

Inhibitory Effects of Vitamin E on Collagen Synthesis and Wound Repair

H. PAUL EHRLICH, PH.D., HAROLD TARVER, PH.D.,
THOMAS K. HUNT, M.D.

*From the Department of Surgery and Department of Biochemistry and Biophysics
at the University of California, San Francisco, California 94122*

VITAMIN E, a name given to a series of related tocopherols which have analogous biological effects, has been advocated as a stimulant to healing. Unfortunately, no controlled studies have been done. Based upon the findings of Lucy and Dingle,⁸ vitamin E is now known to be a lysosomal stabilizer, which would place it in the group of compounds such as glucocorticoids and aspirin which are anti-inflammatory agents^{10, 15} and depressants to healing.^{3, 6} Lysosomal labilizing compounds such as vitamin A, digitonin, testosterone and papain, stimulate collagen synthesis and repair.^{5, 9} Of these compounds, testosterone and vitamin A have been shown to reverse the retarding effects of glucocorticoids on repair.^{2, 3} Stuyvesant and Jolley¹⁴ described vitamin E as an anti-inflammatory agent. Therefore, our previous findings of interaction of cortisol and vitamin A suggest that vitamin E may inhibit inflammation and wound repair and that aspects of this inhibitory effect can be reversed by concurrent administration of vitamin A.

This study was designed to test further the hypothesis that anti-inflammatory agents which stabilize lysosomes (including vitamin E) will inhibit collagen synthesis. Since vitamin E has been described as both an

aid to healing and an anti-inflammatory agent, the study was designed to settle the question by testing the effect of vitamin E upon collagen synthesis and wound repair.

Materials and Methods

Adult male Sprague-Dawley rats weighing 350 to 440 Gm. were maintained in individual cages and were fed water and commercial rat feed ad libitum. No other nutrient supplement was given.

After the rats were anesthetized with ether, their backs were shaved with hair clippers and the entire shaved area was washed with tincture of iodine.

A standard incision, 6 cm. long, was made on the back 1.5 cm. from and parallel to the spinal cord. The wound was closed with a continuous 4-0 stainless steel suture. The wound was left uncovered. Ivalon Intermedic sponge discs 1 cm. in diameter and 0.2 cm. thick were implanted through separate incisions on the ventral side.

The rats were divided into five groups:

Group 1 (controls). Eight rats received no injections. Previous studies in this and other laboratories have shown that sham injections have no effect upon collagen synthesis or healing.¹³

Group 2 (corticoid alone). Six rats received 8 mg. of Depo-Medrol (prednisolone acetate) subcutaneously and 0.2 ml. of sterile peanut oil intramuscularly on days one, three and five. The day of surgery is defined as day one.

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Request reprints: Thomas K. Hunt, M.D.,
Department of Surgery, University of California
Medical Center, San Francisco, California 94122.

TABLE 1. Summary of Dosage Schedule

| Group | No. Rats | Material Injected | Site | Day of Inspection |
|-----------------------|----------|--|---------------|--------------------|
| Group I (Controls) | 8 | None | | |
| Group II | 6 | 8 mg. Depo-Medrol 0.2 ml. peanut oil | S.C. I.M. | 1 1, 3, 5 |
| Group III | 8 | 8 mg. Depo-Medrol 0.2 ml. dl-alpha-tocopherol acetate | S.C. I.M. | 1 1, 3, 5 |
| Group IV | 8 | 0.2 ml. dl-alpha-tocopherol acetate | I.M. | 1, 3, 5 |
| Group V | 8 | 0.2 ml. dl-alpha-tocopherol acetate 0.1 ml. Vitamin A palmitate | I.M. I.M.* | 1, 3, 5 1, 3, 5 |

Subcutaneous injection (S.C.) in the dorsum of the neck.

Intramuscular injection (I.M.) in the thigh.

I.M.* indicates an intramuscular injection in the thigh opposite to that injected with dl-alpha-tocopherol.

Group 3 (corticoids plus vitamin E). Eight rats received 8 mg. of prednisolone acetate subcutaneously and 0.2 ml. (190 IU) of dl-alpha-tocopherol acetate intramuscularly on days one, three, and five.

Group 4 (vitamin E alone). Eight rats received 0.2 ml. of dl-alpha-tocopherol intramuscularly on days one, three, and five.

