Accordingly in the present study thymus gland weight was measured. The prevention or mitigation of the tumor or CY–therapy-induced thymic involution by supplemental vitamin A correlated directly with a prevention of the wound-healing defect. No such correlation was found in the irradiated tumor-bearing rats. In these animals thymic weight was not increased significantly by the supplemental vitamin A, but both wound breaking strength and hydroyproline content of the sponges were increased significantly to the levels of wounded non–tumor-bearing mice.

The regulatory mechanisms for initiating, sustaining, and terminating wound repair are not understood completely. The inflammatory phase is one of the earliest events in wound healing leading to subsequent angiogenesis, fibroblast migration and proliferation, and proteoglycan and collagen deposition and reorganization. The roles of the various inflammatory cell elements and their products in wound healing are under investigation. For almost 60 years it has been recognized that white blood cells play an important role in healing.[29] Neutrophils are not required for apparently normal healing of skin incisions,[30,31] although wound complications such as infection occur more frequently in patients with neutropenia or derangements of neutrophil function.[32] Lymphocyte- and macrophage-secreted monokines stimulate fibroblast migration, replication, and collagen synthesis.[33]

Among the ways supplemental vitamin A affects healing are (1) enhancing the early inflammatory reaction to wounding, including increasing the number of monocytes and macrophages at an injury site[22]; (2) possibly modulating collagenase activity[34] (the accumulation of reparative collagen in the healing wound is the result of a net balance between collagen synthesis and collagenolysis); (3) stimulating epithelial cell[35] and possibly mesenchymal cell (fibroblast) differentiation[36]; and (4) stimulating immune responsiveness.[37] The possible interactions on a molecular level among vitamin A and platelets, platelet-derived growth factors, lymphocytes and lymphocyte-derived growth factors, macrophages and macrophage-derived growth factors, endothelial cells and endothelial cell-derived growth facts, fibroblasts and fibroblast-derived growth factors remain to be elucidated.

The results we report take on an additional importance for clinical practice in light of recent studies of the treatment of stage I, node-negative breast tumor patients.[38–41] The data suggest that many such patients have undiagnosed micrometastases at the time of initial treatment and that a significant number of those patients undergoing their initial treatment would benefit by early initiation of adjuvant hormonal or cytotoxic chemotherapy. Supplemental vitamin A may make early chemotherapy and radiotherapy more feasible by promoting wound healing and lessening the immunosuppression that would otherwise follow the chemotherapy or radiotherapy. Supplemental vitamin A also may enhance the anti-tumor actions of the recommended tumor therapies, effects that have been shown in tumor-bearing rodents.[7,42,43]

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