Group 5 (vitamin E plus vitamin A). Eight rats received 0.2 ml. of dl-alpha-tocopherol intramuscularly on days one, three, and five and 0.1 ml. (5,000 IU) of vitamin A palmitate (Aqualon A) was injected into a separate muscle site on days one, three, and five. The injection schedule is described in Table 1.

TABLE 2. Results—Effects of Vitamin E, Vitamin A, and Glucocorticoids

| No. | Group | Change in Body Weight Gm. | Tensile Strength Gm. ± S.E. | Total Hydroxyproline mcg ± S.E. | Hydroxyproline Dry Weight mcg/mg ± S.E. | Ratio of mcg/gm Hydroxyproline Tensile Strength |
|-----|----------------------------|---------------------------|-----------------------------|---------------------------------|---|---|
| 1 | Control | +17 | 316 ± 13 | 479 ± 26 | 9.4 ± .8 | 1.5 |
| 2 | Corticoid Alone | -59 | 191 ± 15 a | 294 ± 21 b | 8.2 ± .9 c | 1.5 |
| 3 | Corticoid, Vitamin E | -66 | 209 ± 16 a | 274 ± 18 b | 8.2 ± .7 c | 1.5 |
| 4 | Vitamin E alone | +15 | 265 ± 11 a | 418 ± 39 b | 8.2 ± 1.0 c | 1.5 |
| 5 | Vitamin E and Vitamin A | +17 | 364 ± 30 a | 574 ± 26 b | 10.8 ± 1.0 c | 1.5 |

Key: a—Tensile strength of Groups 2, 3, 4 and 5 are statistically different from groups 1 and 2 ($p < 1\%$).

b—The total hydroxyproline content of polyvinyl discs from groups 2, 3 and 4 are statistically less than group 1 and group 5 is statistically more than group 1 ($p < 5\%$).

c—The specific concentration of mcg hydroxyproline per mg. of dry weight is statistically significant for groups 2, 3, 4 and 5 as compared to group 1 ($p < 5\%$).

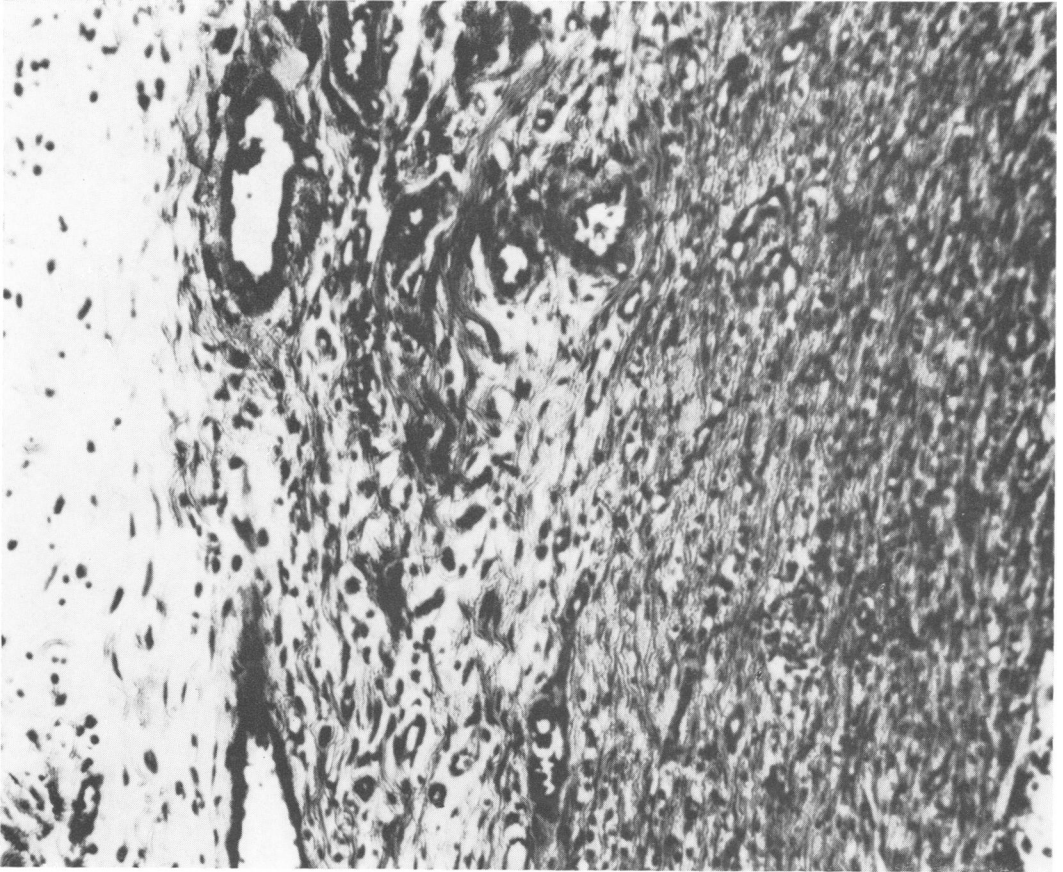


FIG. 1. Histologic appearance of control sponges (Group 1). Outside of sponge is to the right. Inflammatory reaction is somewhat greater than usual.

The tensile strength of the wounds was measured by a Sandblom-Muren Tensiometer by the method reported by Ehrlich and Hunt in 1968.² The polyvinyl sponge implants were carefully removed on day eight and their wet and dry weights were recorded. The dried implants were hydrolyzed in 6N HCl at 110°C. for 24 hours. The hydrolysates were filtered with fiberglass paper filters and their hydroxyproline content was measured with a Technicon AutoAnalyzer by the method of Grant.⁴

Two polyvinyl sponge discs 1 cm. in diameter and 0.1 cm. thick were sandwiched between two silicone discs of stopper material of the same dimensions and were implanted by the method described above. Six rats were divided into three

treatment groups of two rats each. Group 1 (controls) received no injections. Group 2 (vitamin E alone) received 0.2 ml. of dl-alpha-tocopherol acetate intramuscularly on days one, three, and five. Group 3 (vitamin E plus vitamin A) received 0.2 ml. of dl-alpha-tocopherol acetate intramuscularly on days one, three, and five, and 0.1 ml. (5,000 IU) of vitamin A palmitate at a separate intramuscular site on days, one, three and five.

The implants were removed on day eight. The silicone discs were removed from the polyvinyl sponge which was then fixed in buffered 10% formaldehyde. Sections were cut in the circular plane of the disc and were stained with hematoxylin and eosin and Mallory phosphotungstic acid-

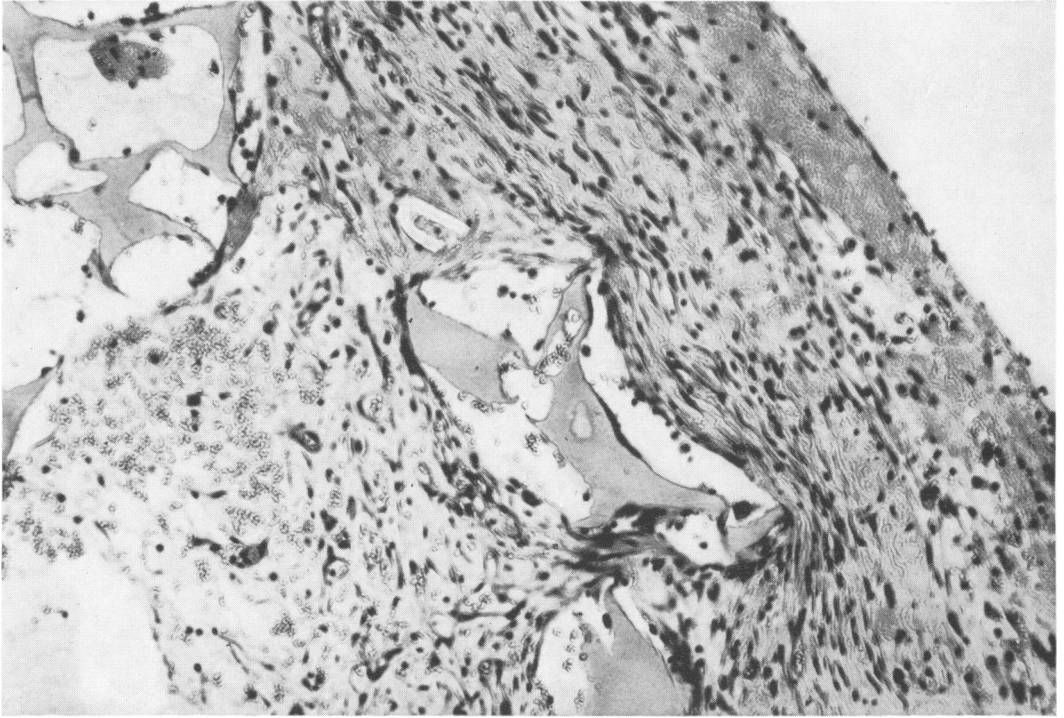


FIG. 2. Histologic appearance of sponge taken from an animal treated by vitamin E alone. Outside of sponge is to the right. Note paucity of cells and vessels. Sponges taken from group treated with cortisol alone could not be distinguished from the group treated with vitamin E alone.

hematoxylin (PTAH) stain. The resulting sections were circular.

Results

The tensile strength, hydroxyproline content, and changes in body weight are summarized in Table 2. When the analysis of variance was used as the statistical method, it was found that corticoids and vitamin E each significantly retarded collagen synthesis and tensile strength at 7 days. Vitamin E did not alter the effects of glucocorticoids. Vitamin A reversed the retarding effect of vitamin E (and has previously been shown to alter the effects of cortisone).² The animals that received vitamin A plus vitamin E "healed" significantly faster than controls.

Both tensile strength and total hydroxyproline were increased. The amount of increase is approximately that to be expected during treatment with vitamin A alone.⁵ No

other explanation is offered. Vitamin E did not affect body weight, but corticoids did induce weight loss. The weight loss does not adequately explain the wound inhibitory effects of glucocorticoids.¹³ There were 1.5 micrograms of hydroxyproline per gram of tensile strength in all groups. This demonstrates the consistent correlation between these two measurements and serves as a useful check on accuracy of data. The reduction in collagen accumulation was apparently responsible for the decrease in wound tensile strength.

A silicone-polyvinyl sponge-silicone disc "sandwich" was used to achieve a unidirectional movement of cells and tissue from the periphery into the center of the polyvinyl sponge disc. Each sponge section contained concentric zones of collagen fibers, capillaries, fibroblasts, inflammatory cells and fibrin. At 7 days the polyvinyl

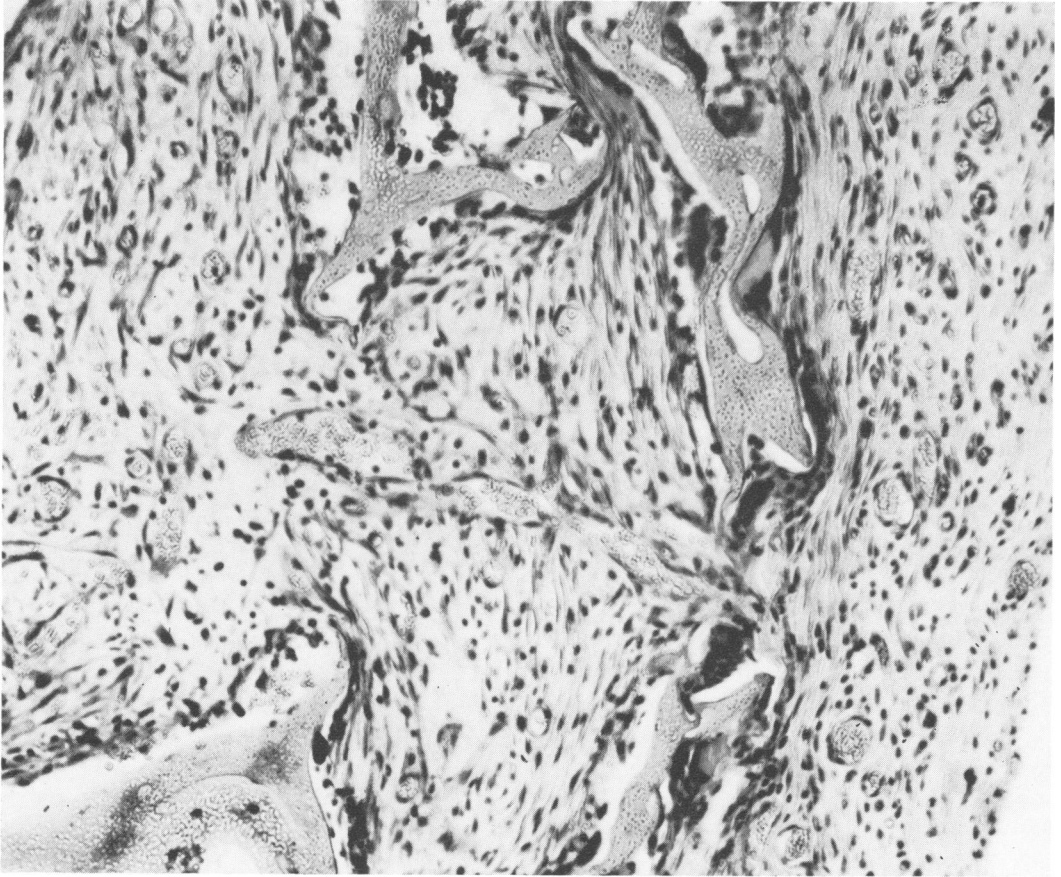


FIG. 3. Sponge taken from animal given both vitamins A and E. Note greater vascularity and cellularity.

sponge from untreated controls contained collagen and a great number of cells and capillaries (Fig. 1). In contrast, the microscopic sections from the group of rats that received vitamin E alone had the appearance of less collagen and fewer cells (Fig. 2). The administration of vitamin A concurrently with vitamin E (Group 3) produced more collagen and a greater number of cells as compared to rats receiving E alone (Fig. 3). The results suggest that the administration of vitamin E inhibits the inflammatory response, reduces the number of fibroblasts, and retards the accumulation of collagen. The implants from rats treated with vitamin E were similar to the implants removed from rats treated with corticoid.

Discussion

We can find no well-controlled published studies demonstrating a beneficial effect of vitamin E on wound healing or collagen synthesis. When given to rats, vitamin E prevents sterility in males and resorption of fetuses in females. In dogs and rabbits a deficiency of vitamin E is responsible for a form of muscular dystrophy. Vitamin E is a biological anti-oxidant which prevents peroxides from accumulating and protects cells from damaging effects of free radicals. Vitamin E also ensures the stability and integrity of biological membranes.⁸

The first studies of the effects of vitamin E on biological membranes showed it to be a lysosomal labilizer *in vitro*, when used in

high pharmacological concentrations.¹ *In vivo* a moderate amount of vitamin E acts as a lysosomal stabilizer. Vitamin E protects lysosomal membranes from the labilizing effects of excessive vitamin A.¹²

Glucocorticoids and aspirin inhibit healing.^{6, 7} Wounds treated with glucocorticoids contain fewer fibroblasts than do untreated control wounds.¹¹ The severity of the effect is related to dose and time sequence. Sandberg¹³ demonstrated that cortisone administered after the second day following injury did not retard healing as measured by collagen accumulation and wound strength. Thus, the effects of glucocorticoids are diminished after inflammation has become established. The action of glucocorticoids apparently depends more upon its anti-inflammatory than on its anti-synthetic action.

These findings lend further support to the idea that lysosomal stabilizers inhibit collagen synthesis and repair, while lysosomal labilizers reverse this retardation. Unlike the corticoid-treated group, the animals treated with vitamin E did not lose weight. It is unlikely that vitamin E and vitamin A have direct effects upon one another *in vivo*. In fact, vitamin E is added to commercial vitamin preparations to prevent oxidation of vitamin A.

The results of this study indicate that vitamin E may have clinical value in modifying scar formation. In this respect, it could prove superior to corticoids by virtue of its lesser side effects.

Summary

Vitamin E inhibited wound healing, as measured by the tensile strength and accumulation of collagen. It did not reverse the retardation of wound healing by glucocorticoids. On the other hand, the retarding effect of vitamin E on healing was reversed by vitamin A. Thus vitamin E acts on healing in a manner similar to that of corticoids.

Possible mechanisms for the inhibitory effects of vitamin E upon collagen synthesis and repair are discussed. Vitamin E may be of clinical value as an agent for modifying undesirable scar formation.

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